European Commission

Combined Draft Renewal Assessment Report prepared according to Regulation (EC) N° 1107/2009
and
Proposal for Harmonised Classification and Labelling (CLH Report) according to Regulation (EC) N° 1272/2008

Glyphosate

Volume 1

Rapporteur Member State: Assessment Group on Glyphosate (AGG) consisting of FR, HU, NL and SE

Version History

When	What
2021/06	Initial RAR

The RMS is the author of the Assessment Report. The Assessment Report is based on the validation by the RMS, and the verification during the EFSA peer-review process, of the information submitted by the Applicant in the dossier, including the Applicant's assessments provided in the summary dossier. As a consequence, data and information including assessments and conclusions, validated and verified by the RMS experts, may be taken from the applicant's (summary) dossier and included as such or adapted/modified by the RMS in the Assessment Report. For reasons of efficiency, the Assessment Report should include the information validated/verified by the RMS, without detailing which elements have been taken or modified from the Applicant's assessment. As the Applicant's summary dossier is published, the experts, interested parties, and the public may compare both documents for getting details on which elements of the Applicant's dossier have been validated/verified and which ones have been modified by the RMS. Nevertheless, the views and conclusions of the RMS should always be clearly and transparently reported; the conclusions from the applicant should be included as an Applicant's statement for every single study reported at study level; and the RMS should justify the final assessment for each endpoint in all cases, indicating in a clear way the Applicant's assessment and the RMS reasons for supporting or not the view of the Applicant.

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Level 1

Glyphosate

1 STATEMENT OF SUBJECT MATTER AND PURPOSE FOR WHICH THIS REPORT HAS BEEN PREPARED AND BACKGROUND INFORMATION ON THE APPLICATION

1.1 CONTEXT IN WHICH THIS DRAFT ASSESSMENT REPORT WAS PREPARED

1.1.1 Purpose for which the draft assessment report was prepared

This renewal assessment report (RAR) has been prepared in accordance with Regulation (EC) No 1107/2009, Commission Implementing Regulation (EU) No 844/2012 and the EFSA Administrative Guidance Document[1] in order to evaluate the application and the collective dossier submitted by Knoell Germany GmbH on behalf of the Glyphosate Renewal Group (GRG) and to allow a decision on the renewal of the approval of the active substance glyphosate.

GRG submitted an application for MRL setting in honey. However, due to a data gap no MRL for honey is proposed.

A proposal for Classification and Labelling is included in this renewal assessment report.

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

Commission appointed with Commission Implementing Regulation (EU) 2019/724 four Member States (France, Hungary, the Netherlands and Sweden) to act jointly as 'rapporteurs' for the renewal of glyphosate. This group of Member States is known as the Assessment Group on Glyphosate (AGG).

1.1.3 EU Regulatory history for use in Plant Protection Products

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

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

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

Glyphosate was included in Annex I of Council Directive 91/414/EEC with Commission Directive 2001/99/EC (OJ L 304/14, 21.11.2001), entering into force on 1 July 2002, with an expiry date of 30 June 2012.

Commission Directive 2010/77/EU extended the expiry date for glyphosate to 31 December 2015.

Commission Implementing Regulation (EU) No 540/2011 arranged glyphosate to be deemed to have been approved under Regulation (EC) No 1107/2009.

First renewal of approval (AIR-2 programme)
In agreement with Article 4 of Regulation (EC) No 1141/2010, Monsanto Europe N.V./S.A. on behalf of the European Glyphosate Task Force submitted an application to Germany as RMS and Slovakia as Co-RMS notifying the intention to renew the existing approval of glyphosate on 24 March 2011.

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

[1] European Food Safety Authority, 2019. Administrative guidance on submission of dossiers and assessment reports for the peer-review of pesticide active substances, EFSA supporting publication 2019:EN-1612. 49 pp. doi:10 2903/sp.efsa.2019.EN-1612

The renewal assessment report, prepared by Germany and Slovakia, was submitted to Commission and EFSA on 20 December 2013.

The International Agency for Research on Cancer (IARC) published a Monograph containing detailed information on its evaluation as regards the carcinogenic potential of glyphosate in July 2015. The Commission mandated EFSA to review the underlying information and to include those findings in its conclusion.

Commission Implementing Regulation (EU) 2015/1885 extended until 30 June 2016 the period of approval of glyphosate to allow the completion of its review.

On 30 October 2015, EFSA sent to the Commission its conclusion on the risk assessment of glyphosate (Conclusion on the peer review of the pesticide risk assessment of the active substance glyphosate (EFSA Journal 2015;13(11):4302)).

Also on 30 October 2015, following a request from the Commission dated 19 November 2014, EFSA sent to the Commission a 'Statement of EFSA on the request for the evaluation of the toxicological assessment of the co-formulant POE-tallowamine' (EFSA Journal 2015;13(11):4303).

Commission Implementing Regulation (EU) 2016/1056 extended until "6 months from the date of receipt of the opinion of the Committee for Risk Assessment of the European Chemicals Agency (ECHA) by the Commission or 31 December 2017, whichever is the earlier" the period of approval of glyphosate to allow the completion of the assessment of the dossier concerning the harmonised classification and the completion of its review. Given that the Opinion of the Committee for Risk Assessment of ECHA was submitted to the Commission on 15 June 2017, the expiry date of glyphosate was extended until 15 December 2017.

The conditions of approval of glyphosate were amended in light of the new scientific and technical knowledge by Commission Implementing Regulation (EU) 2016/1313. Reference is made to the 'Addendum to the Review report for the active substance glyphosate (SANTE/11051/2016, rev 0, 11 July 2016).

On 7 September 2017, following a request from the Commission dated 27 September 2016, EFSA sent to the Commission a conclusion on the potential endocrine disrupting properties of glyphosate (Conclusion on the peer review of the pesticide risk assessment of the potential endocrine disrupting properties of glyphosate. EFSA Journal 2017;15(9):4979).

The Renewal Report on renewal of approval (SANTE/10441/2017, Rev 2, 9 November 2017) was finalised in the meeting of the Standing Committee on 9 November 2017.

The approval of glyphosate in accordance with Regulation (EC) No 1107/2009 was renewed with Commission Implementing Regulation (EU) 2017/2324. The expiry date for glyphosate is 15 December 2022.

Second renewal of approval (AIR-5 programme)

On 10 May 2019, the Commission appointed four Member States (France, Hungary, the Netherlands and Sweden) acting jointly as 'rapporteurs' for the renewal of glyphosate (Commission Implementing Regulation (EU) 2019/724). This group of Member States is known as the Assessment Group on Glyphosate (AGG).

In accordance with Article 1 of Commission Implementing Regulation (EU) No 844/2012, the Glyphosate Renewal Group (GRG, comprising eight companies with Bayer Agriculture BV as lead registrant) submitted before the deadline of 15 December 2019 an application to all members of the AGG. The application was checked by the members of the AGG according to Article 3 of the aforementioned Regulation. The members of the AGG concluded on 31 January 2020 that – after setting a period of 14 days for GRG to submit missing elements which were received in time – the application contained all elements provided for in Article 2 of the aforementioned Regulation.

In accordance with Article 6 of Commission Implementing Regulation (EU) No 844/2012, GRG submitted before the deadline of 15 June 2020, a supplementary dossier to all members of the AGG. On 18 August 2020 – after setting a period of 14 days for GRG to submit missing elements which were received in time – the members of the AGG concluded that the dossier contained all elements provided for in Article 7 of the aforementioned Regulation and that the application was admissible.

1.1.4 Evaluations carried out under other regulatory contexts

The International Agency for Research on Cancer (IARC) published a Monograph containing detailed information on its evaluation as regards the carcinogenic potential of glyphosate in July 2015:
IARC Monographs on the evaluation of carcinogenic risks to humans; volume 112.

In 2016, glyphosate was re-evaluated by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR).

European Chemicals Agency (ECHA) (2017). Opinion of the Committee for Risk Assessment proposing harmonised classification and labelling of glyphosate (ISO); N-(phosphonomethyl)glycine (EC Number: 213-997-4; CAS Number: 1071-83-6).

In 2017, the Canadian Pest Management Regulatory Agency (PMRA) re-evaluated glyphosate (RVD2017 and RVD 2017-01)

In 2019, EFSA reviewed the existing maximum residue levels for glyphosate according to Article 12 of Regulation (EC) No 396/2005 (EFSA reasoned opinion, adopted 27 September 2019, amended 16 March 2020, doi: 10.2903/j.efsa.2019.5862)

In January 2020, the American Environmental Protection Agency (EPA) released an interim decision for glyphosate: US EPA - Glyphosate Interim Registration Review Decision Case Number 0178

1.2 APPLICANT INFORMATION

Applicants:

1	Company:	Bayer Agriculture BV[2] Lead registrant on behalf of the Glyphosate Renewal Group
	Address:	Haven 627 Scheldelaan 460 B-2040 Antwerp Belgium
	Contact:	[redacted] Bayer AG, Crop Science Division Alfred Nobel Str. 50 40789 Monheim am Rhein Germany
	Telephone: Fax: Email:	[redacted]
2	Company:	Barclay Chemicals Manufacturing Ltd.
	Address:	Damastown Way Damastown Industrial Park Mulhuddart Dublin 15 Ireland
	Contact: Telephone: Fax: Email:	[redacted]
3	Company:	CIECH Sarzyna S.A.

[2] In accordance with the new Belgian Code on Companies and Associations, Bayer Agriculture BVBA's legal form will be formally converted into Bayer Agriculture BV in the beginning of August 2020. Other than legal form change, all other details of the company as well as its address will remain unchanged

Address: ul. Wspólna 62
00-684 Warschau
Poland

Contact: [redacted]

Telephone:

Fax: Not available

Email: [redacted]

4 Company: Albaugh Europe SARL

Address: World Trade Center Lausanne
Avenue Gratta-Paille 2
1018 Lausanne
Switzerland

Contact: [redacted]

Telephone:

Fax: N/A

Email: [redacted]

5 Company: Nufarm GmbH & Co KG

Address: St.-Peter-Str. 25
A-4021 Linz
Austria

Contact: [redacted]

Telephone:

Fax: Not available

Email: [redacted]

6 Company: SINON Corporation

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Taichung City 43245
Taiwan (R.O.C.)

Contact: [redacted]

Telephone:

Fax: Not available

Email: [redacted]

7 Company: Industrias Afrasa, S.A.

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

Contact: [redacted]

Telephone:

Fax:

Email:	[redacted]
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Address:	Rosentalstrasse 67 CH-4002 Basel Switzerland
Contact:	[redacted]
Telephone:	
Fax:	Not available
Email:	[redacted]

Consultant and primary contact:

Company:	Knoell Germany GmbH on behalf of the Glyphosate Renewal Group towards the European Competent Regulatory Authorities
Address:	Konrad-Zuse-Ring 25, 68163 Mannheim, Germany
Contact:	[redacted]
Telephone:	
Email:	

1.2.1 Producer or producers of the active substance

CONFIDENTIAL information – Reference is made to Volume 4.

1.2.2 Information relating to the collective provision of dossiers

For the renewal of approval of the active substance glyphosate and its related salts (variants), a task force ("Glyphosate Renewal Group") has been established among the companies listed under 1.2.1. A joint dossier is submitted by Knoell Germany GmbH on behalf of the Glyphosate Renewal Group (GRG).

The submitted joint dossier for the chemical active substance contains data packages of the members of the GRG, as well as new studies commissioned by the GRG.

1.3 IDENTITY OF THE ACTIVE SUBSTANCE

1.3.1 Common name proposed or ISO-accepted and synonyms	Common name (ISO): Glyphosate Related salt-types: Glyphosate-isopropyl-amine-salt Glyphosate-potassium-salt Glyphosate-ammonium-salt Glyphosate-dimethylammonium-salt
1.3.2 Chemical name (IUPAC and CA nomenclature)	
IUPAC	Glyphosate N-(phosphonomethyl)glycine Glyphosate-isopropyl-amine-salt N-(phosphonomethyl)glycine isopropylammonium Glyphosate-potassium-salt N-(phosphonomethyl)glycine monopotassium salt Glyphosate-ammonium-salt N-(phosphonomethyl)glycine monoammonium salt Glyphosate-dimethylammonium-salt N-(phosphonomethyl)glycine dimethylammonium salt
CA	Glyphosate Glycine, N-(phosphonomethyl)- Glyphosate-isopropyl-amine-salt N-(phosphonomethyl)glycine isopropylammonium salt Glyphosate-potassium-salt N-(phosphonomethyl)glycine potassium salt Glyphosate-ammonium-salt N-(phosphonomethyl)glycine ammonium salt Glyphosate-dimethylammonium-salt N-(phosphonomethyl)glycine dimethylammonium salt

1.3.3 Producer's development code number	Bayer uses the following code numbers: For Glyphosate technical material: MON 77973 For Glyphosate, isopropylamine salt: MON 0139 (62% aqueous solution), MON 77209 (dry solid) For Glyphosate, ammonium salt: MON 8750 For Glyphosate, potassium salt: MON 78623 Nufarm uses the following code numbers: Glyphosate Technical: CA2515 & CA3203.
1.3.4 CAS, EEC and CIPAC numbers	
CAS	Glyphosate CAS No.: 1071-83-6 Glyphosate isopropyl-amine-salt CAS No.: 38641-94-0 Glyphosate potassium-salt (monopotassium salt) CAS No.: 39600-42-5 Glyphosate ammonium-salt CAS No.: 114370-14-8 Glyphosate - dimethylammonium salt CAS No.: 34494-04-7
EEC	Glyphosate EC No.: 213-997-4 Glyphosate isopropyl-amine-salt EC No.: 254-056-8 Glyphosate potassium-salt (monopotassium salt) EC No.: Not available Glyphosate ammonium-salt EC No.: Not available Glyphosate - dimethylammonium salt EC No.: Not available
CIPAC	Glyphosate CIPAC No.: 284 Glyphosate isopropyl-amine-salt CIPAC No.: 284.105 Glyphosate potassium-salt (monopotassium salt)

	CIPAC No.: 284.019 Glyphosate ammonium-salt CIPAC No.: 284.007 Glyphosate - dimethylammonium salt CIPAC No.: 284.102

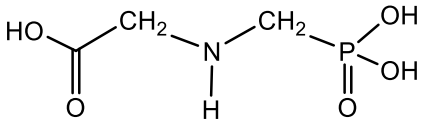
1.3.5 Molecular and structural formula, molecular mass

Molecular formula
Structural formula
Molecular mass

Glyphosate

Molecular formula: $C_3H_8NO_5P$

Structural formula:

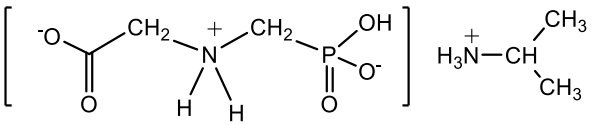


Molecular mass: 169.1 g/mol

Glyphosate isopropyl-amine-salt

Molecular formula:: $C_6H_{17}N_2O_5P$

Structural formula:

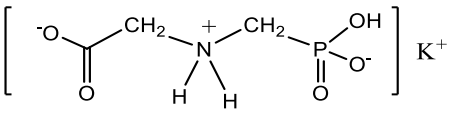


Molecular mass: 228.18 g/mol

Glyphosate potassium-salt (monopotassium salt)

Molecular formula: $C_3H_7KNO_5P$

Structural formula:

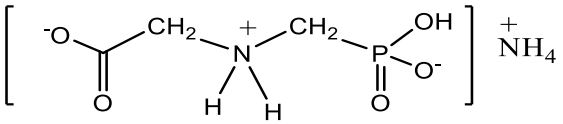


Molecular mass: 207.19 g/mol

Glyphosate - ammonium salt

Molecular formula: $C_3H_{11}N_2O_5P$

Structural formula:

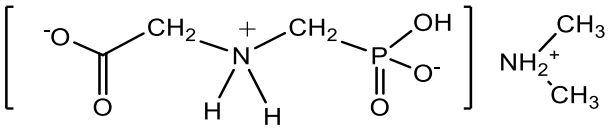


Molecular mass: 186.10 g/mol

Glyphosate - dimethylammonium salt

Molecular formula: $C_5H_{15}N_2O_5P$

Structural formula:



Molecular mass: 214.15 g/mol

1.3.6 Method of manufacture (synthesis pathway) of the active substance	CONFIDENTIAL information - data provided separately (Volume 4)
1.3.7 Specification of purity of the active substance in g/kg	950 g/kg
1.3.8 Identity and content of additives (such as stabilisers) and impurities	
1.3.8.1 Additives	CONFIDENTIAL information - data provided separately (Volume 4)
1.3.8.2 Significant impurities	CONFIDENTIAL information - data provided separately (Volume 4)

1.3.8.3 Relevant impurities

The active substance as manufactured contains the four impurities formaldehyde, N-nitrosoglyphosate (NNG), formic acid and triethylamine which are considered as relevant because of their toxicological properties.

IUPAC name: Formaldehyde
CA name: Formaldehyde

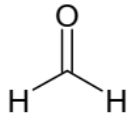
ISO common name: Formaldehyde

CAS No: 50-00-0

EC No: 200-001-8

Molecular formula: CH_2O

Structural formula:



Molecular mass: 30.03 g/mol

Maximum content: 1.0 g/kg

IUPAC name: *N*-nitroso-*N*-(phosphonomethyl)-glycine
CA name: 2-[nitroso(phosphonomethyl)amino]-acetic acid

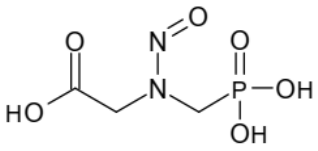
ISO common name: not available

CAS No: 56516-72-4

EC No: not available

Molecular formula: $C_3H_7N_2O_6P$

Structural formula:



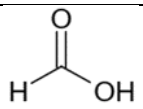
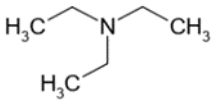
Molecular mass: 198.07 g/mol

Maximum content: 1.0 mg/kg

IUPAC name: Formic acid

CA name: Formic acid

ISO common name: Formic acid
CAS No: 64-18-6
EC No: 200-579-1
Molecular formula: CH2O2

	Structural formula:  Molecular mass: 46.03 g/mol Maximum content: 4 g/kg IUPAC name: Triethylamine CA name: Triethylamine ISO common name: Triethylamine CAS No: 121-44-8 EC No: 204-469-4 Molecular formula: C6H15N Structural formula:  Molecular mass: 101.19 g/mol Maximum content: 2 g/kg
1.3.9 Analytical profile of batches	CONFIDENTIAL information - data provided separately (Volume 4)

1.4 INFORMATION ON THE PLANT PROTECTION PRODUCT

1.4.1 Applicant	
1.4.2 Producer of the plant protection product	Company: Bayer Agriculture BV Address: Haven 627 Scheldelaan 460 B-2040 Antwerp Belgium
1.4.3 Trade name or proposed trade name and producer's development code number of the plant protection product	MON 52276
1.4.4 Detailed quantitative and qualitative information on the composition of the plant protection product	

1.4.4.1 Composition of the plant protection product	CONFIDENTIAL information - data provided separately
1.4.4.2 Information on the active substances	Content of active substance: Glyphosate, pure 360 g/L
1.4.4.3 Information on safeners, synergists and co-formulants	CONFIDENTIAL information - data provided separately
1.4.5 Type and code of the plant protection product	Soluble concentrate (SL)
1.4.6 Function	Herbicide
1.4.7 Field of use envisaged	Currently, MON 52276 has registered uses not only in agriculture, horticulture, orchards and vines, but also in forestry, amenity, weed control of non-cultivated areas, home and garden uses, amongst others. The uses in the representative GAP of this renewal dossier cover uses as pre-sowing, pre-planting and pre-emergence in vegetables and sugar beet, post-harvest, pre-sowing and pre-planting in vegetables and sugar beet, post-emergence of weeds in orchards, vines, vegetables, railway tracks against emerged annual, biennial and perennial weeds as well as cereal volunteers (for post-harvest, pre-sowing, pre-planting). Moreover, uses as spot treatment against invasive species and in vegetables and sugar beet against couch grass are included.
1.4.8 Effects on harmful organisms	Glyphosate is a non-selective herbicidal active substance within the chemical class of glycines, without any soil residual activity. Additionally, EPSPS enzyme does not exist in animals. Glyphosate is taken up by the leaves and other green parts of the plant and is translocated systemically (apoplastic and symplastic) in the whole plant, also in underground parts like roots, rhizomes or stolons. Symptoms of the herbicidal activity are: First signs of wilting occur in annual weeds 4 days and in perennial weeds 7 to 10 days after application of the herbicide. Leaf symptoms are usually detected 7 to 14 days after application, while a complete destruction of the plant takes up to 30 days. As light affects the metabolism via photosynthesis, a higher activity in plants means a better distribution of glyphosate and thus a greater herbicidal effect. Increasing temperatures result in increased biochemical activity and thus in an increased rate of efficacy. Optimum temperatures are 10 to 20 °C. High humidity affects the quality of the leaf surface and thus promotes the uptake of the herbicide.

1.5 DETAILED USES OF THE PLANT PROTECTION PRODUCT

1.5.1 Details of representative uses

PPP (product name/code) **active substance 1** **safener** **synergist**	**MON 52276** **glyphosate as isopropylammonium salt** **-** **-**	**Formulation type:** **Conc. of as 1:** expressed as glyphosate acid, which corresponds to 360 g/L for MON 52276 **Conc. of safener:** **Conc. of synergist:**	**SL** **360 g/L (486 g/L isopropylammonium salt) -** **-** **-**
Applicant: **Zone(s):**	**GRG** **central, southern and northern**	**professional use** **non-professional use**	☒ ☐
Verified by MS:	**y/n**		

1	2	3	4	5	6	7	8	10	11	12	13	14
Use-No.	**Member state(s)**	**Crop and/ or situation** **(crop destination / purpose of crop)**	**F G o r I**	**Pests or Group of pests controlled** **(additionally: developmental stages of the pest or pest group)**	**Application**			**Application rate**			**PHI (days)**	**Remarks:** **e.g. safener/synergist per ha** **e.g. recommended or mandatory tank mixtures**
					Method / Kind	**Timing / Growth stage of crop & season**	**Max. number (min. interval between applications)** **a) per use** **b) per crop/ season**	**kg, L product/ha** **a) max. rate per appl.** **b) max. total rate per crop/season**	**g, kg as/ha** **a) max. rate per appl.** **b) max. total rate per crop/season**	**Water L/ha** **min / max**		
PRE-SOWING, PRE-PLANTING, PRE-EMERGENCE												
1a	EU	Root & tuber vegetables, Bulb vegetables, Fruiting vegetables, Brassica, Leafy vegetables, Stem vegetables, Sugar beet	F	Emerged annual weeds, emerged perennial and biennial weeds BBCH > 13	Tractor mounted broadcast spray	Pre-sowing, Pre-planting, Pre-emergence of the crop	a) 1 b) 1	a) 4 L/ha b) 4 L/ha	a) 1.44 kg as/ha b) 1.44 kg as//ha	100 – 400	N/A	Also applicable to renovation / change of land use applications. Application to 100 % of the field. Use 75 % drift reducing nozzles. Maximum application rate of 1.44 kg as/ha glyphosate in any 12 months period.

1	2	3	4	5	6	7	8	10	11	12	13	14
Use-No.	**Member state(s)**	**Crop and/ or situation** **(crop destination / purpose of crop)**	**F G o r I**	**Pests or Group of pests controlled** **(additionally: developmental stages of the pest or pest group)**	**Application**			**Application rate**			**PHI (days)**	**Remarks:** **e.g. safener/synergist per ha** **e.g. recommended or mandatory tank mixtures**
					Method / Kind	**Timing / Growth stage of crop & season**	**Max. number (min. interval between applications)** **a) per use** **b) per crop/ season**	**kg, L product/ha** **a) max. rate per appl.** **b) max. total rate per crop/season**	**g, kg as/ha** **a) max. rate per appl.** **b) max. total rate per crop/season**	**Water L/ha** **min / max**		
1b	EU	Root & tuber vegetables, Bulb vegetables, Fruiting vegetables, Brassica, Leafy vegetables, Stem vegetables, Sugar beet	F	Emerged annual weeds, emerged perennial and biennial weeds (BBCH 13 – 21)	Tractor mounted broadcast spray	Pre-sowing, Pre-planting, Pre-emergence of the crop	a) 1 b) 1	a) 3 L/ha b) 3 L/ha	a) 1.08 kg as/ha b) 1.08 kg as//ha	100 – 400	N/A	Also applicable to renovation / change of land use applications. Application to 100 % of the field. Use 75 % drift reducing nozzles. Maximum application rate of 1.08 kg as/ha glyphosate in any 12 months period.
1c	EU	Root & tuber vegetables, Bulb vegetables, Fruiting vegetables, Brassica, Leafy vegetables, Stem vegetables, Sugar beet	F	Emerged annual weeds	Tractor mounted broadcast spray	Pre-sowing, Pre-planting, Pre-emergence of the crop	a) 1 b) 1	a) 2 L/ha b) 2 L/ha	a) 0.72 kg as/ha b) 0.72 kg as/ha	100 – 400	N/A	Also applicable to renovation / change of land use applications. Application to 100 % of the field. Use 75 % drift reducing nozzles. Maximum application rate of 0.72 kg as/ha glyphosate in any 12 months period.

1	2	3	4	5	6	7	8	10	11	12	13	14
Use-No.	**Member state(s)**	**Crop and/ or situation** **(crop destination / purpose of crop)**	**F G o r I**	**Pests or Group of pests controlled** **(additionally: developmental stages of the pest or pest group)**	**Application**			**Application rate**			**PHI (days)**	**Remarks:** **e.g. safener/synergist per ha** **e.g. recommended or mandatory tank mixtures**
					Method / Kind	**Timing / Growth stage of crop & season**	**Max. number (min. interval between applications)** **a) per use** **b) per crop/ season**	**kg, L product/ha** **a) max. rate per appl.** **b) max. total rate per crop/season**	**g, kg as/ha** **a) max. rate per appl.** **b) max. total rate per crop/season**	**Water L/ha** **min / max**		
POST-HARVEST, PRE-SOWING, PRE-PLANTING												
2a	EU	Root & tuber vegetables, Bulb vegetables, Fruiting vegetables, Brassica, Leafy vegetables, Stem vegetables, Sugar beet	F	Emerged annual, perennial and biennial weeds	Tractor mounted broadcast spray	Post-harvest, pre-sowing, pre-planting	a) 1 – 2 (28 days) b) 1 – 2 (28 days)	a) 3 – 4 L/ha b) 6 L/ha	a) 1.08 – 1.44 kg as/ha b) 2.16 kg as/ha	100 – 400	N/A	Application to existing row cropland after harvest for removal of remaining crop / stubble and for control of actively growing weeds and mature annual weeds with hardened-off surface Application to 100 % of the field. Use 75 % drift reducing nozzles. Maximum application rate of 2.16 kg as/ha glyphosate in any 12 months period.
2b	EU	Root & tuber vegetables, Bulb vegetables, Fruiting vegetables, Brassica, Leafy vegetables, Stem vegetables, Sugar beet	F	Emerged annual, perennial and biennial weeds	Tractor mounted broadcast spray	Post-harvest, pre-sowing, pre-planting	a) 1 – 3 (28 days) b) 1 – 3 (28 days)	a) 2 – 3 L/ha b) 6 L/ha	a) 0.72 – 1.08 kg as/ha b) 2.16 kg as/ha	100 – 400	N/A	Application to existing row cropland after harvest for removal of remaining crop / stubble and for control of actively growing weeds. Application to 100 % of the field. Use 75 % drift reducing nozzles. Maximum application rate of 2.16 kg as/ha glyphosate in any 12 months period.

1	2	3	4	5	6	7	8	10	11	12	13	14
Use-No.	**Member state(s)**	**Crop and/ or situation** **(crop destination / purpose of crop)**	**F G o r I**	**Pests or Group of pests controlled** **(additionally: developmental stages of the pest or pest group)**	**Application**			**Application rate**			**PHI (days)**	**Remarks:** **e.g. safener/synergist per ha** **e.g. recommended or mandatory tank mixtures**
					Method / Kind	**Timing / Growth stage of crop & season**	**Max. number (min. interval between applications)** **a) per use** **b) per crop/ season**	**kg, L product/ha** **a) max. rate per appl.** **b) max. total rate per crop/season**	**g, kg as/ha** **a) max. rate per appl.** **b) max. total rate per crop/season**	**Water L/ha** **min / max**		
2c	EU	Root & tuber vegetables, Bulb vegetables, Fruiting vegetables, Brassica, Leafy vegetables, Stem vegetables, Sugar beet	F	Emerged annual weeds	Tractor mounted broadcast spray	Post-harvest, pre-sowing, pre-planting	a) 1 – 3 (28 days) b) 1 – 3 (28 days)	a) 2 L/ha b) 6 L/ha	a) 0.72 kg as/ha b) 2.16 kg as/ha	100 – 400	N/A	Application to existing row cropland after harvest for removal of remaining crop / stubble and for control of actively growing annual weeds Application to 100 % of the field. Use 75 % drift reducing nozzles. Maximum application rate of 2.16 kg as/ha glyphosate in any 12 months period.
3a	EU	Root & tuber vegetables, Bulb vegetables, Fruiting vegetables, Brassica, Leafy vegetables, Stem vegetables, Sugar beet	F	Cereal volunteers	Tractor mounted broadcast spray	Post-harvest, pre-sowing, pre-planting	a) 1 b) 1	a) 1.5 L/ha b) 1.5 L/ha	a) 0.54 kg as/ha b) 0.54 kg as/ha	100 – 400	N/A	Application to existing row cropland after harvest for removal of cereal volunteers. Maximum application rate of 0.54 kg as/ha glyphosate in any 12 months period.
3b	EU	Root & tuber vegetables, Bulb vegetables, Fruiting vegetables, Brassica, Leafy vegetables, Stem vegetables, Sugar beet	F	Cereal volunteers	Tractor mounted broadcast spray	Post-harvest, pre-sowing, pre-planting	a) 1 b) 1	a) 1.5 L/ha b) 1.5 L/ha	a) 0.54 kg as/ha b) 0.54 kg as/ha	100 – 400	N/A	Application to existing row cropland after harvest for removal of cereal volunteers once every three years. Maximum application rate of 0.54 kg as/ha glyphosate in any 36 months period.

1	2	3	4	5	6	7	8	10	11	12	13	14
Use-No.	**Member state(s)**	**Crop and/ or situation** **(crop destination / purpose of crop)**	**F G o r I**	**Pests or Group of pests controlled** **(additionally: developmental stages of the pest or pest group)**	**Application**			**Application rate**			**PHI (days)**	**Remarks:** **e.g. safener/synergist per ha** **e.g. recommended or mandatory tank mixtures**
					Method / Kind	**Timing / Growth stage of crop & season**	**Max. number (min. interval between applications)** **a) per use** **b) per crop/ season**	**kg, L product/ha** **a) max. rate per appl.** **b) max. total rate per crop/season**	**g, kg as/ha** **a) max. rate per appl.** **b) max. total rate per crop/season**	**Water L/ha** **min / max**		
POST-EMERGENCE OF WEEDS												
4a	EU	Orchard crops (citrus, stone and pome fruits, kiwi, tree nuts, banana, and table olives)	F	Emerged annual, biennial and perennial weeds	Ground directed, shielded spray, band application	Post-emergence of weeds	a) 1 – 2 (28 days) b) 1 – 2 (28 days)	a) 3 – 4 L/ha b) 8 L/ha	a) 1.08 – 1.44 kg as/ha b) 2.88 kg as/ha	100 – 400	7	Avoid crop contamination during treatment. Maximum application rate of 2.88 kg as/ha treated area glyphosate in any 12 months period. Band application in the rows below the trees or as spot treatments. The treated area represents not more than 50 % of the total orchard area. The application rate with reference to the total orchard surface area is not more than 50 % of the stated dose rate.
4b	EU	Orchard crops (citrus, stone and pome fruits, kiwi, tree nuts, banana, and table olives)	F	Emerged annual, biennial and perennial weeds	Ground directed, shielded spray, band application	Post-emergence of weeds	a) 1 – 3 (28 days) b) 1 – 3 (28 days))	a) 2 – 3 L/ha b) 8 L/ha	a) 0.72 – 1.08 kg as/ha b) 2.88 kg as/ha	100 – 400	7	Avoid crop contamination during treatment. Maximum application rate of 2.88 kg as/ha treated area glyphosate in any 12 months period. Band application in the rows below the trees or as spot treatments. The treated area represents not more than 50 % of the total orchard area. The application rate with reference to the total orchard surface area is not more than 50 % of the stated dose rate.

1	2	3	4	5	6	7	8	10	11	12	13	14
Use-No.	**Member state(s)**	**Crop and/ or situation** **(crop destination / purpose of crop)**	**F G o r I**	**Pests or Group of pests controlled** **(additionally: developmental stages of the pest or pest group)**	**Application**			**Application rate**			**PHI (days)**	**Remarks:** **e.g. safener/synergist per ha** **e.g. recommended or mandatory tank mixtures**
					Method / Kind	**Timing / Growth stage of crop & season**	**Max. number (min. interval between applications)** **a) per use** **b) per crop/ season**	**kg, L product/ha** **a) max. rate per appl.** **b) max. total rate per crop/season**	**g, kg as/ha** **a) max. rate per appl.** **b) max. total rate per crop/season**	**Water L/ha** **min / max**		
4c	EU	Orchard crops (citrus, stone and pome fruits, kiwi, tree nuts, banana, and table olives)	F	Emerged annual weeds	Ground directed, shielded spray, band application	Post-emergence of weeds	a) 1 – 3 (28 days) b) 1 – 3 (28 days)	a) 2 L/ha b) 6 L/ha	a) 0.72 kg as/ha b) 2.16 kg as/ha	100 – 400	7	Avoid crop contamination during treatment. Maximum application rate of 2.16 kg as/ha treated area glyphosate in any 12 months period. Band application in the rows below the trees or as spot treatments. The treated area represents not more than 50 % of the total orchard area. The application rate with reference to the total orchard surface area is not more than 50 % of the stated dose rate.
5a	EU	Vines (table and wine grape, leaves not intended for human consumption)	F	Emerged annual, biennial and perennial weeds	Ground directed, shielded spray, band application	Post-emergence of weeds	a) 1 – 2 (28 days) b) 1 – 2 (28 days)	a) 3 – 4 L/ha b) 8 L/ha	a) 1.08 – 1.44 kg as/ha b) 2.88 kg as/ha	100 – 400	7	Avoid crop contamination during treatment. Maximum application rate of 2.88 kg as/ha treated area glyphosate in any 12 months period. Band application in the rows below the vine stock or as spot treatments. The treated area represents not more than 50 % of the total vineyard area. The application rate with reference to the total vineyard surface area is not more than 50 % of the stated dose rate.

1	2	3	4	5	6	7	8	10	11	12	13	14
Use-No.	**Member state(s)**	**Crop and/ or situation** **(crop destination / purpose of crop)**	**F G o r I**	**Pests or Group of pests controlled** **(additionally: developmental stages of the pest or pest group)**	**Application**			**Application rate**			**PHI (days)**	**Remarks:** **e.g. safener/synergist per ha** **e.g. recommended or mandatory tank mixtures**
					Method / Kind	**Timing / Growth stage of crop & season**	**Max. number (min. interval between applications)** **a) per use** **b) per crop/ season**	**kg, L product/ha** **a) max. rate per appl.** **b) max. total rate per crop/season**	**g, kg as/ha** **a) max. rate per appl.** **b) max. total rate per crop/season**	**Water L/ha** **min / max**		
5b	EU	Vines (table and wine grape, leaves not intended for human consumption)	F	Emerged annual, biennial and perennial weeds	Ground directed, shielded spray, band application	Post-emergence of weeds	a) 1 – 3 (28 days) b) 1 – 3 (28 days)	a) 2 – 3 L/ha b) 8 L/ha	a) 0.72 – 1.08 kg as/ha b) 2.88 kg as/ha	100 – 400	7	Avoid crop contamination during treatment. Maximum application rate of 2.88 kg as/ha treated area glyphosate in any 12 months period. Band application in the rows below the vine stock or as spot treatments. The treated area represents not more than 50 % of the total vineyard area. The application rate with reference to the total vineyard surface area is not more than 50 % of the stated dose rate.
5c	EU	Vines (table and wine grape, leaves not intended for human consumption)	F	Emerged annual weeds	Ground directed, shielded spray, band application	Post-emergence of weeds	a) 1 – 3 (28 days) b) 1 – 3 (28 days)	a) 2 L/ha b) 6 L/ha	a) 0.72 kg as/ha b) 2.16 kg as/ha	100 – 400	7	Avoid crop contamination during treatment. Maximum application rate of 2.16 kg as/ha treated area glyphosate in any 12 months period. Band application in the rows below the vine stock or as spot treatments. The treated area represents not more than 50 % of the total vineyard area. The application rate with reference to the total vineyard surface area is not more than 50 % of the stated dose rate.

1	2	3	4	5	6	7	8	10	11	12	13	14
Use-No.	**Member state(s)**	**Crop and/ or situation** **(crop destination / purpose of crop)**	**F G or I**	**Pests or Group of pests controlled** **(additionally: developmental stages of the pest or pest group)**	**Application**			**Application rate**			**PHI (days)**	**Remarks:** **e.g. safener/synergist per ha** **e.g. recommended or mandatory tank mixtures**
					Method / Kind	**Timing / Growth stage of crop & season**	**Max. number (min. interval between applications)** **a) per use** **b) per crop/ season**	**kg, L product/ha** **a) max. rate per appl.** **b) max. total rate per crop/season**	**g, kg as/ha** **a) max. rate per appl.** **b) max. total rate per crop/season**	**Water L/ha** **min / max**		
6a	EU	Vegetables (Root and tuber vegetables Bulb vegetables, Fruiting vegetables Legume vegetables Leafy vegetables)	F	Emerged annual, biennial and perennial weeds	Inter-row application: ground directed, shielded spray	Crop BBCH < 20	a) 1 b) 1	a) 3 L/ha b) 3 L/ha	a) 1.08 kg as/ha b) 1.08 kg as/ha	100 – 400	60	Avoid crop contamination during treatment. Maximum application rate of 1.08 kg as/ha glyphosate in any 12 months period. Applications are performed between the crop rows. The rate refers to the treated area only, which represents not more than 50 % of the total area. The application rate with reference to the total surface area is not more than 50 % of the stated dose rate
6b	EU	Vegetables (Root and tuber vegetables Bulb vegetables, Fruiting vegetables Legume vegetables Leafy vegetables)	F	Emerged annual weeds	Inter-row application: ground directed, shielded spray	Crop BBCH < 20	a) 1 b) 1	a) 2 L/ha b) 2 L/ha	a) 0.72 kg as/ha b) 0.72 kg as/ha	100 – 400	60	Avoid crop contamination during treatment. Maximum application rate of 0.72 kg as/ha glyphosate in any 12 months period. Applications are performed between the crop rows. The rate refers to the treated area only, which represents not more than 50 % of the total area. The application rate with reference to the total surface area is not more than 50 % of the stated dose rate

1	2	3	4	5	6	7	8	10	11	12	13	14
Use-No.	**Member state(s)**	**Crop and/ or situation** **(crop destination / purpose of crop)**	**F G o r I**	**Pests or Group of pests controlled** **(additionally: developmental stages of the pest or pest group)**	**Application**			**Application rate**			**PHI (days)**	**Remarks:** **e.g. safener/synergist per ha** **e.g. recommended or mandatory tank mixtures**
					Method / Kind	**Timing / Growth stage of crop & season**	**Max. number (min. interval between applications)** **a) per use** **b) per crop/ season**	**kg, L product/ha** **a) max. rate per appl.** **b) max. total rate per crop/season**	**g, kg as/ha** **a) max. rate per appl.** **b) max. total rate per crop/season**	**Water L/ha** **min / max**		
7a	EU	Railroad tracks	F	Emerged annual, biennial and perennial weeds	Ground directed, spray	Post-emergence of weeds	a) 2 (90 days) b) 2 (90 days)	a) 5 L/ha b) 10 L/ha	a) 1.8 kg as/ha b) 3.6 kg as/ha	100 – 400	N/A	Application by spray train Maximum application rate of 3.6 kg as/ha glyphosate in any 12 months period.
7b	EU	Railroad tracks	F	Emerged annual, biennial and perennial weeds	Ground directed, spray	Post-emergence of weeds	a) 1 b) 1	a) 5 L/ha b) 5 L/ha	a) 1.8 kg as/ha b) 1.8 kg as/ha	100 – 400	N/A	Application by spray train Maximum application rate of 1.8 kg as/ha glyphosate in any 12 months period.
8	EU	Invasive species in agricultural and non-agricultural areas	F	Giant hogweed (*Heracleum mantegazzianum*)	Spot treatment (shielded)	Post-emergence of invasive species	a) 1 b) 1	a) 5 L/ha b) 5 L/ha	a) 1.8 kg as/ha b) 1.8 kg as/ha	5 – 400	N/A	Maximum application rate of 1.8 kg as/ha glyphosate in any 12 months period.
9	EU	Invasive species in agricultural and non-agricultural areas	F	Japanese knotweed (*Reynoutria japonica*)	Spot treatment (shielded), cut stem: spray application	Late summer, early fall	a) 1 b) 1	a) 5 L/ha b) 5 L/ha	a) 1.8 kg as/ha b) 1.8 kg as/ha	5 – 400	N/A	Maximum application rate of 1.8 kg as/ha glyphosate in any 12 months period.

1	2	3	4	5	6	7	8	10	11	12	13	14
Use-No.	**Member state(s)**	**Crop and/ or situation** **(crop destination / purpose of crop)**	**F G or I**	**Pests or Group of pests controlled** **(additionally: developmental stages of the pest or pest group)**	**Application**			**Application rate**			**PHI (days)**	**Remarks:** **e.g. safener/synergist per ha** **e.g. recommended or mandatory tank mixtures**
					Method / Kind	**Timing / Growth stage of crop & season**	**Max. number (min. interval between applications)** **a) per use** **b) per crop/ season**	**kg, L product/ha** **a) max. rate per appl.** **b) max. total rate per crop/season**	**g, kg as/ha** **a) max. rate per appl.** **b) max. total rate per crop/season**	**Water L/ha** **min / max**		
10a	EU	Root & tuber vegetables, Bulb vegetables, Fruiting vegetables, Brassica, Leafy vegetables, Stem vegetables, Sugar beet	F	Couch grass (*Elymus repens*)	Spot treatment (shielded)	Post-harvest, pre-sowing, pre-planting	a) 1 b) 1	a) 3 L/ha b) 3 L/ha	a) 1.08 kg as/ha b) 1.08 kg as/ha	100 – 400	N/A	Application to existing row cropland after harvest for removal of couch grass. Maximum application rate of 1.08 kg as/ha glyphosate in any 12 months period. The treated area represents not more than 20 % of the cropland.
10b	EU	Root & tuber vegetables, Bulb vegetables, Fruiting vegetables, Brassica, Leafy vegetables, Stem vegetables, Sugar beet	F	Couch grass (*Elymus repens*)	Spot treatment (shielded)	Post-harvest, pre-sowing, pre-planting	a) 1 b) 1	a) 2 L/ha b) 2 L/ha	a) 0.72 kg as/ha b) 0.72 kg as/ha	100 – 400	N/A	Application to existing row cropland after harvest for removal of couch grass. Maximum application rate of 0.72 kg as/ha glyphosate in any 12 months period. The treated area represents not more than 20 % of the cropland.
10c	EU	Root & tuber vegetables, Bulb vegetables, Fruiting vegetables, Brassica, Leafy vegetables, Stem vegetables, Sugar beet	F	Couch grass (*Elymus repens*)	Spot treatment (shielded)	Post-harvest, pre-sowing, pre-planting	a) 1 b) 1	a) 2 L/ha b) 2 L/ha	a) 0.72 kg as/ha b) 0.72 kg as/ha	100 – 400	N/A	Application to existing row cropland after harvest for removal of couch grass once every three years. Maximum application rate of 0.72 kg as/ha glyphosate in any 36 months period. The treated area represents not more than 20 % of the cropland.

Remarks table heading:	(a)	e g wettable powder (WP), emulsifiable concentrate (EC), granule (GR)
	(b)	Catalogue of pesticide formulation types and international coding system CropLife International Technical Monograph n°2, 6th Edition Revised May 2008
	(c)	g/kg or g/l
	(d)	Select relevant
	(e)	Use number(s) in accordance with the list of all intended GAPs in Part B, Section 0 should be given in column 1
	(f)	No authorization possible for uses where the line is highlighted in grey, Use should be crossed out when the notifier no longer supports this use

Remarks columns:	1	Numeration necessary to allow references
	2	Use official codes/nomenclatures of EU Member States
	3	For crops, the EU and Codex classifications (both) should be used; when relevant, the use situation should be described (e g fumigation of a structure)
	4	F: professional field use, Fn: non-professional field use, Fpn: professional and non-professional field use, G: professional greenhouse use, Gn: non-professional greenhouse use, Gpn: professional and non-professional greenhouse use, I: indoor application
	5	Scientific names and EPPO-Codes of target pests/diseases/ weeds or, when relevant, the common names of the pest groups (e g biting and sucking insects, soil born insects, foliar fungi, weeds) and the developmental stages of the pests and pest groups at the moment of application must be named
	6	Method, e g high volume spraying, low volume spraying, spreading, dusting, drench Kind, e g overall, broadcast, aerial spraying, row, individual plant, between the plants - type of equipment used must be indicated
	7	Growth stage at first and last treatment (BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4), including where relevant, information on season at time of application
	8	The maximum number of application possible under practical conditions of use must be provided
	9	Minimum interval (in days) between applications of the same product
	10	For specific uses other specifications might be possible, e g : g/m³ in case of fumigation of empty rooms See also EPPO-Guideline PP 1/239 Dose expression for plant protection products
	11	The dimension (g, kg) must be clearly specified (Maximum) dose of a s per treatment (usually g, kg or L product / ha)
	12	If water volume range depends on application equipments (e g ULVA or LVA) it should be mentioned under “application: method/kind”
	13	PHI - minimum pre-harvest interval
	14	Remarks may include: Extent of use/economic importance/restrictions

1.5.2 Further information on representative uses

All information is given in the GAP table under 1.5.1.

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

Only an MRL for honey is applied for. For details of uses, reference is made to 1.5.1.

1.5.4 Overview on authorisations in EU Member States

Details of the currently authorized uses (GAPs) for the representative formulation MON 52276 (glyphosate-isopropylammonium SL 486 G) in the EU are listed in the tables below.

The GAP included below is a summary of currently registered uses in Europe and for most countries under review awaiting the evaluation/decision phase of the running 'Article 43 applications'.

Information is taken from the dossier submitted by GRG, and was not verified by the members of the AGG as the Article 43 applications are still ongoing.

PPP (product name/code):	**MON 52276**	**Formulation type:**	**SL [a, b]**
Active substance 1:	**Glyphosate**	**Conc. of as 1:**	**360 g/L expressed as glyphosate-acid[c]**
Safener:	**N/A**	**Conc. of safener:**	**N/A [c]**
Synergist:	**N/A**	**Conc. of synergist:**	**N/A [c]**
		Professional use:	☒
Zone(s):	**Central [d]**	**Non professional use:**	☒
Field of use:	**herbicide**		

MON 52276 Central zone

1	2	3	4	5	6	7	8	9	10	11	12	13	14
Use-No. [e]	**Member state(s)**	**Crop and/ or situation (crop destination / purpose of crop)**	**F, Fn, Fpn G, Gn, Gpn or I**	**Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group)**	**Application**				**Application rate**			**PHI (days)**	**Remarks:**
					Method / Kind	**Timing / Growth stage of crop & season**	**Max. number a) per use b) per crop/ season**	**Min. interval between applications (days)**	**kg or L product / ha a) max. rate per appl. b) max. total rate per crop/season**	**g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season**	**Water L/ha min / max**		**e.g. g safener/synergist per ha [f]**
1	NL, BE, UK, IE, HU, CZ, SI, SK	Pre-plant/Pre-sowing all crops (YACKR)	F	annual and perennial weeds	spraying	actively growing weeds	1 – 3	28	a) 1.50 – 10.00 b) 10.00	a) 0.54 – 3.60 b) 3.60	100 – 400 20 – 40 rotary atomisers	-	
2	NL, UK, IE, HU, SK	Post-plant/pre-emergence (e.g. BEAVA, BEAVC, SOLTU, …)	F	annual and perennial weeds	spraying	up to BBCH 07	1		a) 1.50 – 6.00 b) 6.00	a) 0.54 – 2.16 b) 2.16	100 – 400 20 – 40 rotary atomisers	-	
3a	AT, NL, BE, UK, IE, DE, PL, HU, CZ, SK	Pre-harvest weed control: cereals (TRZAW, TRZAS, TTLWI, TTLSS, HORVS, HORVW,	F	annual and perennial weeds	spraying	> BBCH 87, grain/seed moisture < 30 %	1 – 3		a) 1.00 – 6.00 b) 6.00	a) 0.36 – 2.16 b) 2.16	100 – 400 20 – 40 rotary atomisers	7 – 14	

MON 52276 Central zone

1	2	3	4	5	6	7	8	9	10	11	12	13	14
Use-No. (e)	**Member state(s)**	**Crop and/ or situation** **(crop destination / purpose of crop)**	**F, Fn, Fpn G, Gn, Gpn or I**	**Pests or Group of pests controlled** **(additionally: developmental stages of the pest or pest group)**	**Application**				**Application rate**			**PHI (days)**	**Remarks:** **e.g. g safener/synergist per ha** (f)
					Method / Kind	**Timing / Growth stage of crop & season**	**Max. number a) per use b) per crop/ season**	**Min. interval between applications (days)**	**kg or L product / ha a) max. rate per appl. b) max. total rate per crop/season**	**g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season**	**Water L/ha min / max**		
		SECCW, SECCS, TRZSP, AVESA)											
3b	UK, IE, PL, HU, CZ, SK	Pre-harvest weed control: OSR, mustard, lupine, linseed (e.g. BRSNW, BRSNN, SINAL; BRSNI, SINSS, LUPAL, LIUUT, CNISA)	F	annual and perennial weeds	spraying	> BBCH 87, grain/seed moisture < 30 %	1		a) 1.00 – 5.00 b) 5.00	a) 0.36 – 1.80 b) 1.80	100 – 400 20 – 40 rotary atomisers	7 – 28	
3c	NL, BE, UK, IE, CZ, SK, HU	Pre-harvest weed control: pulses (PHSSS, PIBSS, PIBSA, PHSVX, GLXMA)	F	annual and perennial weeds	spraying	> BBCH 87, grain/seed moisture < 30 %	1		a) 1.00 – 6.00 b) 6.00	a) 0.36 – 2.16 b) 2.16	100 – 400 20 – 40 rotary atomisers	7 – 14	
3d	HU, SK	Pre-harvest weed control: maize, sunflower (ZEAMX, HELAN)	F	annual and perennial weeds	spraying	> BBCH 87, grain/seed moisture < 30 %	1		a) 2.00 – 5.00 b) 5.00	a) 0.72 – 1.80 b) 1.80	100 – 200	10 – 14	
	HU				aerial spraying				a) 2.00 – 5.00 b) 5.00	a) 0.72 – 1.80 b) 1.80	50 – 60		
4a	DE, UK, IE, NL, BE, PL, HU	Pre-harvest desiccation: cereals (TRZAW, TRZAS, TTLWI, TTLSS, HORVS, HORVW, SECCW, SECCS, TRZSP, AVESA)	F	crop desiccation treatment	spraying	> BBCH 87, grain/seed moisture < 30 %	1		a) 1.00 – 6.00 b) 6.00	a) 0.36 – 2.16 b) 2.16	100 – 400	7 – 14	
4b	UK, IE, PL, HU	Pre-harvest desiccation: OSR, mustard, lupine, linseed (e.g. BRSNW, BRSNN, SINAL;	F	annual and perennial weeds	spraying	> BBCH 87, grain/seed moisture < 30 %	1		a) 1.00 – 4.00 b) 4.00	a) 0.36 – 1.44 b) 1.44	100 – 400 20 – 40 rotary atomisers	8 – 28	

MON 52276 Central zone

1	2	3	4	5	6	7	8	9	10	11	12	13	14
Use-No. (e)	**Member state(s)**	**Crop and/ or situation** (crop destination / purpose of crop)	**F, Fn, Fpn G, Gn, Gpn or I**	**Pests or Group of pests controlled** (additionally: developmental stages of the pest or pest group)	**Application**				**Application rate**			**PHI (days)**	**Remarks:** e.g. g safener/synergist per ha (f)
					Method / Kind	**Timing / Growth stage of crop & season**	**Max. number a) per use b) per crop/ season**	**Min. interval between applications (days)**	**kg or L product / ha a) max. rate per appl. b) max. total rate per crop/season**	**g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season**	**Water L/ha min / max**		
		BRSNI, SINSS, LUPAL, LIUUT, CNISA)											
4c	UK, IE, NL, BE, HU	Pre-harvest desiccation: pulses (PHSSS, PIBSS, PIBSA, PHSVX, GLXMA)	F	annual and perennial weeds	spraying	> BBCH 87, grain/seed moisture < 30 %	1		a) 1.00 – 6.00 b) 6.00	a) 0.36 – 2.16 b) 2.16	100 – 400 20 – 40 rotary atomisers	7 – 14	
4d	HU	Pre-harvest desiccation: maize and sunflower (ZEAMX, HELAN)	F	annual and perennial weeds	spraying	> BBCH 87, grain/seed moisture < 30 %	1		a) 2.00 – 3.00 b) 3.00	a) 0.72 – 1.08 b) 1.08	100 – 250	10 – 14	
	HU				aerial spraying				a) 2.00 – 3.00 b) 3.00	a) 0.72 – 1.08 b) 1.08	50 – 60		
5	DE, UK, IE, NL, BE, CZ, HU, PL, SI, SK	Post-harvest/stubble (YSTEG)	F	annual and perennial weeds	spraying	actively growing weeds	1 – 2	60	a) 1.50 – 10.00 b) 10.00	a) 0.54 – 3.60 b) 3.60	100 – 400 20 – 40 rotary atomisers	-	
6	AT, DE, UK, IE, NL, BE, PL,	Set aside/fallow (YBRAC)	F	annual and perennial weeds, woody plants	spraying or handheld equipment	actively growing weeds	1 – 3		a) 1.50 – 10.00 b) 10.00	a) 0.54 – 3.60 b) 3.60	100 – 400 20 – 40 rotary atomisers	-	
7	AT, DE, UK, IE, NL, BE, CZ, SK, SI, HU	Pasture, meadow, grassland (NNNFW)	F	annual and perennial weeds	spraying, wiping	actively growing weeds	1 – 3	60	a) 0.50 – 10.00 b) 10.00	a) 0.18 – 3.60 b) 3.6	100 – 400	5 – 21	
8	AT, DE, UK, IE, NL, BE,	Orchards (e.g. NNNOK, NNNOS)	F	annual and perennial weeds	spraying	actively growing weeds	1 – 3	60	a) 0.50 – 10.00 b) 10.00	a) 0.18 – 3.60 b) 3.60	100 – 400 20 – 40	no – 42	

MON 52276 Central zone

1	2	3	4	5	6	7	8	9	10	11	12	13	14
Use-No. (e)	**Member state(s)**	**Crop and/ or situation** (crop destination / purpose of crop)	**F, Fn, Fpn G, Gn, Gpn or I**	**Pests or Group of pests controlled** (additionally: developmental stages of the pest or pest group)	**Application**				**Application rate**			**PHI (days)**	**Remarks:** e.g. g safener/synergist per ha (f)
					Method / Kind	**Timing / Growth stage of crop & season**	**Max. number a) per use b) per crop/ season**	**Min. interval between applications (days)**	**kg or L product / ha a) max. rate per appl. b) max. total rate per crop/season**	**g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season**	**Water L/ha min / max**		
	CZ, HU, SI, SK										rotary atomisers		
9	AT, DE, BE, CZ, HU, SI, SK	Vineyards (VITSS)	F	annual and perennial weeds	spraying	actively growing weeds	1 – 3		a) 0.50 – 10.00 b) 10.00	a)0.18 – 3.60 b)3.60	100 – 400	no – 35	
10	NL, BE, SK	Crop interrow (e.g. CUMSA, CUUPE, BRSOL, DAUCS, ALLCE, ASPOF, ALLPO, BEAVA, BEAVC, …)	F	annual and perennial weeds	shielded application, spot application	actively growing weeds	1		a) 0.10 – 10.00 b) 10.00	a) 0.036 – 3.60 b) 3.60	200 – 400	-	
11	AT, DE, UK, IE, NL, BE, CZ, SK	In crop weed wiper (e.g. BEAVA, BEAVC, BRSRR, BRSRE, …)	F	annual and perennial weeds	wiping	actively growing weeds, weeds taller than crop	1 – 3	14	33 – 50 %		-	30 or n.a.	
12a	AT, DE, UK, IE, NL, BE, CZ, HU, SI, SK	Forestry: pre-plant (YACKR)	F	annual and perennial weeds	spraying, shielded spray wiping	actively growing weeds	split application		a) 1.50 – 10.00 b) 10.00	a) 0.54 – 3.60 b) 3.60	100 – 400	-	
	SK								33 – 50 %				
	CZ						1		a) 3.3 (undiluted) b) 3.3 (undiluted)	a) 1.188 b) 1.188	Rotary atomisers		

MON 52276 Central zone

1	2	3	4	5	6	7	8	9	10	11	12	13	14
Use-No. (e)	**Member state(s)**	**Crop and/ or situation** **(crop destination / purpose of crop)**	**F, Fn, Fpn G, Gn, Gpn or I**	**Pests or Group of pests controlled** **(additionally: developmental stages of the pest or pest group)**	**Application**				**Application rate**			**PHI (days)**	**Remarks:** **e.g. g safener/synergist per ha** (f)
					Method / Kind	**Timing / Growth stage of crop & season**	**Max. number a) per use b) per crop/ season**	**Min. interval between applications (days)**	**kg or L product / ha a) max. rate per appl. b) max. total rate per crop/season**	**g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season**	**Water L/ha min / max**		
									a) 4.00 (20%) b) 4.00 (20%)	a) 1.188 b) 1.188	Rotary atomisers		
12b	AT, DE, UK, IE, NL, BE, CZ, HU, SI, SK	Forestry: inter row (e.g. NNNWW, YAUFO, YPFLG)	F	annual and perennial weeds	spraying, shielded spray wiping	actively growing weeds	split application		a) 2.00 – 10.00 b) 10.00	a) 0.72 – 3.60 b) 3.60	100 – 400	-	
	SK								33 – 50 %				
	CZ						1		a) 3.3 (undiluted) b) 3.3 (undiluted)	a) 1.188 b) 1.188	Rotary atomisers		
									a) 4.00 (20%) b) 4.00 (20%)	a) 1.44 b) 1.44	Rotary atomisers		
12c	UK, IE, NL, BE, CZ, HU, SI, SK	Forestry: Christmas trees (e.g. NNNWW)	F	annual and perennial weeds	spraying over the top	actively growing weeds, during dormancy of the trees	1		a) 2.00 – 6.00 b) 6.00	a) 0.72 – 2.16 b) 2.16	100 – 400	-	
13a	UK, IE, NL, BE, CZ, HU, SI, SK	Devitalization of stumps, trees and shrubs (e.g. NNNOG, NNNWL, NNNWN, NNNHS,…)	F	woody weeds, shoots, hollow stem weeds (incl. invasive weeds)	spraying, spot application	Freshly cut stem (from flowering till dieback) or application via freshly cut setm	1 – 2	60	a) 5.00 b) 5.00	a) 1.8 b) 1.8	100 – 400	-	
					wiping		1 – 2	60	3.5 – 20 %				
13d	NL			Hollow stem weeds (e.g. Japanese knotweed, bamboo,…)	Pipette injection		1		20 % solution (5 – 10 ml/stem)	-	-	-	

MON 52276 Central zone

1	2	3	4	5	6	7	8	9	10	11	12	13	14
Use-No. (e)	**Member state(s)**	**Crop and/ or situation** **(crop destination / purpose of crop)**	**F, Fn, Fpn G, Gn, Gpn or I**	**Pests or Group of pests controlled** **(additionally: developmental stages of the pest or pest group)**	**Application**				**Application rate**			**PHI (days)**	**Remarks:** **e.g. g safener/synergist per ha** (f)
					Method / Kind	**Timing / Growth stage of crop & season**	**Max. number a) per use b) per crop/ season**	**Min. interval between applications (days)**	**kg or L product / ha a) max. rate per appl. b) max. total rate per crop/season**	**g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season**	**Water L/ha min / max**		
14	AT, DE, UK, IE, NL, BE, CZ, HU, PL, SI, SK	Non-crop areas (incl. dry ditches w/o water flows) (e.g. YNKKX, YNKOB, …)	F	annual and perennial weeds	spraying, spot treatment or shielded application	actively growing weeds	split application		a) 1.50 – 10.00 b) 10.00	a) 0.54 – 3.60 b) 3.60	100 – 400	-	
15	AT, BE, DE, HU	Ornamentals (NNNZZ)	F	annual and perennial weeds	spraying	actively growing weeds	1 – 2		a) 4.00 – 10.00 b) 10.00	a) 1.44 – 3.60 b) 3.60	100 – 400	-	
17	HU	Alfalfa Parasite control (MEDVA)	F	Cuscuta control in Alfalfa	spot application	5 – 7 days after cutting	2	60 d	a) 0.50 – 0. 70 b) 1.40	a) 0.18 – 0.25 b) 0.50	150 – 250	-	
18	UK, IE, CZ, HU, SK	Aquatic use (e.g. PASNO)	F	Aquatic weeds, emerged and floating weeds	spraying	actively growing weeds	split application		a) 5.00 – 10.00 b) 10.00	a) 1.80 – 3.60 b) 3.60	100 – 400	-	
50a	NL, BE, DE, AT, SK	**Home and garden uses** Cultivated areas (e.g. YACKR, NNNZL, NNNZG, CUMSA, LACSA, MABSD, PRNDO,)	Fn	annual and perennial weeds, brush weeds and sapling	Handheld spray or hydraulic knapsack,	actively growing weeds	1 or split application		a) 2.00 – 10.00 b) 10.00	a) 0.72 – 3.60 b) 3.60	100 – 500	no – 42	
50b	NL, BE, DE, AT, SK	**Home and garden uses** Areas not intended to bear vegetation (e.g. YNKKX, YNKOB, …)	Fn	annual and perennial weeds, brush weeds and sapling	Handheld spray or hydraulic knapsack, shielded spray, wiping	actively growing weeds	1 or split application		a) 2.00 – 10.00 b) 10.00	a) 0.72 – 3.60 b) 3.60	100 – 500		

MON 52276 Central zone

1	2	3	4	5	6	7	8	9	10	11	12	13	14
Use-No. (e)	**Member state(s)**	**Crop and/ or situation** (crop destination / purpose of crop)	**F, Fn, Fpn G, Gn, Gpn or I**	**Pests or Group of pests controlled** (additionally: developmental stages of the pest or pest group)	**Application**				**Application rate**			**PHI (days)**	**Remarks:**
					Method / Kind	**Timing / Growth stage of crop & season**	**Max. number a) per use b) per crop/ season**	**Min. interval between applications (days)**	**kg or L product / ha a) max. rate per appl. b) max. total rate per crop/season**	**g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season**	**Water L/ha min / max**		**e.g. g safener/synergist per ha** (f)
50c	NL, BE, DE, AT, SK	**Home and garden uses** Lawn renovation (NNNZW)	Fn	Broad leaved weeds, perennial weeds	Handheld spray or hydraulic knapsack, shielded spray	actively growing weeds	1 or split application		a) 2.00 – 7.00 b) 7.00	a) 0.72 – 2.52 b) 2.52	100 – 500		
50d	NL, BE, SK	**Home and garden uses** Devitalization of stumps, bushes (e.g. NNNOG, NNNWL, NNNWN, NNNHS, …)	Fn	Tree stumps or bushes	Paint brush	Treatment of stump surface immediately after cutting	1 per spot		5 % – 20 % solution				
	SK			Overgrown weeds		Actively growing weeds			33 % – 50 % solution				

Remarks table heading:

(a) e g wettable powder (WP), emulsifiable concentrate (EC), granule (GR)
(b) Catalogue of pesticide formulation types and international coding system CropLife International Technical Monograph n°2, 6th Edition Revised May 2008
(c) g/kg or g/l
(d) Select relevant
(e) Use number(s) in accordance with the list of all intended GAPs in Part B, Section 0 should be given in column 1
(f) No authorization possible for uses where the line is highlighted in grey, Use should be crossed out when the notifier no longer supports this use

Remarks columns:

1 Numeration necessary to allow references
2 Use official codes/nomenclatures of EU Member States
3 For crops, the EU and Codex classifications (both) should be used; when relevant, the use situation should be described (e g fumigation of a structure)
4 F: professional field use, Fn: non-professional field use, Fpn: professional and non-professional field use, G: professional greenhouse use, Gn: non-professional greenhouse use, Gpn: professional and non-professional greenhouse use, I: indoor application
5 Scientific names and EPPO-Codes of target pests/diseases/ weeds or, when relevant, the common names of the pest groups (e g biting and sucking insects, soil born insects, foliar fungi, weeds) and the developmental stages of the pests and pest groups at the moment of application must be named
6 Method, e g high volume spraying, low volume spraying, spreading, dusting, drench Kind, e g overall, broadcast, aerial spraying, row, individual plant, between the plants - type of equipment used must be indicated
7 Growth stage at first and last treatment (BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4), including where relevant, information on season at time of application
8 The maximum number of application possible under practical conditions of use must be provided
9 Minimum interval (in days) between applications of the same product
10 For specific uses other specifications might be possible, e g : g/m³ in case of fumigation of empty rooms See also EPPO-Guideline PP 1/239 Dose expression for plant protection products
11 The dimension (g, kg) must be clearly specified (Maximum) dose of a s per treatment (usually g, kg or L product / ha)
12 If water volume range depends on application equipments (e g ULVA or LVA) it should be mentioned under "application: method/kind"
13 PHI - minimum pre-harvest interval
14 Remarks may include: Extent of use/economic importance/restrictions

PPP (product name/code):	**MON 52276**	**Formulation type:**	**SL [a, b]**
Active substance 1:	**Glyphosate**	**Conc. of as 1:**	**360 g/L expressed as glyphosate-acid [c]**
Safener:	**N/A**	**Conc. of safener:**	**N/A [c]**
Synergist:	**N/A**	**Conc. of synergist:**	**N/A [c]**
		Professional use:	☒
Zone(s):	**Southern [d]**	**Non professional use:**	☒
Field of use:	**herbicide**		

MON 52276 Southern zone

1	2	3	4	5	6	7	8	9	10	11	12	13	14
Use-No. [e]	**Member state(s)**	**Crop and/ or situation (crop destination / purpose of crop)**	**F, Fn, Fpn G, Gn, Gpn or I**	**Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group)**	**Application**				**Application rate**			**PHI (days)**	**Remarks: e.g. g safener/synergist per ha [f]**
					Method / Kind	**Timing / Growth stage of crop & season**	**Max. number a) per use b) per crop/ season**	**Min. interval between applications (days)**	**kg or L product / ha a) max. rate per appl. b) max. total rate per crop/season *rounded***	**g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season**	**Water L/ha min / max**		
Zonal uses (field or outdoor uses, certain types of protected crops)													
1	IT, GR, CY, HR	Pre-plant/Pre-sowing all crops (YACKR)	Fpn	annual and perennial weeds	spraying	actively growing weeds	1		a) 1.00 – 10.0 b) 10.0	a) 0.36 – 3.60 b) 3.60	100 – 400	-	
2	IT	Post-plant/pre-emergence (e.g. BEAVA, BEAVC, SOLTU, …)	Fpn	annual and perennial weeds	spraying	actively growing weeds	1 – 2		a) 1.00 – 10.0 b) 10.0	a) 0.36 – 3.60 b) 3.60	100 – 400	-	

MON 52276 Southern zone

1	2	3	4	5	6	7	8	9	10	11	12	13	14
Use-No. (e)	**Member state(s)**	**Crop and/ or situation** (crop destination / purpose of crop)	**F, Fn, Fpn G, Gn, Gpn or I**	**Pests or Group of pests controlled** (additionally: developmental stages of the pest or pest group)	**Application**				**Application rate**			**PHI (days)**	**Remarks:** e.g. g safener/synergist per ha (f)
					Method / Kind	**Timing / Growth stage of crop & season**	**Max. number a) per use b) per crop/ season**	**Min. interval between applications (days)**	**kg or L product / ha a) max. rate per appl. b) max. total rate per crop/season** *rounded*	**g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season**	**Water L/ha min / max**		
3a	HR	Pre-harvest weed control: cereals (TRZAW, TRZAS, TTLWI, TTLSS, HORVS, HORVW, SECCW, SECCS, TRZSP, AVESA)	F	annual and perennial weeds	spraying	grain moisture < 30 % BBCH 87	1		a) 1.50 – 6.00 b) 6.00	a) 0.54 - 2.16 b) 2.16	100 - 300	7	
5	IT, GR, CY, HR	Post-harvest/stubble (YSTEG)	Fpn	annual and perennial weeds	spraying	actively growing weeds	1 – 2		a) 1.00 – 10.0 b) 10.0	a) 0.36 – 3.60 b) 3.60	100 – 400	-	
6	IT, GR, CY	Set aside/fallow (YBRAC)	Fpn	annual and perennial weeds	spraying	actively growing weeds	1 – 3		a) 1.00 – 10.0 b) 10.0	a) 0.36 – 3.60 b) 3.60	100 – 400	-	
7	IT, HR	Pasture, meadow, grassland (NNNFW)	Fpn	annual and perennial weeds	spraying	actively growing weeds	1 – 3		a) 1.00 – 10.0 b) 10.0	a) 0.36 – 3.60 b) 3.60	100 – 400	no – 7	
8	IT, GR, CY, HR	Orchards (e.gNNNOK, CIDLI, CIDLC, NNNOS, IUGRE, PIAVE, MUBPA, ATICH, ELYCA,),	Fpn	annual and perennial weeds	spraying, handheld equipment, shielded application, spot treatment	actively growing weeds	1 – 5	28 d	a) 1.00 – 10.0 b) 10.0	a) 0.36 – 3.60 b) 3.60	100 – 400	7 – 90	for crop groups and PHIs we refer to the Residue Section of the dRR

MON 52276 Southern zone

1	2	3	4	5	6	7	8	9	10	11	12	13	14
Use-No. (e)	**Member state(s)**	**Crop and/ or situation** (crop destination / purpose of crop)	**F, Fn, Fpn G, Gn, Gpn or I**	**Pests or Group of pests controlled** (additionally: developmental stages of the pest or pest group)	**Application**				**Application rate**			**PHI (days)**	**Remarks:** e.g. g safener/synergist per ha (f)
					Method / Kind	**Timing / Growth stage of crop & season**	**Max. number a) per use b) per crop/ season**	**Min. interval between applications (days)**	**kg or L product / ha a) max. rate per appl. b) max. total rate per crop/season** ***rounded***	**g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season**	**Water L/ha min / max**		
9	IT, GR, CY, HR	Vineyards (VITSS)	Fpn	annual and perennial weeds	spraying, handheld equipment, shielded application, spot treatment	actively growing weeds	1 –3		a) 1.00 – 10.0 b) 10.0	a) 0.36 – 3.60 b) 3.60	100 – 400	7 – 28	
10	IT, GR,CY	Crop interrow (e.g. CUMSA, CUUPE, BRSOL, DAUCS, ALLCE, ASPOF, ALLPO, BEAVA, BEAVC, …)	Fpn	annual and perennial weeds	shielded spray	actively growing weeds	1 –3		a) 1.00 – 10.0 b) 10.0	a) 0.36 – 3.60 b) 3.60	100 – 400	-	for crop groups we refer to the Residue Section of the dRR
12	HR, IT	Forestry: inter row (e.g. NNNWW, YAUFO, YPFLG)	F	annual and perennial weeds	spraying, shielded application, spot treatment	actively growing weeds	1 – 3		a) 1.00 – 10.0 b) 10.0	a) 0.36 – 3.60 b) 3.60	100 – 400	-	
13	HR	Devitalization of stumps, trees and shrubs (e.g. NNNOG, NNNWL, NNNWN, NNNHS,…)	F	tree stumps or bushes	wiping	treatment of stump surface immediately after cutting	1		10 % – 20 %	120 g a.s./m²	-	-	

MON 52276 Southern zone

1	2	3	4	5	6	7	8	9	10	11	12	13	14
Use-No. (e)	**Member state(s)**	**Crop and/ or situation** (crop destination / purpose of crop)	**F, Fn, Fpn G, Gn, Gpn or I**	**Pests or Group of pests controlled** (additionally: developmental stages of the pest or pest group)	**Application**				**Application rate**			**PHI (days)**	**Remarks:** e.g. g safener/synergist per ha (f)
					Method / Kind	**Timing / Growth stage of crop & season**	**Max. number a) per use b) per crop/ season**	**Min. interval between applications (days)**	**kg or L product / ha a) max. rate per appl. b) max. total rate per crop/season** *rounded*	**g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season**	**Water L/ha min / max**		
14	ES, PT, IT, GR, CY, HR	Non-crop areas (incl. dry ditches w/o water flows) (e.g. YNKKX, YNKOB, …)	F	annual and perennial weeds	spraying, spot treatment, shielded application	actively growing weeds	1 – 5		a) 1.00 – 10.0 b) 10.0	a) 0.36 – 3.60 b) 3.60	100 – 400	-	
15	GR, IT	Ornamentals (NNNZZ)	F	annual and perennial weeds	Spraying	actively growing weeds	1 – 2		a) 1.50 – 10.0 b) 10.0	a) 0.54 – 3.60 b) 3.60	200 – 400	-	
16	GR, CY	Parasite control – tobacco (NIOGL)	F	Orobanche ramosa	spraying		2	40 d and 60 d after transplanting	a) 0.40 b) 0.60	a) 0.144 b) 0.216	300 – 400	7d	
18	PT, GR, CY, HR	Aquatic use: enclosed waters, open waters (e.g. PASNO)	F	aquatic plants, emerged weeds	Spraying, spot treatment		1 – 5		a) 1.00 – 10.0 b) 10.0	a) 0.36 – 3.60 b) 3.60	100 – 400	-	
50a	IT, PT	**Home and garden uses** Cultivated areas (e.g. YACKR, NNZL, NNZG CUMSA, LACSA, MABSD, PRNDO, …)	Fn	annual and perennial weeds, brush weeds and sapling	Spraying, spot treatment	actively growing weeds	1 – 3		a) 1.00 – 10.0 b) 10.0	a) 0.36 – 3.60 b) 3.60	200 – 500	-	

MON 52276 Southern zone

1	2	3	4	5	6	7	8	9	10	11	12	13	14
Use-No. (e)	**Member state(s)**	**Crop and/ or situation** (crop destination / purpose of crop)	**F, Fn, Fpn G, Gn, Gpn or I**	**Pests or Group of pests controlled** (additionally: developmental stages of the pest or pest group)	**Application**				**Application rate**			**PHI (days)**	**Remarks:** e.g. g safener/synergist per ha (f)
					Method / Kind	**Timing / Growth stage of crop & season**	**Max. number a) per use b) per crop/ season**	**Min. interval between applications (days)**	**kg or L product / ha a) max. rate per appl. b) max. total rate per crop/season** *rounded*	**g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season**	**Water L/ha min / max**		
50b	IT, PT	**Home and garden uses** Areas not intended to bear vegetation (e.g. YNKKX, YNKOB, …)	Fn	annual and perennial weeds, brush weeds and sapling	Spraying, shielded application, spot treatment	actively growing weeds	1 or split application		a) 2.00–10.00 b) 10.00	a) 0.72–3.60 b) 3.60	100–500		
50c	IT, PT	**Home and garden uses** Lawn renovation (NNNFW)	Fn	annual and perennial weeds, brush weeds and sapling	Spraying, spot treatment	actively growing weeds	1		a) 7.00 b) 7.00	a) 2.52 b) 2.52	200–500	-	

Remarks table heading:

(a) e g wettable powder (WP), emulsifiable concentrate (EC), granule (GR)
(b) Catalogue of pesticide formulation types and international coding system CropLife International Technical Monograph n°2, 6th Edition Revised May 2008
(c) g/kg or g/l
(d) Select relevant
(e) Use number(s) in accordance with the list of all intended GAPs in Part B, Section 0 should be given in column 1
(f) No authorization possible for uses where the line is highlighted in grey, Use should be crossed out when the notifier no longer supports this use

Remarks columns:

1 Numeration necessary to allow references
2 Use official codes/nomenclatures of EU Member States
3 For crops, the EU and Codex classifications (both) should be used; when relevant, the use situation should be described (e g fumigation of a structure)
4 F: professional field use, Fn: non-professional field use, Fpn: professional and non-professional field use, G: professional greenhouse use, Gn: non-professional greenhouse use, Gpn: professional and non-professional greenhouse use, I: indoor application
5 Scientific names and EPPO-Codes of target pests/diseases/ weeds or, when relevant, the common names of the pest groups (e g biting and sucking insects, soil born insects, foliar fungi, weeds) and the developmental stages of the pests and pest groups at the moment of application must be named
6 Method, e g high volume spraying, low volume spraying, spreading, dusting, drench Kind, e g overall, broadcast, aerial spraying, row, individual plant, between the plants - type of equipment used must be indicated
7 Growth stage at first and last treatment (BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4), including where relevant, information on season at time of application
8 The maximum number of application possible under practical conditions of use must be provided
9 Minimum interval (in days) between applications of the same product
10 For specific uses other specifications might be possible, e g : g/m³ in case of fumigation of empty rooms See also EPPO-Guideline PP 1/239 Dose expression for plant protection products
11 The dimension (g, kg) must be clearly specified (Maximum) dose of a s per treatment (usually g, kg or L product / ha)
12 If water volume range depends on application equipments (e g ULVA or LVA) it should be mentioned under "application: method/kind"
13 PHI - minimum pre-harvest interval
14 Remarks may include: Extent of use/economic importance/restrictions

PPP (product name/code):	**MON 52276**	**Formulation type:**	**SL [a, b]**
Active substance 1:	**Glyphosate**	**Conc. of as 1:**	**360 g/L expressed as glyphosate-acid[c]**
Safener:	**N/A**	**Conc. of safener:**	**N/A [c]**
Synergist:	**N/A**	**Conc. of synergist:**	**N/A [c]**
Applicant:		**Professional use:**	☒
Zone(s):	**Northern [d]**	**Non professional use:**	☒
Verified by MS:			
Field of use:	**herbicide**		

MON 52276 – Northern zone

1	2	3	4	5	6	7	8	9	10	11	12	13	14
Use-No. [e]	**Member state(s)**	**Crop and/ or situation** **(crop destination / purpose of crop)**	**F, Fn, Fpn G, Gn, Gpn or I**	**Pests or Group of pests controlled** **(additionally: developmental stages of the pest or pest group)**	**Application**				**Application rate**			**PHI (days)**	**Remarks:**
					Method / Kind	**Timing / Growth stage of crop & season**	**Max. number** **a) per use** **b) per crop/ season**	**Min. interval between applications (days)**	**kg or L product / ha** **a) max. rate per appl.** **b) max. total rate per crop/season**	**g or kg as/ha** **a) max. rate per appl.** **b) max. total rate per crop/season**	**Water L/ha** **min / max**		**e.g. g safener/synergist per ha [f]**
1	DK, SE, NO, FI, LV, LT	Pre-plant/Pre-sowing all crops (YACKR)	F	annual and perennial weeds	Hydraulic spray, tractor, knapsack	actively growing weeds	1		a) 1.00 – 8.00 b) 8.00	a) 0.36 – 2.88 b) 2.88	100 – 200 Knapsack: 250 – 500	-	
2	DK, SE, NO, FI, LV, LT	Post-plant/pre-emergence (e.g. BEAVA, BEAVC, SOLTU, …)	F	annual and perennial weeds	Hydraulic spray, tractor, knapsack	actively growing weeds	1		a) 1.00 – 8.00 b) 8.00	a) 0.36 – 2.88 b) 2.88	100 – 200 Knapsack: 250 – 500	-	

MON 52276 – Northern zone

1	2	3	4	5	6	7	8	9	10	11	12	13	14
Use-No. (e)	**Member state(s)**	**Crop and/ or situation** **(crop destination / purpose of crop)**	**F, Fn, Fpn G, Gn, Gpn or I**	**Pests or Group of pests controlled** **(additionally: developmental stages of the pest or pest group)**	**Application**				**Application rate**			**PHI (days)**	**Remarks:** **e.g. g safener/synergist per ha** (f)
					Method / Kind	**Timing / Growth stage of crop & season**	**Max. number** **a) per use** **b) per crop/ season**	**Min. interval between applications (days)**	**kg or L product / ha** **a) max. rate per appl.** **b) max. total rate per crop/season**	**g or kg as/ha** **a) max. rate per appl.** **b) max. total rate per crop/season**	**Water L/ha** **min / max**		
3a	DK, NO, FI, EE, LT, LV, SE	Pre-harvest weed control: cereals (TRZAW, TRZAS, TTLWI, TTLSS, HORVS, HORVW, SECCW, SECCS, TRZSP, AVESA)	Fpn	annual and perennial weeds	Hydraulic spray, tractor,	grain moisture < 30 % BBCH 87	1		a) 2.00 – 4.00 b) 4.00	a) 0.72 – 1.44 b) 1.44	100 – 200	7	
3b	DK, SE, FI, EE, LT, LV, NO	Pre-harvest weed control: OSR, mustard, lupine, linseed (e.g. BRSNW, BRSNN, SINAL; BRSNI, SINSS, LUPAL, LIUUT, CNISA)	Fpn	annual and perennial weeds	Hydraulic spray, tractor,	grain moisture < 30 % BBCH 87	1		a) 2.00 – 4.00 b) 4.00	a) 0.72 – 1.44 b) 1.44	100 – 200	10	
3c	DK, LT, LV, FI, NO, SE	Pre-harvest weed control: pulses (PHSSS, PIBSS, PIBSA, PHSVX, GLXMA)	F	annual and perennial weeds	Hydraulic spray, tractor,	70 % of pods ripe BBCH 87	1		a) 2.00 – 4.00 b) 4.00	a) 0.72 – 1.44 b) 1.44	100 – 200	10	
3e	DK, NO, SE	Pre-harvest weed control: pre-cut grass (LOLSS, FESSS, POASS)	F	annual and perennial weeds	Hydraulic spray, tractor,	> BBCH 61	1		a) 3.00 – 4.00 b) 4.00	a) 1.08 – 1.44 b) 1.44	100 – 200	10	

MON 52276 – Northern zone

1	2	3	4	5	6	7	8	9	10	11	12	13	14
Use-No. (e)	**Member state(s)**	**Crop and/ or situation** (crop destination / purpose of crop)	**F, Fn, Fpn G, Gn, Gpn or I**	**Pests or Group of pests controlled** (additionally: developmental stages of the pest or pest group)	**Application**				**Application rate**			**PHI (days)**	**Remarks:** e.g. g safener/synergist per ha (f)
					Method / Kind	**Timing / Growth stage of crop & season**	**Max. number** a) per use b) per crop/ season	**Min. interval between applications (days)**	**kg or L product / ha** a) max. rate per appl. b) max. total rate per crop/season	**g or kg as/ha** a) max. rate per appl. b) max. total rate per crop/season	**Water L/ha** min / max		
4a	DK, NO, FI, EE, LT, LV, SE	Pre-harvest desiccation: cereals (TRZAW, TRZAS, TTLWI, TTLSS, HORVS, HORVW, SECCW, SECCS, TRZSP, AVESA)	Fpn	annual and perennial weeds	Hydraulic spray, tractor,	grain moisture < 30 % BBCH 87	1		a) 2.00 – 4.00 b) 4.00	a) 0.72 – 1.44 b) 1.44	100 – 200	7	
4b	DK, SE, FI, EE, LT, LV, NO	Pre-harvest desiccation: OSR, mustard, lupine, linseed (e.g. BRSNW, BRSNN, SINAL; BRSNI, SINSS, LUPAL, LIUUT, CNISA)	Fpn	annual and perennial weeds	Hydraulic spray, tractor,	grain moisture < 30 % BBCH 8787	1		a) 2.00 – 4.00 b) 4.00	a) 0.72 – 1.44 b) 1.44	100 – 200	10	
4c	DK, LT, LV, FI, SE, NO	Pre-harvest desiccation: pulses (PHSSS, PIBSS, PIBSA, PHSVX, GLXMA)	F	annual and perennial weeds	Hydraulic spray, tractor,	70 % of pods ripe BBCH 87	1		a) 2.00 – 4.00 b) 4.00	a) 0.72 – 1.44 b) 1.44	100 – 200	10	
5	DK, SE, NO, FI, EE, LT, LV	Post-harvest/stubble (YSTEG)	Fpn	annual and perennial weeds	Hydraulic spray, tractor, knapsack	actively growing weeds	1		a) 1.00 – 8.00 b) 8.00	a) 0.36 – 2.88 b) 2.88	100 – 200 Knapsack: 250 – 500	-	
6	DK, SE, NO, FI, EE, LT, LV	Set aside/fallow (YBRAC)	Fpn	annual and perennial weeds	Hydraulic spray, tractor, knapsack	actively growing weeds	1		a) 1.00 – 8.00 b) 8.00	a) 0.36 – 2.88 b) 2.88	100 – 200 Knapsack: 250 – 500	-	

MON 52276 – Northern zone

1	2	3	4	5	6	7	8	9	10	11	12	13	14
Use-No. (e)	**Member state(s)**	**Crop and/ or situation** (crop destination / purpose of crop)	**F, Fn, Fpn G, Gn, Gpn or I**	**Pests or Group of pests controlled** (additionally: developmental stages of the pest or pest group)	**Application**				**Application rate**			**PHI (days)**	**Remarks:** e.g. g safener/synergist per ha (f)
					Method / Kind	**Timing / Growth stage of crop & season**	**Max. number** a) per use b) per crop/ season	**Min. interval between applications (days)**	**kg or L product / ha** a) max. rate per appl. b) max. total rate per crop/season	**g or kg as/ha** a) max. rate per appl. b) max. total rate per crop/season	**Water L/ha** min / max		
7	DK, SE, NO, FI, EE, LV, LT	Pasture, meadow, grassland (NNNFW)	Fpn	annual and perennial weeds	Hydraulic spray, tractor, knapsack	actively growing weeds	1		a) 1.00 – 8.00 b) 8.00	a) 0.36 – 2.88 b) 2.88	100 – 200 Knapsack: 250 – 500	-	
8	DK, SE, NO, FI, EE, LT, LV	Orchards (e.g. MABSD, PYUCO, MSPGE, PRNDO, PRNCE, PRNAV, RIBNI, RIBRU, RIBUC, VACCO, CYLAV, CSNNS, IUGRE)	Fpn	annual and perennial weeds	Hydraulic spray, tractor, knapsack	actively growing weeds	1 - 3		a) 1.00 – 8.00 b) 10.00	a) 0.36 – 2.88 b) 3.60	100 – 200 Knapsack: 250 – 500		
10	FI, LV, LT	Crop interrow (e.g. CUMSA, CUUPE, BRSOL, DAUCS, ALLCE, ASPOF, ALLPO, BEAVA, BEAVC, …)	F	annual and perennial weeds	Hydraulic spray, knapsack	actively growing weeds	1		a) 1 50 – 3.00 b) 3.00	a) 0.54 – 1.08 b) 1.08	100 – 200	-	
11	SE, FI, NO, LV, LT	In crop weed wiper (e.g. BEAVA, BEAVC, BRSRR, BRSRE, …)	F	annual and perennial weeds	weed wiper	actively growing weeds, weeds min 20 cm taller than crop	1		30 – 50 % solution			-	
12a, 12b, 12c	DK, SE, FI, LT, LV, NO, EE	Forestry (e.g. NNNWW, YAUFO, YPFLG)	Fpn	annual and perennial weeds	Hydraulic spray, tractor, knapsack	actively growing weeds	1 – 3		a) 2.00 – 8.00 b) 10.00	a) 0.72– 2.88 b) 3.60	100 – 200 Knapsack: 250 – 500	-	

MON 52276 – Northern zone

1	2	3	4	5	6	7	8	9	10	11	12	13	14
Use-No. (e)	**Member state(s)**	**Crop and/ or situation** (crop destination / purpose of crop)	**F, Fn, Fpn G, Gn, Gpn or I**	**Pests or Group of pests controlled** (additionally: developmental stages of the pest or pest group)	**Application**				**Application rate**			**PHI (days)**	**Remarks:** e.g. g safener/synergist per ha (f)
					Method / Kind	**Timing / Growth stage of crop & season**	**Max. number** a) per use b) per crop/ season	**Min. interval between applications (days)**	**kg or L product / ha** a) max. rate per appl. b) max. total rate per crop/season	**g or kg as/ha** a) max. rate per appl. b) max. total rate per crop/season	**Water L/ha** min / max		
13a	DK, SE, NO, FI, LT, LV	Devitalisation of stumps, trees and shrubs (e.g. NNNOG, NNNWL, NNNWN, NNNHS,…)	F	Trees stumps or bushes	paint brush	treatment of stump surface immediately after cutting	1		20 % – 30 % solution			-	
14	DK, SE, NO, FI, EE, LT, LV	Non crop areas (e.g. YNKKX, YNKOB, …)	Fpn	annual and perennial weeds	Hydraulic spray, knapsack	actively growing weeds	1 – 3		a) 1.00 – 8.00 b) 10.00	a) 0.36 – 2.88 b) 3.60	100–300 Knapsack: 350 – 500	-	

Remarks table heading:

(a) e g wettable powder (WP), emulsifiable concentrate (EC), granule (GR)
(b) Catalogue of pesticide formulation types and international coding system CropLife International Technical Monograph n°2, 6th Edition Revised May 2008
(c) g/kg or g/l
(d) Select relevant
(e) Use number(s) in accordance with the list of all intended GAPs in Part B, Section 0 should be given in column 1
(f) No authorization possible for uses where the line is highlighted in grey, Use should be crossed out when the notifier no longer supports this use

Remarks columns:

1 Numeration necessary to allow references
2 Use official codes/nomenclatures of EU Member States
3 For crops, the EU and Codex classifications (both) should be used; when relevant, the use situation should be described (e g fumigation of a structure)
4 F: professional field use, Fn: non-professional field use, Fpn: professional and non-professional field use, G: professional greenhouse use, Gn: non-professional greenhouse use, Gpn: professional and non-professional greenhouse use, I: indoor application
5 Scientific names and EPPO-Codes of target pests/diseases/ weeds or, when relevant, the common names of the pest groups (e g biting and sucking insects, soil born insects, foliar fungi, weeds) and the developmental stages of the pests and pest groups at the moment of application must be named
6 Method, e g high volume spraying, low volume spraying, spreading, dusting, drench Kind, e g overall, broadcast, aerial spraying, row, individual plant, between the plants - type of equipment used must be indicated
7 Growth stage at first and last treatment (BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4), including where relevant, information on season at time of application
8 The maximum number of application possible under practical conditions of use must be provided
9 Minimum interval (in days) between applications of the same product
10 For specific uses other specifications might be possible, e g : g/m³ in case of fumigation of empty rooms See also EPPO-Guideline PP 1/239 Dose expression for plant protection products
11 The dimension (g, kg) must be clearly specified (Maximum) dose of a s per treatment (usually g, kg or L product / ha)
12 If water volume range depends on application equipments (e g ULVA or LVA) it should be mentioned under "application: method/kind"
13 PHI - minimum pre-harvest interval
14 Remarks may include: Extent of use/economic importance/restrictions

Use description (all zones):

1. Pre-plant/ pre-sowing
Preparation of the seed bed. Application in spring or autumn (but mainly in spring) before sowing or planting. Crops are not present in the ground. Examples: wheat, maize, oil seed rape, sugar beet

2. Post-plant/ pre-emergence
Seeds have been sown/crops planted but crops have not emerged (up to BBCH 08, before the ground breaks). Seeds are present in the soil (in contrast to use 1 (pre-plant)). Application usually in spring. Examples: Maize, sunflower, sugar beets, spring crops, potatoes

3. Pre-harvest, weed control
a. cereals
b. oil seeds
c. pulses
d. maize, sunflower
   - (a-d) Mature crops (≥ BBCH 87, grain moisture content < 30 %) are treated over the top to control perennial and difficult to control weeds long-term (e.g. couch, *Cirsium avensis*). Post-harvest treatment would be ineffective because weeds would be cut during harvest and therefore would not have enough leaf surface to allow efficient control. Example: cereals, pulses, oil seed rape.

e. grass
   - Pre-cut / Pre-harvest grass (Grass over the top treatment (> BBCH 61) 10 d before cutting. Hay and silage can be fed to cattle; to control perennial and difficult to control weeds long-term (e.g. *Cirsium avensis*). Post-harvest treatment would be ineffective because weeds would be cut during harvest and therefore would not have enough leaf surface to allow efficient control. Application in summer.

4. Pre-harvest, desiccation treatments
a. cereals
b. oil seeds
c. pulses
d. maize, sunflower

Mature crops (≥ BBCH 87, grain moisture content < 30 %) are treated over the top to facilitate uniform maturity/desiccation of the crops. Examples: lodged cereals, pulses, oil seed rape

5. Post-harvest/stubble
Control of volunteers, annual and perennial weeds, also after shallow soil cultivation. Application usually in autumn before preparation of succeeding crop. Examples: all arable crops

6. Set aside, fallow
Annual and perennial weeds control for maintenance of fallow. Preparation of the seed bed from fallow land that was temporarily removed from agricultural production. Application any time during vegetation period

7. Pasture, meadow, grassland (pre-plant, renovation or selective control of weeds)
- Pre-plant: Seed bed preparation for pasture seeding (also see use 1)
- Renovation: Control existing pasture and prepare for seeding new pasture (avoid livestock feeding)
- Selective control of weeds: spot treatment, single weed treatment of perennial or noxious weeds (avoid livestock feeding on noxious weeds, e.g. ragwort)

8. Orchards
Annual and perennial weeds control whilst maintaining ground cover to minimize competition, erosion and soil moisture loss. To control a wide range of annual and perennial weeds that otherwise become very well established. Foliar ground application. Inter-row (between rows), intra-row (within rows) or spot application. Avoid contact with orchard trees. Examples: pome fruits, stone fruits, nuts, citrus, olives, tropical fruits etc.

9. Vineyards
Annual and perennial weeds control whilst maintaining ground cover to minimize competition, erosion and soil moisture loss. To control a wide range of annual and perennial weeds that otherwise become very well established. Foliar ground application. Inter-row (between rows), intra-row (within rows) or spot application. Avoid contact with vineyard trees.

10. In crop inter-row
Shielded foliar ground application between the crops (inter-row). Avoid drift and contact with crops. Example: maize, vegetables (onions, leek, carrots, cucumber, …), cotton, tobacco, rice levees

11. In crop, weed wiper
Selective application on weeds taller than the crop canopy. Reduced application volume and chemical use with increase precision of application. Examples: bolter control in arable crops e.g. sugar beet, turnips

12. Forestry (incl. pre-plant, inter-row, spot application, nurseries, Christmas trees and fire break)
a. **pre-plant:** Seed or transplant bed preparation, see pre-plant use (use 1)
b. **inter-row, spot application, nurseries, fire break, stumps** see inter-row (use 8), Spot application: single weed treatment, Nurseries: inter-row application in tree nurseries (usually in protected environments), Thinning of established trees, injection; Stumps: wiping application on cut tree stumps to control re-growth, Fire break: Ground application to control vegetation in fire breaks
c. **Christmas trees:** over the top application on Christmas trees during dormant stage of leaders (Nov – Feb), control of perennial weeds

13. Devitalisation of stumps, trees and shrubs (incl. invasive weeds control)
a. Wiping or hand spraying (spot treatment) application to control re-growth of trees and woody weeds
b. **Devitalisation of vines and brambles :** Spraying, with tunnel sprayer – in autumn (vines >BBCH 91)
c. **Ecoplug** : Direct insertion of a plastic plug containing the glyphosate dry product into the tree stump or the standing tree

14. Non-crop areas (industrial sites, amenity (pathways, urban areas, motorways, walkways, land immediately adjacent to aquatic area, dry ditches, field edges, railways, pavements, airports, cemeteries, sport and recreation areas etc.)
Broad spectrum long-term control of unwanted vegetation

15. Ornamental plants, lawn
See pre-plant, inter-row and spot treatment
Renovation of public and sport lawns
Nurseries

16. Parasite control (all situations)
Orobanche ramosa control in tobacco

17. Alfalfa
Application during dormancy of the crop (winter), low level application to mainly control grasses or spot application

18. Aquatic use: Enclosed waters, open waters
Spraying over the water to control aquatic, noxious, invasive and alien weeds and emergent floating plants to enable water habitat management and to improve water flow

50. Amateur users
Plant Protection Products used in personal home and garden areas, Concentrates, Ready-to-use formulations, Gels
a. In cropped area. Before, after planting, or between crops (e.g. in ornamental culture, pome fruit, vegetable beds, flower beds, under trees, vineyards....)
b. Areas not indented to bear vegetation: e.g., along fences, permanent and temporary uncultivated areas, hard surfaces... (spot treatment): non-cropped areas, open hard surfaces, gravel, temporary uncultivated areas
c. Lawn renovation
d. Against regrowth of trees (tree stump and bushes) : treatment done on the stump surface by wiping immediately after cutting
e. Control of hollow stem weeds: treatment by injection into the freshly cut stem

Level 2

Glyphosate

2 SUMMARY OF ACTIVE SUBSTANCE HAZARD AND OF PRODUCT RISK ASSESSMENT

Summary of methodology proposed by the applicant for literature review and for all sections

A literature search for glyphosate and its metabolites (*aminomethyl)phosphonic acid* (AMPA), *N-acetyl-AMPA*, *N-acetyl-glyphosate*, *(hydroxymethyl)phosphonic acid* (HMPA), *N-methyl-AMPA*, *N-glyceryl-AMPA*, *N-malonyl-AMPA*, *methylphosphonic acid* and *N-methylglyphosate*) was carried out by the applicant according to the requirements stated in the EFSA Guidance document "*Submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) 1107/2009"* (EFSA Journal 2011;9(2):2092).

The literature search was conducted accessing 11 bibliographic databases (AGRICOLA, BIOSIS, CABA, CAPLUS, EMBASE, ESBIOBASE, MEDLINE, TOXCENTER, FSTA, PQSCITECH, SCISEARCH) in order to identify scientific peer-reviewed open literature published within the 10 years prior to the renewal dossier submission (2010-2020). Top-up literature search was conducted to cover the period directly before the dossier submission, e.g. between January 2020 and June 2020.
Upon removal of duplicates 12,178 articles in total were identified. All 12,178 articles were subsequently assessed for their relevance at title/abstract level.
A total of 10,558 articles were identified as "non-relevant" in the rapid assessment (e.g. publications dealing with chemical synthesis, efficacy, analytical methods etc.) and excluded from further evaluation.

The overall results of the search for sections relevant for the environmental or human safety assessment are presented below.

Section	Number of articles found	Rapid assessment (title/abstract level)		Detailed assessment (full-text level)	
		Non-relevant articles	Potentially relevant / unclear relevance	Non-relevant articles	Relevant articles
Ecotoxicology	1614	1039	575	412	163
E-fate	1147	842	305	132	173
Residues	491	420	71	30	41
Toxicology	1550	881	669	313	356
Total	**4802**	**3182**	**1620**	**887**	**733**

Separate literature searches were conducted for relevant publications on endocrine disruption and on biodiversity, which are presented in detail in Vol.3 CA B.6.10., and in Vol.3 CP B.9, respectively.

The articles identified relevant by the applicant as well as additional articles found or identified as relevant by the RMS are evaluated further in Volumes 3 of the RAR for each section.

2.1 IDENTITY

2.1.1 Summary or identity

EU agreed minimum purity of glyphosate is 950 g/kg (95.0 % w/w) according to Commission Implementing Regulation (EU) 2017/2324.
There are no additives intentionally added to the glyphosate technical.
Impurities N-nitroso-glyphosate (NNG) and formaldehyde have been identified as being of (eco)toxicological relevance according to Commission Implementing Regulation (EU) 2017/2324. Two new relevant impurities have been identified trimethylamine and formic acid. In consequence the reference specifications has been revised.
The level of NNG, formaldehyde, trimethylamine and formic acid in glyphosate technical are less than 1 mg/kg, 1 g/kg, 2 g/kg and 4 g/kg, respectively. Data gap have been identified for several sources claimed.
Depending on the manufacturing process of glyphosate technical material, different impurities might be present as residue of starting materials or by-products from glyphosate synthesis. Please refer to the individual Volumes 4 for

respective information on different glyphosate technical sources from the Glyphosate Renewal Group.

2.2 PHYSICAL AND CHEMICAL PROPERTIES [EQUIVALENT TO SECTION 7 OF THE CLH REPORT TEMPLATE]

2.2.1 Summary of physical and chemical properties of the active substance

Glyphosate acid and its related salt variations are white (crystalline) powders without odour, with the exception of glyphosate DMA salt which cannot be isolated. Glyphosate DMA salt (~62 % solution) is a yellow liquid with a waxy odour. Melting point of glyphosate acid is 189.5 °C and the other related salt variations range from 110 to 164 °C for IPA salt, 219.8 °C for K salt the NH4 salt decomposed at 190 °C before melting. Glyphosate and is variants all decompose exothermically before boiling. Glyphosate and its salt variations are not volatile substances. Glyphosate acid has moderate solubility and its salt variations have high water solubility, which shows moderate to high pH dependence. All partition coefficients in octanol/water of glyphosate acid and its salt variations are at a negative level. Glyphosate acid and its related salt variations are not highly flammable, not auto-flammable, not explosive and have no oxidising properties.

Table 1: Summary of physicochemical properties of the active substance

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	White solid without characteristic odour	[redacted] (1997) Report no. RJ2400B KCA 2.3/001	Visual
Melting/freezing point	Melting point: 189.5 °C	[redacted], [redacted] (1989)	Measured
Boiling point	The boiling point is not applicable because glyphosate and its salts decompose during melting	-	Statement
Relative density	$D^{20}_4 = 1.70$	[redacted] (1997) Report no. RJ2400B KCA 2.14/02	Measured
Vapour pressure	Vapour pressure: 1.31 × 10-5 Pa (25 °C)	[redacted] (1991) Report no. 6611-676/2-A KCA 2.2/001	Measured
Surface tension	72.2 mN/m at 20 °C (1 g/L aqueous solution) Glyphosate acid is not surface active.	[redacted] (1997) Report no. RJ2401B KCA	Measured

Property	Value	Reference	Comment (e.g. measured or estimated)
		2.12/001	
Water solubility	The solubility of glyphosate acid at pH 5 and pH 7 at 20 ºC was determined to be greater than 100 g/L. The solubility of glyphosate at pH 9 at 20 ºC was 171 g/L.	[redacted] (2020a) Report no. 139K-101 KCA 2.5/001	Measured
Partition coefficient n-octanol/water	Log P_{ow} = -5.39 at 25 °C (at pH buffers at 5) Log P_{ow} = -6.28 at 25 °C (at pH buffers at 7) Log P_{ow} = -5.83 at 25 °C (at pH buffers at 9) Note: sufficient log Pow data for the metabolites included in the residue definition is also available. See Vol 3 CA B2.	[redacted] (2020a) Report no. 139K-102 KCA 2.7/001	Measured
Henry's law constant	Henry's law constant is re-calculated based on vapour pressure 1.31 × 10-5 Pa (25 °C) and water solubility > 100 g/L (20 °C) Henry's law constant: < 2.21 × 10^{-8} Pa·m^3·mol^{-1}	-	Calculation
Flash point	Flash point is not required, as glyphosate acid is a solid	-	Statement
Flammability	The test item glyphosate, technical substance is not a readily combustible solid	[redacted] (2019) Report no. PS20190309-1 KCA 2.9/001	Measured
Explosive properties	Statement: Glyphosate acid is not explosive, the substance does not contain any chemically instable or highly energetic groups that might lead to an explosion. Result can be extrapolated to CLP regulation	[redacted]. (1984) Report no. 122377 KCA 2.11/002	Statement
Self-ignition temperature	The test item is not classified as "self-heating substance" according to UN Test N.4 and chapter 2.11 of the GHS and CLP regulations.	[redacted] (2019) Report no. PS20190309-2 KCA 2.9/002	Measured
Oxidising properties	Glyphosate acid is not an oxidising substance. Result can be extrapolated to CLP regulation	[redacted] (1997) Report no. RJ2401B KCA 2.13/001	Measured

<table>
<tr><th>Property</th><th>Value</th><th>Reference</th><th>Comment (e.g. measured or estimated)</th></tr>
<tr><td>Solubility in organic solvents and identity of relevant degradation products</td><td>At 20 °C:
Heptane: < 0.6 mg /L
Octan-1-ol: < 0.6 mg /L
Methanol: 10 mg /L
Xylenes: < 0.6 mg /L
Ethyl acetate: < 0.6 mg /L
Acetonitrile: 0.8 mg /L
1,2-dichloroethane: < 0.6 mg /L

Acetone: 0.078 g/L
Dichloromethane: 0.233 g/L
Ethyl acetate: 0.012 g/L
Hexane: 0.026 g/L
Methanol: 0.231 g/L
Propane-2-ol: 0.02 g/L
Toluene: 0.036 g/L</td><td>[redacted]
(1997)
Report no. RJ2401B
KCA 2.6/001

[redacted]
(1991)
Report no. 6759-676/5
KCA 2.6/002</td><td>Measured</td></tr>
<tr><td>Dissociation constant</td><td>At 20 °C:
pKa1 = 2.34 ± 0.11
pKa2 = 5.73 ± 0.10

At 25 °C:
pKa1 = 2.74
pKa2 = 5.63
pKa3 = 10.2</td><td>[redacted]
(1995)
Report no. 141828
KCA 2.8/001

[redacted]
(1995)
Report no. 95-044-1020
KCA 2.8/002</td><td>Measured</td></tr>
<tr><td>Viscosity</td><td>Not required as glyphosate acid is a solid</td><td>-</td><td>Statement</td></tr>
<tr><td>Spectra (UV/VIS, IR, NMR, MS), molar extinction at relevant wavelengths, optical purity</td><td><table><tr><th>pH conditions</th><th>UV/VIS maximum</th><th>pH conditions</th></tr><tr><td>Neutral (pH 7.19)</td><td>200</td><td>122</td></tr><tr><td>Acidic (pH 1.99)</td><td>200</td><td>760</td></tr><tr><td>Basic (pH 10.29)</td><td>200</td><td>712</td></tr></table>The highest absorbance for all samples was observed at 200 nm</td><td>[redacted]
(1997)
Report no. RJ2400B
KCA 2.4/002</td><td>Measured</td></tr>
</table>

2.2.1.1 Evaluation of physical hazards [equivalent to section 8 of the CLH report template]

2.2.1.1.1 **Explosives** *[equivalent to section 8.1 of the CLH report template]*

Table 2: Summary table of studies on explosive properties

Method	Results	Remarks	Reference
Statement	Not explosive	-	[redacted] (1984) Report no. 122377

2.2.1.1.1.1 Short summary and overall relevance of the provided information on explosive properties

Glyphosate acid does not contain chemical groups associated with explosive properties, see table A6.1 in Appendix 6 of the UN RTDG.

2.2.1.1.1.2 Comparison with the CLP criteria

Fulfil the screening criteria in 2.1.4.3 (a), i.e. absence of chemical groups associated with explosive properties.

2.2.1.1.1.3 Conclusion on classification and labelling for explosive properties

Not classified as explosive.

2.2.1.1.2 **Flammable gases (including chemically unstable gases)** *[equivalent to section 8.2 of the CLH report template]*

Table 3: Summary table of studies on flammable gases (including chemically unstable gases)

Method	Results	Remarks	Reference
-	-	-	-

2.2.1.1.2.1 Short summary and overall relevance of the provided information on flammable gases (including chemically unstable gases)

Not applicable, the substance is not a gas.

2.2.1.1.2.2 Comparison with the CLP criteria

Not applicable.

2.2.1.1.2.3 Conclusion on classification and labelling for flammable gases

Not classified as flammable gas.

2.2.1.1.3 **Oxidising gases** *[equivalent to section 8.3 of the CLH report template]*

Table 4: Summary table of studies on oxidising gases

Method	Results	Remarks	Reference
-	-	-	-

2.2.1.1.3.1 Short summary and overall relevance of the provided information on oxidising gases

Not applicable, the substance is not a gas.

2.2.1.1.3.2 Comparison with the CLP criteria

Not applicable.

2.2.1.1.3.3 Conclusion on classification and labelling for oxidising gases

Not classified as oxidising gas.

2.2.1.1.4 **Gases under pressure** ***[equivalent to section 8.4 of the CLH report template]***

Table 5: Summary table of studies on gases under pressure

Method	Results	Remarks	Reference
-	-	-	-

2.2.1.1.4.1 Short summary and overall relevance of the provided information on gases under pressure

Not applicable, the substance is not a gas under pressure.

2.2.1.1.4.2 Comparison with the CLP criteria

Not applicable.

2.2.1.1.4.3 Conclusion on classification and labelling for gases under pressure

Not classified as gas under pressure.

2.2.1.1.5 **Flammable liquids** ***[equivalent to section 8.5 of the CLH report template]***

Table 6: Summary table of studies on flammable liquids

Method	Results	Remarks	Reference
-	-	-	-

2.2.1.1.5.1 Short summary and overall relevance of the provided information on flammable liquids

Not applicable, the substance is not a liquid.

2.2.1.1.5.2 Comparison with the CLP criteria

Not applicable.

2.2.1.1.5.3 Conclusion on classification and labelling for flammable liquids

Not classified as flammable liquid.

2.2.1.1.6 **Flammable solids** ***[equivalent to section 8.6 of the CLH report template]***

Table 7: Summary table of studies on flammable solids

Method	Results	Remarks	Reference
UN test N.1	Not flammable	-	[redacted] (2019) Report no. PS20190309-1

2.2.1.1.6.1 Short summary and overall relevance of the provided information on flammable solids

Experimental test demonstrated that the pure substance is not flammable.

2.2.1.1.6.2 Comparison with the CLP criteria

The results of the the experimental test (substance not flammable) do not fulfil the criteria in table 2.7.1.

2.2.1.1.6.3 Conclusion on classification and labelling for flammable solids

Not classified as flammable solid.

2.2.1.1.7 Self-reactive substances *[equivalent to section 8.7 of the CLH report template]*

Table 8: Summary table of studies on self-reactivity

Method	Results	Remarks	Reference
-	-	-	-

2.2.1.1.7.1 Short summary and overall relevance of the provided information on self-reactive substances

Glyphosate acid does not contain chemical groups associated with explosive or sel reactive properties properties, see tables A6.1 and A6.3 in Appendix 6 of the UN RTDG.

2.2.1.1.7.2 Comparison with the CLP criteria

The criteria in 2.8.4.2 (a) are fulfilled, therefore glyphosate does not warrant classification.

2.2.1.1.7.3 Conclusion on classification and labelling for self-reactive substances

Not classified as self-reactive.

2.2.1.1.8 Pyrophoric liquids *[equivalent to section 8.8 of the CLH report template]*

Table 9: Summary table of studies on pyrophoric liquids

Method	Results	Remarks	Reference
-	-	-	-

2.2.1.1.8.1 Short summary and overall relevance of the provided information on pyrophoric liquids

Not applicable, the substance is not a liquid.

2.2.1.1.8.2 Comparison with the CLP criteria

Not applicable.

2.2.1.1.8.3 Conclusion on classification and labelling for pyrophoric liquids

Not applicable.

2.2.1.1.9 Pyrophoric solids *[equivalent to section 8.9 of the CLH report template]*

Table 10: Summary table of studies on pyrophoric solids

Method	Results	Remarks	Reference
-	-	-	-

2.2.1.1.9.1 Short summary and overall relevance of the provided information on pyrophoric solids

Experience in manufacture or handling shows that glyphosate does not ignite spontaneously in contact with air at normal temperatures.

2.2.1.1.9.2 Comparison with the CLP criteria

No pyrophoric properties was observed experimentally.

2.2.1.1.9.3 Conclusion on classification and labelling for pyrophoric solids

Not classified as pyrophoric solid.

2.2.1.1.10 **Self-heating substances** *[equivalent to section 8.10 of the CLH report template]*

Table 11: Summary table of studies on self-heating substances

Method	Results	Remarks	Reference
UN test N.4	Not self heating substance		[redacted] (2019) Report no. PS20190309-2

2.2.1.1.10.1 Short summary and overall relevance of the provided information on self-heating substances

The UN N.4 test was negative, thefore glyphosate has no self-heating properties.

2.2.1.1.10.2 Comparison with the CLP criteria

Glyphosate does not fulfil the criteria self-heating substances, see table 2.11.1.

2.2.1.1.10.3 Conclusion on classification and labelling for self-heating substances

Not classified as self-heating solid.

2.2.1.1.11 **Substances which in contact with water emit flammable gases** *[equivalent to section 8.11 of the CLH report template]*

Table 12: Summary table of studies on substances which in contact with water emit flammable gases

Method	Results	Remarks	Reference
-	-	-	-

2.2.1.1.11.1 Short summary and overall relevance of the provided information on substances which in contact with water emit flammable gases

Knowledge of the substance and experimental studies show the substance does not emit flammable gases when in contact with water. In addition, glyphosate chemical structure does not contain metals or metalloids.

2.2.1.1.11.2 Comparison with the CLP criteria

Glyphosate fulfils the criteria in 2.12.4.1 (a) and (b), therefore no classification is warranted.

2.2.1.1.11.3 Conclusion on classification and labelling for substances which in contact with water emit flammable gases

Not classified as a substance that emits flammable gas in contact with water.

2.2.1.1.12 **Oxidising liquids** *[equivalent to section 8.12 of the CLH report template]*

Table 13: Summary table of studies on oxidising liquids

Method	Results	Remarks	Reference
-	-	-	-

2.2.1.1.12.1 Short summary and overall relevance of the provided information on oxidising liquids

Not applicable as the substance is not a liquid.

2.2.1.1.12.2 Comparison with the CLP criteria

Not applicable.

2.2.1.1.12.3 Conclusion on classification and labelling for oxidising liquids

Not classified as oxidising liquid.

2.2.1.1.13 Oxidising solids *[equivalent to section 8.13 of the CLH report template]*

Table 14: Summary table of studies on oxidising solids

Method	Results	Remarks	Reference
EEC A.17	Not oxidizing	-	[redacted] (1997) Report no. RJ2401B

2.2.1.1.13.1 Short summary and overall relevance of the provided information on oxidising solids

The negative A.17 result is not sufficient to conclude the substance is not oxidizing. The chemical structure contains oxygen atoms which are not bonded only to carbon or hydrogen.

2.2.1.1.13.2 Comparison with the CLP criteria

Glyphosate does not fulfil the criteria in 2.14.4.1 (b) and should have been tested according to UN O.1 method. The negative A.17 test provides supporting information that glyphosate is not oxidising, however it is not sufficient to conclude on the classification.

2.2.1.1.13.3 Conclusion on classification and labelling for oxidising solids

Not classified as oxidizing due to lack of data.

2.2.1.1.14 Organic peroxides *[equivalent to section 8.14 of the CLH report template]*

Table 15: Summary table of studies on organic peroxides

Method	Results	Remarks	Reference
-	-	-	-

2.2.1.1.14.1 Short summary and overall relevance of the provided information on organic peroxides

Not applicable as the substance does not contain peroxides.

2.2.1.1.14.2 Comparison with the CLP criteria

Not applicable.

2.2.1.1.14.3 Conclusion on classification and labelling for organic peroxides

Not classified as organic peroxides.

2.2.1.1.15 **Corrosive to metals** ***[equivalent to section 8.15 of the CLH report template]***

Table 16: Summary table of studies on the hazard class corrosive to metals

Method	Results	Remarks	Reference
-	-	-	-

2.2.1.1.15.1 Short summary and overall relevance of the provided information on the hazard class corrosive to metals

The glyphosate melting point was determined to be 189.5 °C, which is above the cut off criteria of 55 °C for testing.

2.2.1.1.15.2 Comparison with the CLP criteria

No corrosiveness to metals is expected for this substance as its melting point is above 55 °C.

2.2.1.1.15.3 Conclusion on classification and labelling for corrosive to metals

Not classified as corrosive to metals.

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

The appearance of the product is a clear yellow homogeneous liquid free from visible suspended matter and sediment, with amine odour. It is not explosive, has no oxidising properties. The product has no flash point up to boiling point. It has a self-ignition temperature of 440 ± 5 °C. In aqueous solution, it has a pH value 4.83 at room temperature. There is no effect of low and high temperature on the stability of the formulation, since after 7 days at 0 °C and 14 days at 54 °C, neither the active ingredient content and relevant impurities formaldehyde and NNG, nor the physical properties changed. The ambient temperature shelf-life studies (two years and five years at 20 °C) show no significant changes in physical properties and on the content of active ingredient and relevant impurities formaldehyde and NNG. Therefore, a shelf life of up to five years at ambient temperature can be considered for the product MON 52276. Its physical characteristics are acceptable for a soluble concentrate formulation.

2.3 DATA ON APPLICATION AND EFFICACY

2.3.1 Summary of effectiveness

Glyphosate acts as a post-emergence herbicide and is only taken up by the green parts of already emerged plants. It has systemic action and is effective against a range of weed growth stages. Glyphosate is non-selective, hence used for the control of a broad range of annual, biennial and perennial monocotyledonous and dicotyledonous weeds.

Glyphosate is one of the most utilized herbicides within the European Union (EU). Commercial glyphosate products have registered uses in agriculture, horticulture, forestry, viticulture, amenity, weed control of non-cultivated areas, home and garden uses and aquatic weed control. The substance has become an important part of current practices for the control of weeds and invasive species, including Integrated Weed Management (IWM) programs and in Conservation Agriculture.

The applicant submitted several reports addressing the efficacy of glyphosate, comparison to chemical/non-chemical alternatives, the need for effective control, and socio-economic value of glyphosate etc.. The reports address use in agriculture, in Conservation Agriculture, for railways, and for the control of invasive species. However, considering that glyphosate is approved and authorisations of plant protection products containing glyphosate have been evaluated according to the Uniform Principles (Regulation (EC) No 546/2011), detailed data related to efficacy is not required at this stage. Furthermore, assessments of the value of active substances or socio-economic analyses are not part of the assessment of applications for (renewal of) approval of active substances under Regulation (EC) No 1107/2009. Most of the reports submitted have therefore only been briefly presented in Vol 3, B.3 (CA). It is acknowledged though, that all reports may be of interest in a wider context.

2.3.2 Summary of information on the development of resistance

The first case of reported resistance to glyphosate in Europe was recorded in 2004 for *Conyza bonariensis* in orchards in Spain. In Europe there are about 30 confirmed glyphosate resistance cases reported, mainly for *Conyza* spp. and *Lolium* spp., but recently cases of resistance for *H. murinum* subsp. *leporinum*, *Bromus madritensis* and *Bromus rubens* were reported from Spain, and for *Eleusine indica* in Italy (http://weedsceince.org). The majority of confirmed cases of resistance within Europe are found in perennial crop situations or railways, with only two cases in arable crops (wheat).

Globally, 50 different species/sub-species have been confirmed as having weed populations resistant to glyphosate (www.weedscience.org summary sheet "Glyphosate Resistant Weeds"). The first glyphosate resistant population was identified in 1996 in Australia.

Glyphosate is classified by HRAC (Herbicide Resistance Action Committee) in group 9 (Inhibition of Enolpyruvyl Shikimate Phosphate Synthase), in Legacy HRAC this was group G. The mode of action of glyphosate is unique, which provides an alternative solution to control weeds and plays a role to manage the development of resistance of weeds to other chemical herbicide with a different mechanism of action.

The applicant provided a management strategy to avoid resistance for consideration at national (or regional) level.

2.3.3 Summary of adverse effects on treated crops

Glyphosate is a non-selective herbicide, taken up by green tissue of the leaves and stems of treated plants. It is transported systemically (via apoplastic and symplastic pathways) throughout the plant including the roots, rhizomes and stolons but especially to areas of metabolic activity within the plant (sinks).

In case of pre-planting or pre-sowing applications there are no crops on the treated area.
Pre-planting covers uses on stubble as well as on seedbed preparations. The waiting period between the last application and the sowing or (trans-)planting of the succeeding crops is 3 days. If the instructions are kept no crop damage is expected to occur.
Weeds in orchards and vines can be treated throughout the growing season (inter-row or around the stem) provided that the trees or vines are well developed (woody stems). During application care must be taken not to spray the green parts of the crops (shoots, leaves) as they could be damaged. The active substance can also damage saplings younger than 2-3 years which have no lignified trunk, therefore it is recommended not to use the product in new plantations younger than 2-3 years.
In vegetables an inter-row application should take place before BBCH 20. The spray application must be ground directed and shielded. If the instructions are kept no crop damage is expected to occur.

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

There were no reported observations on other undesirable or unintended side-effects.

2.4 FURTHER INFORMATION

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

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

2.4.2 Summary of procedures for destruction or decontamination

Adequate information on destruction or decontamination is available.

2.4.3 Summary of emergency measures in case of an accident

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

2.5 METHODS OF ANALYSIS

2.5.1 Methods used for the generation of pre-authorisation data

2.5.1.1 Analysis of the active substance as manufactured

Analytical methods have been provided for the analysis of glyphosate and its impurities. These relevant suitable methods have been validated by checking the parameters linearity, precision, accuracy, specificity and interference. For several methods used for the determination of relevant impurities, the LOQs are not fully validated.

2.5.1.2 Formulation analysis

A fully validated analytical method for the determination of the glyphosate in the representative SL formulation has been developed according to current guidelines.
Analytical methods for the determination of the relevant impurities formaldehyde and N-Nitrosoglyphosate in the representation SL formulation have been provided. However, the precision data have been addressed only with the analysis of standard solutions. An analysis using fortified samples should have been performed. This is considered as a data garp. Moreover, no methods for the determination of the new relevant impurities (formic acid and trimethylamine) have been provided. This is also considered as a data gap.

2.5.1.3 Methods for Risk Assessment

In support of physical and chemical properties tests

All studies in this section were evaluated for analytical validation data, and summarised if available. The methods used in peer-reviewed physical and chemical properties studies which are still relied upon for re-approval of the active substance glyphosate, are considered acceptable as fit for purpose to support the respective studies concerned. Analytical summaries for new studies are provided and considered valid.

In support of efficacy studies

No analytical data are submitted in support of efficacy studies.

In support of toxicological studies

All studies in this section were evaluated for analytical validation data, and summarised if available. The methods used in peer-reviewed toxicological studies which are still relied upon for re-approval of the active substance glyphosate, are assessed and for the majority of methods are considered acceptable as fit-for-purpose to support the respective studies concerned. Analytical summaries for new or not previously submitted studies are provided and the majority of methods are considered acceptable as fit-for-purpose.

In support of operator, worker, resident and bystander exposure studies

No analytical data are submitted in support of exposure studies.

In support of residue studies

All studies in this section were evaluated for analytical validation data and summarised if available. The methods used in peer-reviewed residue studies which are still relied upon for re-approval of the active substance glyphosate, for the most, they can be considered as validated to support the respective studies concerned. However for some of methods several deficiencies have been noted such as the demonstration of the derivatisation efficiency. Without this information, these methods cannot be considered as validated. The stability studies are considered as fit for purpose. Analytical summaries for new studies are provided.

In support of environmental fate studies

All studies in this section were evaluated for analytical validation data, and summarised if available. The methods used in peer-reviewed environmental fate studies which are relied upon for re-approval of the active substance glyphosate, for the most, considered acceptable as fit-for-purpose to support the respective studies concerned except

two methods for which validation data available do not allow to validate them. Analytical summaries for new or not previously submitted studies are provided.

In support of ecotoxicology studies

All studies in this section were evaluated for analytical validation data, and summarised if available. Some of the methods used in peer-reviewed ecotoxicology studies which are still relied upon for re-approval of the active substance glyphosate, are assessed and for the majority of methods are considered acceptable as fit for purpose to support the respective studies concerned. For some study reports, no validation data are available. Therefore, these methods cannot be considered as fit for purpose. Analytical summaries for new or not previously submitted studies are provided and some are considered acceptable as fit-for-purpose. For some studies, the analytical report is not available or very limited data are available, therefore these methods cannot be considered as fit for purpose.

2.5.2 Methods for post control and monitoring purposes

Plants and plant products

New analytical monitoring methods (based on HPLC-MS/MS) for the determination of glyphosate and AMPA in plant matrices with high water content (sugar beet tops), high oil content (undelinted cotton seeds, soybean seeds), dry (corn grain and corn stover) and fruits with high acid content (oranges) and *N*-acetylglyphosate in plant matrices with high water content (corn forage), high oil content (soybean and canola seed), dry (corn grain) and fruits with high acid content (oranges) have been developed. Independent laboratory validations are available. The methods with an LOQ of 0.05 mg/kg for glyphosate and AMPA and 0.025 mg/kg for *N*-acetylglyphosate are suitable to be used for monitoring/enforcement purposes. Concerning the extraction efficiency, the solvents used in the analytical method and the metabolism studies have considered identical.

Food of animal origin

New analytical monitoring methods (based on HPLC-MS/MS) for the determination of glyphosate and AMPA or *N*-acetylglyphosate in animal matrices (meat, fat, liver, milk, egg) have been developed and validated. An independent laboratory validation is available. The methods, with an LOQ of 0.025 mg/kg for glyphosate, AMPA and *N*-acetylglyphosate, are suitable to be used for monitoring/enforcement purposes. Concerning the extraction efficiency, the solvents used in the analytical method and the metabolism studies have been considered as identical.

A new method for monitoring purposes of glyphosate and AMPA (based on HPLC-MS/MS) in honey was developed. An independent laboratory validation is available. The method, with an LOQ of 0.025 mg/kg for glyphosate and AMPA, is suitable to be used for monitoring/enforcement purposes. The extraction efficiency of the method is not demonstrated for honey. Therefore, the analytical method for honey is not considered as validated.

Soil

A new analytical monitoring method (based on HPLC-MS/MS) for the determination of glyphosate and AMPA in soil has been developed and validated. An ILV is not required. The method with an LOQ of 0.05 mg/kg for each analyte is suitable to be used for monitoring/enforcement purposes.

Water

An already evaluated analytical monitoring method (based on HPLC-MS/MS) for the determination of glyphosate and AMPA in surface, ground and drinking water is submitted. The method was validated by an independent laboratory for the analysis of both analytes in drinking water. The method with an LOQ of 0.03 µg/L for each analyte is suitable to be used for monitoring/enforcement purposes. However, the validation of the derivatisation efficiency should be provided.

Air

An already evaluated analytical monitoring method (based on GC-MS) for the determination of glyphosate in air is submitted. An ILV is not required. The method with an LOQ of 5 µg/m³ is suitable to be used for monitoring/enforcement purposes. However, the validation of the derivatisation efficiency should be provided

Body fluids and tissues

A new analytical monitoring method (based on HPLC-MS/MS) for the determination of glyphosate and AMPA in urine has been developed and validated. An ILV is not required. The method with an LOQ of 0.01 mg/L for each analyte is suitable to be used for monitoring/enforcement purposes.

2.6 EFFECTS ON HUMAN AND ANIMAL HEALTH

2.6.1 Summary of absorption, distribution, metabolism and excretion in mammals [equivalent to section 9 of the CLH report template]

Table 17: Summary table of toxicokinetic studies

Method	Results	Remarks	Reference
No guideline GLP Deviations: None Study acceptable Sprague Dawley rats, males and females, 4/sex/dose Glyphosate, batch 11493988, purity 97.7% 14-day repeated dose, dietary applied, at 75 and 400 mg/kg bw	Systemic exposure (C_{max}, AUC) increased in dose dependent manner and independent of gender Plasma elimination within 48 h independent of dose $t_{1/2}$ of 11-13 h No AMPA detected at 75 mg/kg bw. AMPA exposure at 400 mg/kg bw approx. 0.6% AMPA $t_{1/2}$ = 7.3 h	None	Report No. 00050502 (2020)
Performed according OECD 417 GLP Deviations: None Study acceptable Sprague Dawley rats, male females, 5/sex/dose (excretion, plasma concentration), 12/sex/dose (tissue distribution), 8/sex/dose (biliary excretion). Glyphosate, batch 08808TG and H95D161A, purity 96 % and 95.3 %, respectively Single oral dose at 1 and 100 mg/kg bw; 1 mg/kg bw/day (for biliary excretion)	Limited absorption with C_{max} of 0.02-0.04 µg/mL at 1 mg/kg bw and 7.6-8.9 µg/mL at 100 mg/kg bw. T_{max} 4-8 h Widespread but limited distribution. Apart from GI tract and carcass, highest amount in kidney and bone <1% metabolised independent of dose Elimination almost complete within 48 h 25-35% and 53-55% excreted in urine at 1 and 100 mg/kg bw, respectively. 62-73% and 41-42% in faeces at 1 and 100 mg/kg bw respectively. Negligible biliary excretion, 0.3-0.8% No gender differences noted	None	Report No. 1413/2-1011 (1996)

Method	Results	Remarks	Reference
OECD 417 (1984) GLP Deviations: None Study acceptable Alpk:APfSD rats, males and females, 5/sex/dose. Glyphosate, batch Y04707/045, purity 99.2% Single oral dose at 10 mg/kg bw	Limited absorption (11-13%) and widespread distribution <0.6% present in tissues after 72 h. Highest amount in bone, GI tract, carcass, kidney and liver Urinary excretion mainly completed within 24 h No gender differences noted	None	Report No. [redacted]/P/4940 (1996)
OECD 417 (1984) GLP Deviations: None Study acceptable Alpk:APfSD rats, males and females, 5/sex/dose. Glyphosate, batch Y04707/048, purity 99.5% Single oral dose at 1000 mg/kg bw.	Limited absorption (17-18%) and widespread distribution <0.6% present in tissues after 72 h Urinary excretion mainly completed within 24 h No gender differences noted	None	Report No. [redacted]/P/4942 (1996)
OECD 417 (1984) GLP Deviations: None Study acceptable Alpk:APfSD rats, males and females, 5/sex/dose. Glyphosate, batch Y04707/045, purity 99.2% Repeated oral dose at 10 mg/kg bw	Limited absorption (11%) Widespread distribution with <0.5% present in tissues after 72 h Similar pattern as for single oral dose No evidence of accumulation	None	Report No. [redacted]/P/4944 (1996)
OECD 417 (1984) GLP Deviations: low animal number **Study unacceptable** Alpk:APfSD rats, males and females, 2/sex/dose. Glyphosate, batch Y04707/045, purity 99.2%	Rapid excretion predominantly in faeces followed by urine with <0.2% in exhaled air Whole body autoradiography confirmed distribution and elimination observed in other studies	None	Report No. [redacted]/P/4943 (1996)

Method	Results	Remarks	Reference
Single oral dose at 10 mg/kg bw			
OECD 417 (1984) GLP Deviations: None Study acceptable Alpk:AP_fSD rats, males and females, 2/sex/dose (bile cannulation), 15/dose/sex (biotransformation) Glyphosate, batch Y04707/048, purity 99.5% Single oral dose at 1000 mg/kg bw (bile cannulation)	Absorbed dose (21-22%) excreted in urine Bile excretion negligible Glyphosate mainly excreted unchanged with trace amounts of AMPA in urine.	Biotransformation investigated in pooled data from Report No. [redacted]/P/4940; [redacted]/P/4942; [redacted]/P/4944.	Report No. [redacted]/P/5058 (1996)
Deviations: Reporting and methodological deficiencies (e.g. radioactivity was only investigated in the organs, tissues and carcass in one group receiving a low intravenous dose. Metabolites only investigated in urine and not faeces). **Study supplementary** Sprague-Dawley rats, males and females, 4/sex (group I to III) and 8 females (group IV) Glyphosate, batch UN-NO:1759, 32140, purity 98% Group I Single *i.v.* dose of 0.2 mg/kg bw/day Group II Single low oral dose of 0.2 mg/kg bw/day Group III Single high oral dose of 200 mg/kg bw/day Group IV Single *i.v.* dose of 0.2 mg/kg bw/day Group I to III: urine and faeces sampling Group IV: blood and organ sampling	Low absorption (11-15%) and independent of dose Bioavailability (F) approx. 12% Limited tissue sampled with highest levels detected in kidneys No metabolites detected in urine at 200 mg/kg bw Excretion mainly completed within 24 h	None.	Report No. 9202/95 (part 1), [redacted] 038/94 (part 2)
OECD 417 (1984) GLP Deviations: None Study acceptable	Absorbed dose (19-30%) similar for both sex and independent of dose	None.	Report No. [redacted] 332/951256 (1995)

Method	Results	Remarks	Reference
Sprague-Dawley rats, males and females, 9/dose/sex (plasma concentration, 6/dose/sex (distribution), 5/dose/sex (excretion) Glyphosate, batch 061221, purity 98.9% Single oral dose at 10 or 600 mg/kg bw	Peak plasma concentrations at 2-6 h, t½ of 6-8 h Tissue distribution independent of gender and dose. Highest concentration detected in GI tract, kidneys, muscle, bone and plasma Glyphosate mainly excreted unchanged. Main metabolite AMPA (0.1-2%) Rapid excretion with most excreted within 48 h. Within 7 days, 19-23% and 29-30% excreted in urine at 10 and 600 mg/kg bw, respectively and 75-84% and 74-75% excreted in faeces at 10 and 600 mg/kg bw, respectively. <0.2% of dose detected in exhaled air		
US-EPA FIFRA 85-1 GLP Deviations: Preliminary study; only 3 animals at single dose and limited focus Acceptable as preliminary study only Sprague-Dawley rats, males, 3/dose Glyphosate, batch 206-JaK-25-1, purity 98.6% Single oral dose at 30 mg/kg bw	C_{max} of 0.7-1.8 µg/mL and T_{max} within 4 h. Non-detectable levels at 12 h Widespread but limited distribution into tissues. Highest concentration 10 h post dose in bone, bone marrow, cartilage, GI tract, kidney, urinary tract and nasal mucosa. At 24 h negligible tissue concentrations except bone and bone marrow	Preliminary study	Report No. 6365-676/1
Performed according OECD 417 GLP Deviations: None Study acceptable Sprague-Dawley rats, males and females, 5/dose/sex Glyphosate, batch 206-JaK-25-1, purity 98.6% Single *i.v.* dose at 30 mg/kg bw, single oral dose at 30 or 1000 mg/kg bw and 14-day repeated dose at 30 mg/kg bw	Absorption pattern similar after single low and high oral dose and repeated oral dose (22-35% in urine) Widespread tissue distribution with low residues. Highest amount in bone. Concentration increased with dose dependent but independent of frequency (no accumulation) No metabolites detected in urine or faeces. Rapid excretion (most within 24 h (oral) and 4 hours (*i.v.*).	None	Report No. 7006-676/2

Method	Results	Remarks	Reference
	Renal excretion dominant after *i.v.* administration		
Performed according OECD 417 GLP Deviations: None Study acceptable Sprague-Dawley rats, males and females, 5/dose/sex (urine, faeces, tissues), 3/dose/sex (blood) Glyphosate, batch not reported, purity 99.8% Single dose at 10 mg/kg bw (*i.v.* or oral), single dose at 1000 mg/kg bw (oral), 14 day repeated dose at 10 mg/kg bw (oral)	Absorption low (30-36%) and independent of dose and frequency Tissue residues low (slightly higher in males). Highest amount in bone. Concentration dose dependent and no sign of accumulation Faecal excretion major route of elimination irrespective of dose, dosing frequency and gender. Renal excretion dominant after *i.v.* administration	Part 2 in report No. [redacted]-7206	Report No. [redacted]-7215
Performed according OECD 417 GLP Deviations: None Study acceptable Sprague-Dawley rats, males and females, 5/dose/sex (urine, faeces, tissues), 3/dose/sex (blood) Glyphosate, batch not reported, purity 99.8% Single dose at 10 mg/kg bw (*i.v.* or oral), single dose at 1000 mg/kg bw (oral), 14 day repeated dose at 10 mg/kg bw (oral)	Mainly excreted unmetabolized (98.5-99.3%). AMPA <1% excreted; N-nitroso-glyphosate 0.06-0.2% excreted; N-acetylglyphosate 0.1% excreted; unknown metabolites 0.5% excreted Results independent of dose, method or frequency of dosing.	Part 1 in report No. [redacted]-7215	Report No. [redacted]-7206
No guideline GLP Deviations : not applicable Study acceptable Cryo-preserved pooled male and female hepatocytes from human, rat (Sprague-Dawley), mouse (CD-1), dog (beagle), rabbit (New Zealand White, only female) 0.5 x 10^6 viable cells/mL Glyphosate, batch 6848SXD008-2, purity 98.3%	No human specific metabolites detected. Mainly unmetabolized glyphosate detected (97%).	None	Report No. S19-04081

Method	Results	Remarks	Reference
Single exposure at 1 and 10 µM for 0 ,60 and 120 min in 1000 µL.			

2.6.1.1 Short summary and overall relevance of the provided toxicokinetic information on the proposed classification(s)

Following intravenous dosing excretion was predominantly via urine (Report No 9202/95, Report No 7006-672/2 and Report No [redacted]-7215) and the extent of biliary excretion in rats was low (< 0.1 %) irrespective of dose or sex (Report No 1413/2-1011 and [redacted]/P/5058), therefore following oral administration the amount excreted via the urinary route was considered absorbed.

Glyphosate is absorbed from the gastrointestinal tract with peak plasma levels (T_{max}) ranging from 0.5 to 8 h (Report No 00050502, 1413/2/1011 and [redacted]332/951256) after exposure. Absorption after oral application is limited and independent of dose, exposure duration and sex. Approximately 10 – 35% of the glyphosate dose is absorbed and excreted predominantly unchanged in the urine.

An overview of the percentage of glyphosate absorbed for the acceptable studies is included in the table below. These values are based on the amounts found in urine and cage wash. A few studies also include bile cannulated rats. However, excretion via bile was concluded to be a negligible excretion route and is therefore not included.

Report number	Dose level tested	Percentage absorbed
1413/2-1011	1 or 100 mg/kg bw, single oral dose Vehicle: deionised water	1 mg/kg bw: 24.9% in males, 34.9% in females 100 mg/kg bw: 53.3% in males, 55.0% in females
[redacted]/P/4940	10 mg/kg bw, single oral dose Vehicle: deionised water	13.3% in males and 11.1% in females
[redacted]/P/4942	1000 mg/kg bw, single oral dose Vehicle: deionised water	16.9% in males and 17.8% in females
[redacted]/P/4944	10 mg/kg bw/day, repeated oral dose Vehicle: deionised water	10.8% in males and 10.9% in females
[redacted]/P/5058	1000 mg/kg bw/day single oral dose Vehicle: deionised water	22.7% in males and 21.4% in females
[redacted] 332/951256	10 or 600 mg/kg bw, single oral dose Vehicle: Water, solubility was increased by addition of sodium hydrogen carbonate	10 mg/kg bw: 22.5% in males and 19.4% in females 600 mg/kg bw: 30.3% in males and 29.5% in females
7006-676/2	30 mg/kg bw, single *i.v.* dose 30 or 1000 mg/kg bw, single oral dose	30 mg/kg bw, *i.v.* dose: 92.9% in males, 97.4% in females 30 mg/kg bw, single oral dose: 35.9% in males, 38.2% in females

Report number	Dose level tested	Percentage absorbed
	30 mg/kg bw/day, repeated oral dose Vehicle: 0.9 % w/v sodium chloride solution in water	30 mg/kg bw, repeated dose: 39.4% in males, 39.8% in females 1000 mg/kg bw, single oral dose: 42.6% in males, 39.1% in females
[redacted]-7215	10 mg/kg bw, single *i.v.* dose 10 or 1000 mg/kg bw, single oral dose 10 mg/kg bw/day, repeated oral dose Vehicle: sterile saline	10 mg/kg bw, single *i.v.* dose 79.9% in males, 75.8% in females 10 mg/kg bw, single oral dose 29.9% in males, 24.5% in females 1000 mg/kg bw, single oral dose 21.7% in males, 22.3% in females 10 mg/kg bw, repeated dose 31.7% in males, 25.1% in females

The oral absorption values do not show any sex differences nor a clear difference between the low and the high dose tested, although some studies (1413/2-1011 and [redacted] 322/951256) do seem to indicate slightly higher absorption at higher dose levels while the other two studies in which two doses were tested do not (7006-676/2 and [redacted]-7215). In general, repeated exposure did result in higher absorption values.
During the first approval an oral absorption value of 30% was derived. This was lowered to 20% during the previous EU evaluation as this value was in the middle of the values that were derived in the various studies. The oral absorption values ranged between 10.8% to 55% for the single oral dose studies, with a mean of 27% (sexes and different dose levels combined). Therefore, the absorption value of 20% is still considered to be acceptable.

Elimination of ingested glyphosate via faeces and urine is rapid and is nearly complete within 48 h as shown in the majority of studies. The major route of excretion for the unabsorbed fraction is via the faeces and via the urine for the absorbed fraction. The pulmonary route of elimination is negligible (< 0.2%; Report NO 5.1.1/010).

The systemic available amount (given as AUC) correlates with the dose level upon single gavage application. Repeated dietary application resulted in comparably lower maximum plasma concentrations. Maximum blood plasma concentration in rats after repeated 14-day dietary application of 72 and 385 mg glyphosate/kg bw/day were 0.84 and 5.31 µg/mL for male and 0.64 and 4.69 µg/mL for female rats, respectively (Report No 00050502). After a single gavage application of 1 and 100 mg glyphosate/kg bw maximum plasma concentrations of 0.02 and 8.91 µg/mL for male rats and 0.036 and 7.63 µg/mL for female rats (Report No 1413/2-1011). After a single gavage application of 10 and 600 mg glyphosate/kg bw maximum plasma concentrations of 0.22 and 26 µg/mL for male rats and 0.28 and 29 µg/mL for female rats were determined (Report No [redacted] 332/951256).

Several studies investigated distribution of glyphosate at different dose levels into the organs and tissues after oral dose (e.g. Report No. [redacted]/P/4942, [redacted]/P/4944, [redacted] 332/951256 and 7006-676/2). The absorbed glyphosate distributes rapidly, however, only low levels were found in organs and tissues at termination. After a period of 3 - 7 days following oral administration, total body burden accounted for less than 1% of the applied radioactivity. The highest levels were measured in bone, followed by kidney and liver. There is no evidence of a potential for accumulation in animals based on residue analysis in organs and tissues after 3 - 7 days. Elimination from bone is slower than from other tissues. However, the amount of radiolabel in bone after 7 days after a single oral dose was relatively low at 0.02 - 0.03% of the applied dose. The pattern of absorption, distribution and elimination was not significantly changed either by single high doses administered or by repeated administration of low doses. Similarly, the pattern of absorption, distribution and elimination was irrespective of the sex.
Elimination from blood and plasma was rapid with no evidence of accumulation in blood cells. A biphasic pattern of elimination of radiolabel in plasma has been suggested from the plasma radiolabel in a range of studies and terminal half-lives were 6 – 12 h and independent of dose level (Report No [redacted] 332/951256 and 6365-676/1). The terminal half-lives were comparable (11 and 13 h at low and high dose, respectively) when glyphosate was applied via diet at 14 consecutive days (Report No. 00050502).
Detection of radiolabelled material in plasma was negligible after 24 h and not detected at 168 h upon single application. Upon repeated application for 14 consecutive days via diet blood plasma concentrations were higher but rapidly declined within 48 h.

Metabolism

In the rat metabolism studies, the metabolism of glyphosate is very limited. Most of the parent is eliminated unchanged and a small amount, just under 0.5% of the applied dose, is eliminated as aminomethylphosphonic acid (AMPA). Low AMPA concentrations were detected in faeces and urine upon intravenous application of glyphosate ([redacted]-7206). Following 14 days of dietary administration of 72 and 385 mg/kg bw/day glyphosate to rats no AMPA was detected in plasma of the rats at the low dose. AMPA was only detected in plasma at 385 mg/kg bw/day and only accounted for 0.6% of the systemic exposure (AUC0-48h) for glyphosate (Report No. 00050502). Maximum blood plasma concentration of AMPA in rats after repeated dietary application for 14 consecutive days of 385 mg glyphosate/kg bw/day was about 0.04 µg/mL, which was reached at a T_{max} of 0.5 h. The half-life of AMPA was approximately 7 h.

Metabolism of glyphosate by other mammals was also confirmed to be limited based on an *in vitro* comparative metabolism study. At least 97% of the applied radioactivity was identified as glyphosate when cryo-preserved hepatocytes from human, rat, dog, mouse and rabbit were incubated with 14C-glyphosate. No AMPA was detected in hepatocytes of any species at any time point and likewise no human unique metabolites were detected (Report No. S19-04081). Overall, glyphosate metabolism in mammals has been shown to be very limited.

Human data
Reliable kinetic data obtained in humans are scarce. One study investigated the half-life of glyphosate from human urine samples collected from amenity horticulture workers using glyphosate based pesticide products (Connolly *et al*., 2019a (refer to Vol 3 CA B.6.9.8.3)). Urine samples from seven participants (6 males, 1 female) performing eight application work tasks were analyzed. Per participant 28 individual spot urine samples were analyzed (3 to 4 spot urine samples per task). Based on these samples, the study authors derived an average glyphosate half-life of approximately 5.5 to 10 hours. However, it should be noted that there was limited standardization (different products used, quantity of pesticides applied per task varied and different application methods and different sampling times were used). The pharmacokinetic analysis revealed first order kinetics but due to the collection of urine samples over a limited period of time (19-26 hours) multi-phasic kinetics may not have been identified.

In addition, a public literature study is available in which 13 poisoning incidents with glyphosate-based herbicides in France (Zouaoui *et al.*, 2012) were analysed. **This publication was evaluated during the previous assessment of glyphosate by RMS DE. However, it is not re-submitted by the applicant.** For the process under Regulation (EC) No 1107/2009, the applicant is requested to provide the study and an assessment. For the process under the Regulation (EC) No 1272/2008, the applicant is asked to submit the missing information during the public consultation period. This study showed that there is at least strong evidence that biotransformation of ingested glyphosate to AMPA is very limited also in man. The glyphosate:AMPA ratio in blood analyses varied between 12:1 and 6933:1 with a median value of 235:1. In urine, with data from 7 cases available, the individual ratios ranged from 243:1 to 7863:1 with a median of 422:1. These ratios were independent from the severity of symptoms or a fatal outcome.

2.6.2 Summary of acute toxicity

2.6.2.1 Acute toxicity - oral route [equivalent to section 10.1 of the CLH report template]

Table 18: Summary table of animal studies on acute oral toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Value LD_{50}	Reference
OECD 420 (2001), GLP Signatures are missing in the study report (GLP statement, quality assurance unit) **Study not acceptable**	Rat, RccHanTM:Wistar Female 5/dose	Glyphosate technical Batch: 04062014 Purity: 85.79%	2000 mg/kg bw Single oral dose	Not determined due to unacceptability of the study, however no mortality observed.	CA 5.2.1/001 Report no.: 41401853 (2014)

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Value LD_{50}	Reference
OECD 425 (2008), GLP No significant deviations Study acceptable	Rat, RjHan:WI Female 3/dose	Glyphosate technical Batch: 569753 (BX20070911) Purity: 96.3%	5000 mg/kg bw Single oral dose	>5000 mg/kg bw Clinical signs: none	CA 5.2.1/002 Report no.: 10/218-001P (2011)
OECD 423 (2001), GLP No deviations Study acceptable	Rat albino, CD / Crl:CD(SD) Female 6/dose	Glyphosate technical grade Batch: 2009051501 Purity: 96.4%	2000 mg/kg bw Single oral dose	>2000 mg/kg bw Clinical signs: none	CA 5.2.1/003 Report no.: 24874 (2010)
OECD 423 (2001), GLP No deviations Study acceptable	Rat albino, CD / Crl:CD(SD) Female 6/dose	Glyphosate technical grade Batch: 20090506 Purity: 97.3%	2000 mg/kg bw Single oral dose	>2000 mg/kg bw Clinical signs: none	CA 5.2.1/004 Report no.: 24602 (2010)
OECD 423 (2001), GLP No deviations Study acceptable	Rat albino, CD / Crl:CD(SD) Female 6/dose	Glyphosate technical grade Batch: 20080801 Purity: 98.8%	2000 mg/kg bw Single oral dose	>2000 mg/kg bw Clinical signs: none	CA 5.2.1/005 Report no.: 23910 (2009)
OECD 423 (2001), GLP No significant deviations Study acceptable	Rat, HanRcc: WIST (SPF) Female 6/dose	Glyphosate technical Batch: GI-1045 Purity: 96.66%	2000 mg/kg bw Single oral dose	> 2000 mg/kg bw Clinical signs: none	CA 5.2.1/006 Report no.: C22864 (2009)
OECD 425 (2008), GLP No significant deviations Study acceptable	Rat albino, Sprague-Dawley Female 3/dose	Glyphosate tech grade mixed 5-batch Batch: 080704-1 thru 5 Purity: 96.40%	5000 mg/kg bw Single oral dose	> 5000 mg/kg bw Clinical signs: activity decrease, diarrhoea, piloerection, polyuria and salivation	CA 5.2.1/007 Report no.: 12170-08 (2009)
OECD 423 (2001), GLP No significant deviations Study acceptable	Rat albino, Wistar Hannover Female 6/dose	Glyphosate technical Batch: 20070606 Purity: 98.05%	2000 mg/kg bw Single oral dose	> 2000 mg/kg bw Clinical signs: none	CA 5.2.1/008 Report no.: [redacted]-3996.305.475.07 (2008)
OECD 425 (2001), GLP No significant	Rat, HanRcc:WIST (SPF) Female 3/dose	Glyphosate technical material Batch: 0507 Purity: 96.1%	5000 mg/kg bw Single oral dose	> 5000 mg/kg bw Clinical signs: ruffled fur,	CA 5.2.1/009 Report no.: B02755 (2007)

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Value LD_{50}	Reference
deviations Study acceptable				hunched posture	
OECD 423 (2001), GLP No significant deviations Study acceptable	Rat, HanRcc:WIST (SPF) Female 6/dose	Glyphosate Technical (NUP 05068) Batch: 200609062 Purity: 95.1%	2000 mg/kg bw Single oral dose	> 2000 mg/kg bw Clinical signs: slightly ruffled fur	CA 5.2.1/010 Report no.: B02272 (2007)
OECD 425 (2001), GLP No significant deviations Study acceptable	Rat albino, Sprague-Dawley derived Female 3/dose	Glyphosate Acid Technical Batch: 040205 Purity: 97.23%	5000 mg/kg bw Single oral dose	> 5000 mg/kg bw Clinical signs: diarrhoea, anogenital and facial staining, and/or reduced faecal volume	CA 5.2.1/011 Report no.: 15274 (2005)
US EPA OPPTS 870.1100 (in accordance with OECD guidelines), GLP No significant deviations Study acceptable	Rat albino, Sprague-Dawley derived Male and female 5/sex/dose	NUP5a99 62 % glyphosate MUP Batch: Drum Sample E Purity: 62% (IPA salt)	5000 mg/kg bw Single oral dose (not corrected for purity)	> 5000 mg/kg bw Clinical signs (females only): diarrhoea or soft faeces, anogenital staining	CA 5.2.1/012 Report no.: 7907 (1999)
OECD 401, GLP No significant deviations Study acceptable	Rat, Alpk:APfSD (Wistar-derived) Male and female 5/sex/dose	Glyphosate acid (technical) Batch: P24 Purity: 95.6%	5000 mg/kg bw Single oral dose Red or mottled areas in the lungs or thymus in three males and two females	> 5000 mg/kg bw	CA 5.2.1/013 Report no.: [redacted]/P/4660 (1996)
JMAFF 59 NohSan No. 4200 (in accordance with OECD guidelines), GLP No significant deviations Study acceptable	Mouse, Crj:CD-1(ICR) Male and female 5/sex/dose	MON 0139 Batch: LBRV-11092 Purity: 62.34% (IPA salt)	5000 mg/kg bw Single oral dose	> 5000 mg/kg bw Slight retardation in body weight gain in males	CA 5.2.1/014 Report no.: B-3101 (1995)
OECD 401 (1987), GLP No significant deviations Study acceptable	Rat, Sprague-Dawley (Crj:CD), SPF Male and female 5/sex/dose	Glyphosate technical, Code: HR-001 Batch: 940908-1 Purity: 95.68%	5000 mg/kg bw Single oral dose	> 5000 mg/kg bw Clinical signs: decreased spontaneous motor activity, salivation	CA 5.2.1/015 Report no.: [redacted] 94-0134 (1995)

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Value LD_{50}	Reference
OECD 401 (1987), GLP No significant deviations Study acceptable	Mouse, ICR (Crj:CD-1) Male and female 5/sex/dose	Glyphosate technical, Code: HR-001 Batch: 940908-1 Purity: 95.68%	5000 mg/kg bw Single oral dose	> 5000 mg/kg bw Clinical signs: decreased spontaneous motor activity, sedation, crouching position Slight body weight loss in one male.	CA 5.2.1/016 Report no.: [redacted] 94-0133 (1995)
OECD 401 (1987), GLP Body weights only recorded once prior to start Individual body weights and clinical signs not reported. On day of dosing, animals observed for 1-2 hours after dosing only. Study acceptable but with restrictions	Rat, Sprague-Dawley Male and female 5/sex/dose	Glyphosate acid technical Batch: 1073 Purity: 97.6%	2000 mg/kg bw Single oral dose	> 2000 mg/kg bw Necroscopy: congested lungs, splenomegaly and centrilobular hepatic congestion	CA 5.2.1/017 Report no.: 00917 (1995)
OECD 401 (1987), GLP Body weights only recorded once prior to start Individual body weights and clinical signs not reported. On day of dosing, animals observed for 1-2 hours after dosing only. Study acceptable but with restrictions.	Rat, Sprague-Dawley Male and female 5/sex/dose	[redacted] Glyphosate 62 % IPA Batch: 940950 Purity: 62% (IPA salt)	2000 mg/kg bw Single oral dose	> 2000 mg/kg bw (not corrected for purity) Necroscopy: lung congestion, splenomegaly, hepatomegaly with centrilobular congestion and subcapsular renal petechiae	CA 5.2.1/018 Report no.: 00926 (1995)
US EPA Subdivision F, 81-1 (in accordance with OECD guidelines), GLP No significant deviations	Rat, Sprague-Dawley Male and female 5/sex/dose	T1586.3 Glyphosate Technical 95% Batch: - Purity: 95%	5000 mg/kg bw Single oral dose	> 5000 mg/kg bw Clinical signs: piloerection, subdued behaviour and hunched appearance	CA 5.2.1/019 Report no.: 10670 (1995)

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Value LD_{50}	Reference
Study acceptable					
EPA OTS 798.1175, EPA OPP 81-1 (in accordance with OECD guidelines), GLP No significant deviations Study acceptable **Remark RMS:** **<u>For the process under Regulation (EC) No 1107/2009,</u> the applicant is requested to justify why for the same batch different conclusions are drawn regarding the purity and the acceptability of acute toxicity studies. <u>For the process under the Regulation (EC) No 1272/2008, the applicant is asked to submit the missing information during the public consultation period</u>** **CA 5.2.1/020 acceptable** **CA 5.2.3/016 acceptable** **CA 5.2.4/012 supportive due to low purity** **CA 5.2.5/015 supportive due to low purity** **CA 5.2.6/016 acceptable**	Rat, Sprague-Dawley Male and female 5/sex/dose	Glyphosate Premix Batch: 290-JaK-146-4 Purity: 46.1% (glyphosate), 62.2% (glyphosate IPA salt)	5000 mg/kg bw Single oral dose	> 5000 mg/kg bw Clinical signs: none	CA 5.2.1/020 Report no.: 545/37 (1994)

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Value LD_{50}	Reference
OECD 401 (1987), GLP No significant deviations Study acceptable	Rat, Sprague-Dawley Male and female 5/sex/dose	Glyphosate Technical 95% Batch: - Purity: 95%	2000 mg/kg bw Single oral dose	> 2000 mg/kg bw Clinical signs: none	CA 5.2.1/021 Report no.: 710/14 (1994)
OECD 401 (1987), GLP No significant deviations Study acceptable	Rat, Wistar, Male and female 5/sex/dose	Glyphosate Technical Batch: 36300892 Purity: 97.2%	5000 mg/kg bw Single oral dose	> 5000 mg/kg bw Body weight: statistically significant decrease in weight gain during 2nd week (males only) Necroscopy: heart weight significantly lower (males only).	CA 5.2.1/022 Report no.: [redacted]-94-401/R (1994)
OECD 401 (1987), GLP No significant deviations Study acceptable	Mouse, Crl:CD-1 (ICR) Male and female 5/sex/dose	Glyphosate Technical Batch: - Purity: -	2000 mg/kg bw Single oral dose	> 2000 mg/kg bw Clinical signs: piloerection, hunched posture, hypoactivity	CA 5.2.1/023 Report no.: 940020 (1994)
OECD 401 (1987), GLP No significant deviations Study acceptable	Rat, Sprague-Dawley Male and female 5/sex/dose	Glyphosate technical Batch: L3258 Purity: -	2000 mg/kg bw Single oral dose	> 2000 mg/kg bw Clinical signs: none	CA 5.2.1/024 Report no.: 134/37 (1992)
OECD 401 (1987), GLP No significant deviations Study acceptable	Mice, Bom:NMRI Male and female 5/sex/dose	Glyphosate Technical (PMG) Batch: 206-JaK-25-1 Purity: 98.6%	2000 mg/kg bw Single oral dose	> 2000 mg/kg bw Clinical signs: piloerection, sedation	CA 5.2.1/025 Report no.: 12321 (1991)
OECD 401 (1987), GLP Significant deviations: dose volume 25 mL/kg bw; day of clinical observations not provided. Study acceptable but with	Rat, Wistar Male and female 5/sex/dose	Glyphosate Technical Batch: 60 Purity: 96.80%	2500, 5000, 7500 mg/kg bw Single oral dose	> 7500 mg/kg bw Mortality: 2/5 males and 2/5 females at 7500 mg/kg bw Clinical signs: lethargy, ataxia and dyspnoea. Body weight gain affected.	CA 5.2.1/026 Report no.: [redacted].874.AOR (1991)

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Value LD_{50}	Reference
restrictions					
OECD 401 (1987), GLP Significant deviations: dose volume 25 mL/kg bw; day of clinical observations not provided, animals 14 weeks old Study acceptable but with restrictions	Mice, Swiss albino Male and female 5/sex/dose	Glyphosate Technical Batch: 60 Purity: 96.80%	2500, 5000, 7500 mg/kg bw Single oral dose	> 7500 mg/kg bw combined and in females > 5000 mg/kg bw in males Mortality: 1/5 males at 2500 mg/kg bw; 1/5 males and 1/5 females at 5000 mg/kg bw; 3/5 males and 1/5 females at 7500 mg/kg bw Clinical signs: lethargy, urine incontinence ataxia and dyspnoea. Body weight gain affected.	CA 5.2.1/027 Report no.: [redacted].875.AOM (1991)
OECD 401 (1987), GLP Significant deviations: no fasting after dosing, clinical signs not reported individually Study acceptable but with restrictions	Rats, CD Male and female 5/sex/dose	Glyphosate technical Batch: 0190 A Purity: 98.1%	3000, 5000, 8000 mg/kg bw Single oral dose	> 8000 mg/kg bw Clinical signs: decreased activity, abnormal body posture, abnormal gait, abnormal limb position	CA 5.2.1/028 Report no.: [redacted]-900823B (1990)
OECD, EEC, EPA guidelines (not further specified), GLP No significant deviations Study acceptable	Rat, Sprague-Dawley Male and female 5/sex/dose	Glyphosate Technical (PMG) Batch: 206-JaK-25-1 Purity: 98.6%	5000 mg/kg bw Single oral dose	> 5000 mg/kg bw Clinical signs: piloerection, reduced activity, ataxia	CA 5.2.1/029 Report no.: 5883 (1989)
OECD 401 (1987), GLP No significant deviations Study acceptable	Rat, KFM-Han., Wistar Male and female 5/sex/dose	Glyphosate technical (IPA salt) Batch: unknown Purity: 62% (IPA salt); 46% (glyphosate equivalents)	2000 mg/kg bw Single oral dose	> 2000 mg/kg bw Clinical signs: none	CA 5.2.1/030 Report no.: PRO439 / 238050 (1989)
EPA 81-1 (in accordance to	Rat, Sprague-Dawley	Glyphosate	5000 mg/kg bw	> 5000 mg/kg bw	CA 5.2.1/031 Report no.:

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Value LD_{50}	Reference
OECD 420), GLP No significant deviations Study acceptable	Male and female 5/sex/dose	Batch: XLI-55 Purity: 97.76%	Single oral dose	Clinical signs: diarrhoea, apparent urinary incontinence, and hair loss on the abdomen	88.2053.007 (1988)
EPA 81-1 (in accordance to OECD 420), GLP No significant deviations Study acceptable	Rat, Sprague-Dawley Male and female 5/sex/dose	Glyphosate (MON 8750) Batch: XLG-255 Purity: 90.8%	2222, 5000, 7500 mg/kg bw Single oral dose	4613 mg/kg bw (males: 5904 mg/kg bw; females 2222-5000 mg/kg bw) Mortality: 1/5 males and 5/5 females at 5000 mg/kg bw; 4/5 males and 5/5 females at 7500 mg/kg bw Main clinical signs: ataxia, decreased activity, diarrhea and labored breathing Abnormal changes at necroscopy in the lungs, stomach, and intestines.	CA 5.2.1/032 Report no.: [redacted]-86-431/9308A (1987)
EPA (in accordance to OECD guidelines), GLP No significant deviations Study acceptable	Rat, Sprague-Dawley Male and female 5/sex/dose	Glyphosate (MON 8722) Batch: XLG-256 Purity: 70.7%	5000 mg/kg bw Single oral dose	> 5000 mg/kg bw Clinical signs: ataxia, decreased activity, diarrhoea, rectal sores	CA 5.2.1/033 Report no.: [redacted]-86-430/93087A (1987)
No guideline, no GLP Deviations: substantial information missing, no guidelines, no GLP **Study not acceptable**	Mouse, ICR strain Male and female 15/sex/dose	64 % SN750721 technical liquid Batch: unknown Purity: 64%	4125, 4625, 5125, 5625, 6125 mg/kg bw Single oral dose	Not determined due to unacceptability of the study, however indicated to be >4125 mg/kg bw Clinical signs: immobility, tremor, hyperaemia of the ears	CA 5.2.1/034 Report no.: [redacted]58ao1 (1987)
No guideline, no GLP	Mouse, ICR strain Male and female	41 % SN750721	3125, 3625, 4125, 4625, 5125 mg/kg	Not determined due to	CA 5.2.1/035 Report no.:

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Value LD_{50}	Reference
Deviations: substantial information missing, no guidelines, no GLP **Study not acceptable**	5/sex/dose	Batch: unknown Purity: 41%	bw Single oral dose	unacceptability of the study, however indicated to be above 3125 mg/kg bw. Clinical signs: immobility, tremor, hyperaemia of the ears	[redacted]58ao2 (1987)
No guideline, no GLP Deviations: substantial information missing, no guidelines, no GLP **Study not acceptable**	Mouse, Kasauli Male and female 5/sex/dose	Glyphosate Technical Batch: R&D sample (9-7-83) Purity: 95%	2000, 3000, 4000, 5000 mg/kg bw Single oral dose	Not determined due to unacceptability of the study, however no mortality at 2000 mg/kg bw. Clinical signs: ataxia, loss of muscle tone	CA 5.2.1/036 Report no.: TOX95-51812 (1983)
No guideline, no GLP Deviations: substantial information missing, no guidelines, no GLP, study report of very low quality and at times unreadable. **Study not acceptable**	Albino rats Male and female 5/sex/dose	Glyphosate tech. Batch: unknown Purity: unknown	1250, 2500, 5000 mg/kg bw Single oral dose	Not determined due to unacceptability of the study, however no mortality observed. Clinical signs: slight ataxia	CA 5.2.1/037 Report no.: Not given (1983)
No guideline, no GLP Deviations: substantial information missing, no guidelines, no GLP **Study not acceptable**	Rat, Sprague-Dawley (Crl:CD® (SD)BR) Male and female 5/sex/dose	MON 0139 Batch: SSRT-11012 Purity: unknown	5000 mg/kg bw Single oral dose	Not determined due to unacceptability of the study, however no treatment related mortality occurred. Necroscopy: pale coloured kidneys, bilateral hydronephrosis.	CA 5.2.1/038 Report no.: 800257 (1981)
No guideline (similar to OECD 420), no GLP Significant deviations: no	Rat, Wistar Male and female 5/sex/dose	Glyphosate Technical Batch: XHI-180 Purity: 99%	2500, 3500, 5000, 7000, 9900 mg/kg bw Single oral dose	5600 mg/kg bw Main clinical signs: ataxia, convulsions, muscle tremors,	CA 5.2.1/039 Report no.: [redacted]-77-428 (1979)

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Value LD_{50}	Reference
fasting after dosing, clinical signs not reported individually, age of animals not specified Study not acceptable				red nasal discharge, clear oral discharge, urinary staining of the abdomen, soft stool, piloerection, lethargy, and faecal staining of the abdomen. Weight gain slightly affected. Abnormal changes at necroscopy in the lungs, abdomen, kidneys, stomach and intestines. Oral/nasal discharge.	

Table 19: Summary table of human data on acute oral toxicity

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No poisoning cases with non-formulated glyphosate alone were reported. A short discussion with respect to glyphosate-based formulation is provided in the text below. No data relevant for classification purposes is considered available from humans.				

Table 20: Summary table of other studies relevant for acute oral toxicity

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No information from other studies is considered relevant for classification purposes with respect to acute oral toxicity.				

2.6.2.1.1 Short summary and overall relevance of the provided information on acute oral toxicity

There were 39 acute oral toxicity studies in rat and mice of which 27 studies were concluded to in accordance with OECD test guidelines and to be fully acceptable. All 39 studies reported LD_{50} values that were above 2000 mg/kg bw. The most commonly occurring clinical signs were ataxia, diarrhoea, decreased activity, piloerection, hunched posture and anogenital staining. Reduced body weight gain was also noted in a few studies. There were no differences noted between glyphosate administered as an acid or as a salt.

No studies or case reports are available in which humans would have been exposed to the active ingredient itself. However, over the course of time, a large number of poisoning incidents have been reported that were due to accidental or intentional intake of glyphosate-based herbicides (mostly oral, in very few cases by inhalation). Refer to Vol 1 section 2.6.9 for an overview. In most cases, the actual exposure remained unknown. Furthermore, it is not possible to clearly distinguish between effects due to glyphosate and those caused by co-formulants. Therefore, reports on poisoning incidents in humans are not appropriate to be used for the purpose of classification and labelling of glyphosate for acute oral toxicity.

2.6.2.1.2 Comparison with the CLP criteria regarding acute oral toxicity

The classification criteria for acute oral toxicity under Regulation 1272/2008 are as followed:
Category 1: $\leq$ 5 mg/kg bw
Category 2: 5 mg/kg bw < ATE $\leq$ 50 mg/kg bw
Category 3: 50 mg/kg bw < ATE $\leq$ 300 mg/kg bw

Category 4: 300 mg/kg bw < ATE ≤ 2000 mg/kg bw

Since all acute oral toxicity studies indicated a LD_{50} value of >2000 mg/kg bw no classification is required.

2.6.2.1.3 Conclusion on classification and labelling for acute oral toxicity

Glyphosate does not need to be classified for acute oral toxicity according to the CLP Regulation (EU) No. 1272/2008.

Not classified – Conclusive but not sufficient for classification.

2.6.2.2 Acute toxicity - dermal route [equivalent to section 10.2 of the CLH report template]

Table 21: Summary table of animal studies on acute dermal toxicity

Method, guideline, deviations[1] if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Value LD_{50}	Reference
OECD 402 (1987), GLP No significant deviations Study acceptable	Rat; RjHan:WI Males and females 5/dose/sex	Glyphosate technical Batch: 569753 Purity: 96.3% Vehicle: water	5000 mg/kg bw Single dermal dose Exposure: 24 hours	>5000 mg/kg bw in males and females Clinical signs: none	CA 5.2.2/001 Report no.: 10/218-002P (2011)
OECD 402 (1987), GLP Deviations: Occlusive dressing used Study acceptable	Rat; Crl:CD(SD) Males and females 5/dose/sex	Glyphosate technical Batch: 2009051501 Purity: 96.4% Vehicle: *Aqua ad injectabilia*	2000 mg/kg bw Single dermal dose Exposure: 24 hours	> 2000 mg/kg bw in males and females Clinical signs: none	CA 5.2.2/002 Report no.: 24876 (2010)
OECD 402 (1987), GLP Deviations: Occlusive dressing used Study acceptable	Rat; Crl:CD(SD) Males and females 5/dose/sex	Glyphosate technical Batch: 20090506 Purity: 97.3% Vehicle: *Aqua ad injectabilia*	2000 mg/kg bw Single dermal dose Exposure: 24 hours	> 2000 mg/kg bw in males and females Clinical signs: none	CA 5.2.2/003 Report no.: 24604 (2010)
US EPA OPPTS (1998), GLP No significant deviations from OECD 402 Study acceptable	Rats; Sprague-Dawley Males and females 5/dose/sex	Glyphosate tech grade mixed 5-batch Batch: 080704-1 thru 5 Purity: 96.71% Vehicle: deionised water	5050 mg/kg bw Single dermal dose Exposure: 24 hours	>5050 mg/kg bw in males and females Body weight: 2 animals lost or failed to gain weight during Day 7-14	CA 5.2.2/004 Report no.: 12171-08 (2009)
OECD 402 (1987), GLP	Rats; HanRcc:WIST (SPF)	Glyphosate technical	2000 mg/kg bw Single dermal dose	> 2000 mg/kg bw in males and females	CA 5.2.2/005 Report no.: C22875

Method, guideline, deviations[1] if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Value LD_{50}	Reference
Deviations: Low female bodyweight at start Study acceptable	Males and females 5/dose/sex	Batch: GI-1045 Purity: 96.66% Vehicle: purified water	Exposure: 24 hours	Skin observations: very slight erythema in 4 females on Day 4-10,11,12 and scabs in 2 females on Day 9-11	(2009)
OECD 402 (1987), GLP Deviations: Occlusive dressing Study acceptable	Rats; Crl:CD(SD) Males and females 5/dose/sex	Glyphosate technical Batch: 20080801 Purity: 98.8% Vehicle: *Aqua ad injectabilia*	2000 mg/kg bw Single dermal dose Exposure: 24 hours	> 2000 mg/kg bw in males and females Clinical signs: none	CA 5.2.2/006 Report no.: 23912 (2009)
OECD 402 (1987), GLP Deviations: Low female bodyweight at start Study acceptable	Rat; Wistar Hannover Males and females 5/dose/sex	Glyphosate technical Batch: 20070606 Purity: 98.05% Vehicle: deionised water	2000 mg/kg bw Single dermal dose Exposure: 24 hours	> 2000 mg/kg bw in males and females Body weight: 2 females lost or failed to gain weight during Day 7-14	CA 5.2.2/007 Report no.: [redacted]-3996.310.456.07 (2008)
OECD 402 (1987), GLP Deviations: Low female bodyweight at start Study acceptable	Rat; HanRcc:WIST (SPF) Males and females 5/dose/sex	Glyphosate technical (NUP 05068) Batch: 200609062 Purity: 95.1% Vehicle: polyethylene glycol 300 (PEG 300)	2000 mg/kg bw Single dermal dose Exposure: 24 hours	> 2000 mg/kg bw in males and females Clinical signs: none	CA 5.2.2/008 Report no.: B02283 (2007)
OECD 402 (1987), GLP Deviations: Low female bodyweight at start Study acceptable	Rat; HanRcc:WIST (SPF) Males and females 5/dose/sex	Glyphosate technical material Batch: 0507 Purity: 96.1% Vehicle: purified water	5000 mg/kg bw Single dermal dose Exposure: 24 hours	>5000 mg/kg bw in males and females Clinical signs: none	CA 5.2.2/009 Report no.: B02766 (2007)
OECD 402 (1987), GLP Deviations: Low female bodyweight at start, humidity and air changes not reported	Rat; Sprague-Dawley derived, albino Males and females 5/dose/sex	Glyphosate acid technical Batch: 040205 Purity: 97.23% Vehicle: distilled water	5000 mg/kg bw Single dermal dose Exposure: 24 hours	>5000 mg/kg bw in males and females Clinical signs: none	CA 5.2.2/010 Report no.: 15275 (2005)

Method, guideline, deviations[1] if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Value LD_{50}	Reference
Study acceptable					
OECD 402 (1987), GLP Deviations: Specific age animals not reported, no observations in first 30 min, occlusive dressing used Study acceptable	Rat; Alpk:APfSD (Wistar-derived) Males and females 5/dose/sex	Glyphosate acid Batch: P24 Purity: 95.6% Vehicle: deionised water	2000 mg/kg bw Single dermal dose Exposure: 24 hours	>2000 mg/kg bw in males and females Clinical signs: slight erythema on Day1-2 in 1 male and small scabs in 1 female on Day2-7	CA 5.2.2/011 Report no.: [redacted]/P/4664 (1996)
OECD 402 (1987), GLP Deviations: Lower age and bodyweight at start, solvent control included, occlusive dressing used Study acceptable	Rat; specific pathogen free SD rats (Crj:CD) Males and females 5/dose/sex	Glyphosate technical (Code HR-001) Batch: 940908-1 Purity: 95.68% Vehicle: deionised water	2000 mg/kg bw Single dermal dose Exposure: 24 hours	>2000 mg/kg bw in males and females Clinical signs: None	CA 5.2.2/012 Report no.: 94-0154 (1995)
OECD 402 (1987), GLP Deviations: Individual animal data missing, occlusive dressing used, age animals not reported, low bodyweight at start and not frequently recorded during study, air changes not specified Acceptable but with restrictions	Rat; Sprague-Dawley Males and females 5/dose/sex	Glyphosate acid technical Batch: 1073 Purity: 97.6% Vehicle: Cotton seed oil (500 mg/mL)	2000 mg/kg bw Single dermal dose Exposure: 24 hours	>2000 mg/kg bw in males and females Splenomegaly and centrilobular hepatic congestion in males and females	CA 5.2.2/013 Report no.: 00917 (1995)
OECD 402 (1987), GLP Deviations: Individual animal data missing, occlusive dressing used, age animals not reported, low bodyweight at start and not frequently recorded during study, air changes	Rat; Sprague-Dawley Males and females 5/dose/sex	Glyphosate 62% IPA salt Batch: 940950 Purity: 61.8% Vehicle: none	2000 mg/kg bw Single dermal dose Exposure: 24 hours	>2000 mg/kg bw in males and females Severe lung congestion, splenomegaly, hepatomegaly with centrilobular congestion, and subcapsular renal petechiae in males and females	CA 5.2.2/014 Report no.: 00926 (1995)

Method, guideline, deviations[1] if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Value LD_{50}	Reference
not specified Acceptable but with restrictions					
No guideline reported, Not GLP Deviations: limited reporting, treated area to small **Study unacceptable**	Rat; Sprague-Dawley Males and females 5/dose/sex	Glyphosate technical Batch: not reported Purity: 95% Vehicle: suspended (50% w/w) in natrosol (1% w/w in water)	2000 mg/kg bw Single dermal dose Exposure: 24 hours	No mortality observed, but no LD50 derived as study is unacceptable Clinical signs: None	CA 5.2.2/015 Report no.: T1586.3.A (1994)
OECD 402 (1987), GLP Deviations: Skin moistened with oil pre-treatment, limited reporting, no individual data **Study unacceptable**	Rat; Sprague-Dawley Males and females 5/dose/sex	Glyphosate 62% IPA Batch: 9409/50 Purity: 95% Skin pre-treated with arachis oil prior to treatment	2000 mg/kg bw Single dermal dose Exposure: 24 hours	No mortality observed, but no LD50 derived as study is unacceptable Clinical signs: None	CA 5.2.2/016 Report no.: 710/15 (1994)
OECD 402 (1981) Not GLP (QA available) Deviations: size exposed skin, amount of vehicle and age animals not reported, no observations in first 30 min, occlusive dressing used, low bodyweight at start **Study unacceptable**	Rat; LATI/Wistar Males and females 5/dose/sex	Glyphosate technical Batch: 36300892 Purity: 99.6% Vehicle: water	2000 mg/kg bw Single dermal dose Exposure: 24 hours	No mortality observed, but no LD50 derived as study is unacceptable Clinical signs: None	CA 5.2.2/017 Report no.: [redacted]-94-402/R (1994)
OECD 402 (1981) GLP Deviations: purity and stability of test substance not reported, animals 10-14 weeks old instead of 8-10 weeks Study acceptable	Rat, Sprague-Dawley Males and females 5/dose/sex	Glyphosate technical Batch: L3258 Purity: not reported Vehicle: distilled water	2000 mg/kg bw Single dermal dose Exposure: 24 hours	>2000 mg/kg bw in males and females Clinical signs: None	CA 5.2.2/018 Report no.: 134/38 (1992)

Method, guideline, deviations[1] if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Value LD_{50}	Reference
but with restrictions					
OECD 402 (1987) GLP Deviations: occlusive dressing used, low bodyweight at start, animals 14 weeks old instead of 8-10 weeks Study acceptable	Rat, Wistar Males and females 5/dose/sex	Glyphosate technical Batch: 60 Purity: 96.8% Vehicle: distilled water	2500 and 5000 mg/kg bw Single dermal dose Exposure: 24 hours	>5000 mg/kg bw in males and females Clinical signs: bodyweight reduction Day 7-14 in one female both dose groups	CA 5.2.2/019 Report no.: [redacted].876.ADR (1991)
OECD 402 (1981) GLP Deviations: occlusive dressing used, >20% variation in male bodyweight, age animals not reported and transient reduction of room temperature, air changes not specified, no justification for choice and use vehicle included Study acceptable	Rat, CD Males and females 5/dose/sex	Glyphosate technical Batch: 0190 A Purity: 98.1% Vehicle: 0.9% saline	3000, 5000 and 8000 mg/kg bw Single dermal dose Exposure: 24 hours	>8000 mg/kg bw in males and females Clinical signs: None	CA 5.2.2/020 Report no.: [redacted]-900823A (1990)
OECD 402 GLP Deviations: occlusive dressing used, purity, amount vehicle not reported, low bodyweight at start, air changes not specified Study acceptable	Rat, Sprague-Dawley Males and females 5/dose/sex	Glyphosate technical (PMG) Batch: 206-Jak-25-1 Purity: 98.6% (from CA 5.2.1/25) Vehicle: water	2000 mg/kg bw Single dermal dose Exposure: 24 hours	>2000 mg/kg bw in males and females Clinical signs: piloerection and reduced activity 30min – 1day post dosing and scab formation Day 2-14. Bodyweight reduction Day 7-14 in one female	CA 5.2.2/021 Report no.: 5884 (1989)
Guideline unknown GLP status unknown No study report available	Rat, Wistar Males and females 5/dose/sex	Glyphosate 62% IPA	2000 mg/kg bw Single dermal dose	LD_{50} > 2000 mg/kg bw (limit test)	CA 5.2.2/022 Report no.: 238061 (1989)

Method, guideline, deviations[1] if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Value LD_{50}	Reference
Study not acceptable as study report is not available.					
US EPA (1984) GLP Deviations: occlusive dressing used, individual data and age animals not reported, air changes not specified, no justification for choice and use vehicle included, observation time on the day of application not specified Study acceptable but with restrictions	Rabbit; New Zealand White Males and females 5/dose/sex	Glyphosate Batch: XLI-55 Purity: 97.76% Vehicle: physiological saline	5000 mg/kg bw Single dermal dose Exposure: 24 hours	>5000 mg/kg bw in males and females Mortality: 1 female animal Clinical signs: diarrhoea, anorexia and soft stool, bodyweight reduction in one male animal on Day7-14	CA 5.2.2/023 Report no.: 88.2053.008 (1988)
US EPA (1982) GLP Deviations: occlusive dressing used, individual data and age animals not reported, air changes not specified, no justification for choice and use vehicle included Study acceptable but with restrictions	Rabbit, New Zealand White Males and females 5/dose/sex	MON 8722 Batch: XLG-256 Purity: 70.7% Vehicle: physiological saline	5000 mg/kg bw Single dermal dose Exposure: 24 hours	>5000 mg/kg bw in males and females Clinical signs: soft stool	CA 5.2.2/024 Report no.: 9307A (1987)
US EPA (1982) GLP Deviations: occlusive dressing used, individual data and age animals not reported, air changes not specified, no justification for choice and use vehicle included	Rabbit, New Zealand White Males and females 5/dose/sex	MON 8750 Batch: XLG-255 Purity: 90.8% Vehicle: physiological saline	5000 mg/kg bw Single dermal dose Exposure: 24 hours	>5000 mg/kg bw in males and females Mortality: one female animal Day 3 Clinical signs: anorexia (in the female that died), slight weight loss (in two males), decreased activity and soft stools	CA 5.2.2/025 Report no.: 9308A (1987)

Method, guideline, deviations[1] if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Value LD_{50}	Reference
Study acceptable but with restrictions					
No guideline reported Not GLP Deviations: limited reporting, individual data not available, bodyweights not determined post dosing and no gross necropsy performed, solvent control included **Study unacceptable**	Rabbit; Albino NWS Males and Females 2/dose/sex	Glyphosate technical Batch: R&D Sample (8.7.83.) Purity: 95% Vehicle: no data	2000 mg/kg bw Single dermal dose Exposure: 24 hours	No mortality observed, but no LD50 derived as study is unacceptable Clinical signs: slight erythema at site of treatment	CA 5.2.2/026 Report no.: not reported (1983)
No guideline reported Not GLP Deviations: limited reporting, skin abraded prior to administration, occlusive dressing used, individual data not available **Study unacceptable**	Rabbit; New Zealand White Males and females 5/dose/sex	MON 0139 Batch: SSRT-11012 Purity: not reported Vehicle: none	5000 mg/kg bw Single dermal dose Exposure: 24 hours	No mortality observed, but no LD50 derived as study is unacceptable Clinical signs: fur stained with diarrheal faeces	CA 5.2.2/027 Report no.: 800258 (1981)

Table 22: Summary table of human data on acute dermal toxicity

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No human data available on glyphosate-poisoning by the dermal route.				

Table 23: Summary table of other studies relevant for acute dermal toxicity

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No information from other studies is considered relevant for classification purposes with respect to acute dermal toxicity.				

2.6.2.2.1 Short summary and overall relevance of the provided information on acute dermal toxicity

There were 27 acute dermal toxicity studies in rats and rabbits of which 22 were performed with glyphosate acid and 5 with glyphosate salts. Of the 22 studies performed with glyphosate acid, 20 studies were conducted in rat 20

while only 2 studies were conducted in rabbit. Of the 5 studies performed with glyphosate salt, 2 and 3 studies were performed in rat and rabbit, respectively. Of the total of 27 studies, 20 studies were concluded to in accordance with OECD test guidelines and to be fully acceptable (acceptable or acceptable but with restrictions). All 27 studies reported LD_{50}-values above 2000 or 5000 mg/kg bw irrespective of the test substance (glyphosate acid or salt) and the species (rat or rabbit). Apart from two isolated mortalities (report nos. 88.2053.008 and 9308A), there were no deaths. In both studies, a total of 10 animals were treated with a limit dose of 5000 mg/kg bw; neither death was considered test item related. A few studies reported clinical signs such as body weight loss, diarrhoea or soft stool and slight local effects.

Poisoning incidents by the dermal route with non-formulated glyphosate in humans were not reported and therefore no human data is available for the purpose of classification and labelling of glyphosate for acute dermal toxicity.

2.6.2.2.2 Comparison with the CLP criteria regarding acute dermal toxicity

The classification criteria for acute oral toxicity under Regulation 1272/2008 are as followed:
Category 1: $\leq$ 50 mg/kg bw
Category 2: 50 mg/kg bw < ATE $\leq$ 200 mg/kg bw
Category 3: 200 mg/kg bw < ATE $\leq$ 1000 mg/kg bw
Category 4: 1000 mg/kg bw < ATE $\leq$ 2000 mg/kg bw

Since all acute dermal toxicity studies indicated a LD_{50} value of >2000 or > 5000 mg/kg bw no classification is required.

2.6.2.2.3 Conclusion on classification and labelling for acute dermal toxicity

Glyphosate does not need to be classified for acute dermal toxicity according to the CLP Regulation (EU) No. 1272/2008.

Not classified – Conclusive but not sufficient for classification.

2.6.2.3 Acute toxicity - inhalation route [equivalent to section 10.3 of the CLH report template]

Table 24: Summary table of animal studies on acute inhalation toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, form and particle size (MMAD)	Dose levels, duration of exposure	Value LC_{50}	Reference
OECD 403 (2009) GLP No significant deviations Study acceptable	Rat; Wistar RjHan Males and females 5/dose/sex	Glyphosate technical Batch: 614034 (20100609\Milled) Purity: 96.9% MMAD: 3.65µm GSD: 2.24µm	5.04 mg/L air Nose-only exposure for 4 hours	>5.04 mg/L air in males and females Mortality: 1/10 Clinical signs: laboured and noise respiration, increased respiratory rate, gasping, sneezing, decreased activity, thin body appearance	CA 5.2.3/001 Report no: 11/054-004P (2011)
OECD 403 (1981) GLP	Rat; CD/Crl:CD Males and females	Glyphosate TC Batch 20090506 Purity: 97.3%	5.18 mg/L air Nose-only exposure for 4 hours	>5.18 mg/L air in males and females	CA 5.2.3/002 Report no: 24603 (2010)

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, form and particle size (MMAD)	Dose levels, duration of exposure	Value LC_{50}	Reference
Deviations: MMAD >4µm; GSD >3 Study considered acceptable, but with restrictions	5/dose/sex	MMAD: 4.633µm GSD: 3.02µm		No mortality. Clinical signs: slight tremor and dyspnea up to 3h post dosing	
OECD 403 (1981) GLP Deviations: MMAD >4µm Study considered acceptable, but with restrictions	Rat; CD/Crl:CD Males and females 5/dose/sex	Glyphosate TC Batch 2009051501 Purity: 96.4% MMAD: 4.197µm GSD: 2.64µm	5.02 mg/L air Nose-only exposure for 4 hours	>5.02 mg/L air in males and females No mortality. Clinical signs: slight ataxia, tremor and dyspnoea up to 3h post dosing	CA 5.2.3/003 Report no: 24875 (2010)
OECD 403 (1981) GLP Deviations: MMAD not calculated Study considered acceptable, but with restrictions	Rat; albino CD/Crl:CD Males and females 5/dose/sex	Glyphosate TC Batch 20080801 Purity: 98.8% MMAD: - GSD: -	5.12 mg/L air Nose-only exposure for 4 hours	>5.12 mg/L air in males and females No mortality. Clinical signs: slight dyspnoea and ataxia up to 60min post dosing	CA 5.2.3/004 Report no: 23911 (2009)
OECD 403 (1981) GLP Deviations: MMAD >4µm; GSD >3, female bodyweight slightly outside range Acceptable but with restrictions	Rat; HsdRccHan:WIST Males and females 5/dose/sex	Glyphosate Tech Batch: GI-1045 Purity: 96.66% MMAD: 5.25µg GSD: 3.35µg	5.04 mg/L air Nose-only exposure for 4 hours	>5.04 mg/L air in males and females Clinical signs: increased respiratory rate upon removal from the chamber up to 1h post dosing	CA 5.2.3/005 Report no: 2743/0001 (2009)
US EPA GLP Deviations: concentration low for limit test, GSD not calculated, low female bodyweight at start, humidity outside the	Rat; Sprague-Dawley Males and females 5/dose/sex	Glyphosate Tech Grade Mixed 5-Batch Batch: 080704-1 thru 5 Purity: 96.71%; 96.40% MMAD: 2.6 µg GSD: -	2.24 mg/L air Nose-only exposure for 4 hours	>2.24 mg/L air in males and females Clinical signs: piloerection and decreased activity up to day 4 post dosing	CA 5.2.3/006 Report no: 12107-08 (2009)

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, form and particle size (MMAD)	Dose levels, duration of exposure	Value LC_{50}	Reference
specified range on occasion Study acceptable but with restrictions					
OECD 403 (1981) GLP Deviations: MMAD far beyond 4µm **Study unacceptable**	Rat; Wistar Hannover Males and females 5/dose/sex	Glyphosate Technical Batch: 20070606 Purity: 98.05% MMAD: 18.555-19.901µm GSD: 2.869-2.914µm	5.211 mg/L air Nose-only exposure for 4 hours	No mortality observed, but no LC50 derived as study is unacceptable Clinical signs: wheezing and dyspnoea from Day1-4, bodyweight decrease in males on Day1	CA 5.2.3/007 Report no: [redacted]-3996.309.377.07 (2008)
OECD 403 (1981) GLP No significant deviations Study acceptable	Rat; HanRcc:WIST Males and females 5/dose/sex	Glyphosate Technical (NUP 05068) Batch: 200609062 Purity: 95.1% MMAD: 2.95-3.05µm GSD: 2.73-2.97µm	3.252 mg/L air Nose-only exposure for 4 hours and 30 minutes	>3.252 mg/L air in males and females Clinical signs: deep respiration, rales and salivation, transient bodyweight reductions	CA 5.2.3/008 Report no: B02327 (2007)
OECD 403 GLP Deviations: concentration low for limit test, GSD not reported, humidity and air changes not reported Study acceptable but with restrictions	Rat; Sprague-Dawley derived Males and females 5/dose/sex	Glyphosate Acid Technical Batch: 040205 Purity: 97.23% MMAD: 2.5µm GSD: -	2.04 mg/L air Nose-only exposure for 4 hours	>2.04 mg/L air in males and females Clinical signs: None	CA 5.2.3/009 Report no: 15276 (2005)
OECD 403 (1981) GLP No significant deviations Study acceptable	Rat; Sprague-Dawley Males and females 5/dose/sex	MON 78623 Batch: GLP-0306-14124-F Purity: 47.2% (57.8% potassium salt of glyphosate)	2.21 and 5.27 mg/L air Nose-only exposure for 4 hours	>5.27 mg/L air in males and females Clinical signs: transient incidences of congested breathing and dark material	CA 5.2.3/010 Report no: 3044.969 (2004)

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, form and particle size (MMAD)	Dose levels, duration of exposure	Value LC_{50}	Reference
		MMAD: 2.9;3.8µm GSD: 2.18;2.20µm		around the facial area and few faeces, slight transient bodyweight loss	
OPPTS 870.1300 (1998) GLP Deviations: whole body exposure Study acceptable	Rat; Sprague-Dawley derived Males and females 5/dose/sex	NUP5a99 62 % glyphosate MUP Batch: Drum Sample E Purity: 62% MMAD: 2.6µm GSD: 1.72µm	2.08 mg/L air Whole body exposure for 4 hours and 15 minutes	>2.08 mg/L air in males and females Clinical signs: ocular and nasal discharge, hunched posture and hypoactivity during dosing	CA 5.2.3/011 Report no: 7909 (1999)
OECD 403 (1981) GLP No significant deviations Study acceptable	Rat; Alpk:APfSD Males and females 5/dose/sex	Glyphosate acid Batch: P25 Purity: 95.6% MMAD: 3.57, 3.03µm GSD: 2.91, 3.41µm	2.47 and 4.43 mg/L air Nose-only exposure for 4 hours	>4.43 mg/L air in males and females Mortality: 0/10 at 2.47 mg/L air; 4/10 at 4.43 mg/L air Clinical signs: salivation, irregular breathing and auditory hypoaesthesia, breathing irregularities, reduced righting reflex, shaking, splayed gait	CA 5.2.3/012 Report no: [redacted]/P/4882 (1996)
OECD 403 (1981) GLP Deviations: whole body exposure chamber, MMAD >4µm, inconsistency in reported lot number and purity Study acceptable but with restrictions	Rat; F344/DuCri Males and females 5/dose/sex	Glyphosate TC Batch: T941209 Purity: 97.56% MMAD: 4.8µm GSD: 1.7µm	5.48 mg/L air Whole body exposure for 4 hours	>5.48 mg/L air in males and females Clinical signs: wetted and soiled fur	CA 5.2.3/013 Report no: [redacted] 94-0155 (1995)
OECD 403 (1981) GLP	Rat; Sprague-Dawley Males and females	Glyphosate technical Batch: -	5.35 mg/L air Nose-only exposure for 4 hours	>5.35 mg/L air in males in females	CA 5.2.3/014 Report no: 710/16 (1994)

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, form and particle size (MMAD)	Dose levels, duration of exposure	Value LC_{50}	Reference
Deviations: MMAD >4µm Study acceptable but with restrictions	5/dose/sex	Purity: 95% MMAD: 4.4µm GSD: 0.47µm		Clinical signs: ptosis, brown staining, hunched posture and piloerection.	
OECD 403 (1981) Not GLP Deviations: MMAD not measured, mode of exposure not stated **Study unacceptable**	Rat; Wistar Males and females 5/dose/sex	Glyphosate Batch: 36300892 Purity: 97.2% MMAD: - GSD: -	2.876 mg/L air Exposure for 4 hours	No mortality observed, but no LC50 derived as study is unacceptable Clinical signs: none	CA 5.2.3/015 Report no: [redacted]-94-403/R (1994)
OECD 403 (1981) GLP No significant deviations Study acceptable **Remark RMS:** **<u>For the process under Regulation (EC) No 1107/2009,</u> the applicant is requested to justify why for the same batch different conclusions are drawn regarding the purity and the acceptability of acute toxicity studies. <u>For the process under the Regulation (EC) No 1272/2008, the applicant is asked to submit the missing information during the public</u>**	Rat; Sprague-Dawley Males and females 5/dose/sex	Glyphosate Premix Batch: 290-JaK-146-4 Purity: 62.2% as glyphosate isopropylamine salt; 46.1% as glyphosate MMAD: 1.1µm GSD: 0.57µm	4.24 mg/L air Nose-only exposure for 4 hours	>4.24 mg/L air in males and females Clinical signs: hunched posture, piloerection and wet fur	CA 5.2.3/016 Report no: 545/39 (1994)

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, form and particle size (MMAD)	Dose levels, duration of exposure	Value LC_{50}	Reference
consultation period. **CA 5.2.1/020 acceptable** **CA 5.2.3/016 acceptable** **CA 5.2.4/012 supportive due to low purity** **CA 5.2.5/015 supportive due to low purity** **CA 5.2.6/016 acceptable**					
OECD 403 (1987) GLP Deviations: Whole body exposure, MMAD not calculated, low limit concentration **Study unacceptable**	Rat; Wistar Males and females 5/dose/sex	Glyphosate Technical Batch: 60 (code: FSG 03090 H/05 March 90) Purity: 96.8% MMAD: - GSD: -	0.644 mg/L air Whole body exposure for 4 hours	No mortality observed, but no LC50 derived as study is unacceptable Clinical signs: nasal irritation	CA 5.2.3/017 Report no: [redacted].877.AIN (1991)
OECD 403 (1981) GLP Deviations: MMAD not calculated, purity not reported **Study unacceptable**	Rat; Sprague-Dawley Males and females 5/dose/sex	Glyphosate Technical Batch: 206-JAK-25-1 Purity: - MMAD: - GSD: -	4.98 mg/L air Snout-only exposure for 4 hours	No mortality observed, but no LC50 derived as study is unacceptable Clinical signs: slightly subdued up to Day 1	CA 5.2.3/018 Report no: 5993 (1989)
OECD 403 (1981) GLP Deviations: MMAD and GSD not specifically reported (claimed to be <3µm) Study acceptable but with restrictions	Rat: Wistar Males and females 5/dose/sex	Glyphosate Technical Batch:21/39 Purity: 62 % in water equivalent to 46% (w/w) of N-phosphonomethylglycine acid	4.1, 4.42, 6.49 mg/L air Nose-only exposure for 4 hours	>6.49 mg/L air in males and females Clinical signs: nose bleeding and ruffled fur	CA 5.2.3/019 Report no: 238105 (1989)

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, form and particle size (MMAD)	Dose levels, duration of exposure	Value LC_{50}	Reference
No guideline stated GLP Deviations: MMAD >4µm, low limit concentration, whole body exposure, certificate of analysis not attached Study acceptable but with restrictions	Rat; Sprague-Dawley Males and females 5/dose/sex	MON 8750 Technical Batch: XLH-270 Purity: 85.52% MMAD: 4.2µg GSD: 1.8µg	1.9 mg/L air Whole body exposure for 4 hours	>1.9 mg/L air in males and females Clinical signs: hypoactivity, red/brown perinasal encrustation on Day1-2	CA 5.2.3/020 Report no: [redacted]-87-228 (1988)
No guideline followed GLP Deviations: whole body exposure, low limit concentration Study acceptable but with restrictions	Rat; Sprague-Dawley Males and females 5/dose/sex	Rodeo Herbicide Batch: LHRO-12010 X Purity: 53.8 % Isopropylamine salt of glyphosate, 46.2 % Inert MMAD: 3.1µg GSD: 1.9µg	1.3 mg/L air Whole body exposure for 4 hours	>1.3 mg/L air in males and females Mortality: 1/5 females and 0/5 males Clinical signs: yellow/brown nasal discharge, focal and or generalized loss of hair, transient bodyweight decreases, abnormal color and focus in kidney and focus in lungs and thymus, alopecia of the skin and hydrometra in uterus	CA 5.2.3/021 Report no: L-6582 (1987)
No guideline followed Not GLP Deviations: limited reporting, purity and stability test substance not reported, individual data not reported	Rat; species not specified Males and females 5/dose/sex	Glycel 41 SL Batch: - Purity: - MMAD: 3.8, 4.0, 3.9µg GSD: -	4.5 mg/L air Nose and mouth exposure for 4 hours	No mortality observed, but no LC50 derived as study is unacceptable Clinical signs: none	CA 5.2.3/022 Report no: not given (1983)

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, form and particle size (MMAD)	Dose levels, duration of exposure	Value LC_{50}	Reference
Study unacceptable					

Table 25: Summary table of human data on acute inhalation toxicity

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No human data available on glyphosate-poisoning by the inhalation route.				

Table 26: Summary table of other studies relevant for acute inhalation toxicity

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No information from other studies is considered relevant for classification purposes with respect to acute inhalation toxicity.				

2.6.2.3.1 Short summary and overall relevance of the provided information on acute inhalation toxicity

There are 22 acute inhalation toxicity studies of which 17 studies were concluded to be in accordance with OECD test guidelines and to be acceptable (acceptable or acceptable but with restrictions). Of the 22 studies performed in rats, 15 studies were performed with glyphosate acid and seven studies with glyphosate salts. In several studies the limit dose of 5 mg/L was difficult to achieve. Of the acceptable studies, only 10 studies dosed at concentrations at or above the limit dose of 5 mg/L.

The LC_{50} derived in these studies were all above the limit of 5 mg/L. The most commonly occurring clinical signs included laboured breathing, decreased activity, slight ataxia, tremors, dyspnoea, hunched postures and piloerection.

Poisoning incidents by the inhalation route with non-formulated glyphosate in humans were not reported and therefore no human data is available for the purpose of classification and labelling of glyphosate for acute inhalation toxicity.

2.6.2.3.2 Comparison with the CLP criteria regarding acute inhalation toxicity

The classification criteria for acute oral toxicity under Regulation 1272/2008 are as followed:
Category 1: ≤ 0.05 mg/L
Category 2: 0.05 mg/L < ATE ≤ 0.5 mg/L
Category 3: 0.5 mg/L < ATE 1.0 mg/L
Category 4: 1.0 mg/L < ATE $\leq$ 5.0 mg/L

The overall LC_{50} value for glyphosate is >5 mg/L. Therefore, glyphosate does not meet the criteria for classification for acute inhalation toxicity.

2.6.2.3.3 Conclusion on classification and labelling for acute inhalation toxicity

Glyphosate does not need to be classified for acute inhalation toxicity according to the CLP Regulation (EU) No. 1272/2008.

Not classified – Conclusive but not sufficient for classification.

2.6.2.4 Skin corrosion/irritation [equivalent to section 10.4 of the CLH report template]

Table 27: Summary table of animal studies on skin corrosion/irritation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Results - Observations and time point of onset[2] - Mean scores/animal - Reversibility	Reference
OECD 404 (2002) GLP Deviations: occlusive dressing Study acceptable	Rabbit; New Zealand White Males 3/dose	Glyphosate technical Batch: 569753 (BX20070911) Purity: 96.3%	0.5g, 4 hours, occlusive Vehicle: not specified	No mortality, clinical signs or effect on bodyweight Erythema: 0.11 Oedema: 0.0 Reversible by 48 hours	CA 5.2.4/001 Report no: 10/218-006N (2011)
OECD 404 (2002) GLP Deviations: Himalayan rabbits, air changes not specified Study acceptable	Rabbit; Himalayan Males 3/dose	Glyphosate TC Batch: 20090506 Purity: 97.3%	0.5g, 4 hours, semi-occlusive Vehicle: purified water for injection	No mortality, clinical signs or effect on bodyweight Erythema: 0.0 Oedema: 0.0	CA 5.2.4/002 Report no: 24605 (2010)
OECD 404 (2002) GLP Deviations: Himalayan rabbits, air changes not specified Study acceptable	Rabbit; Himalayan Males 3/dose	Glyphosate TC Batch: 2009051501 Purity: 96.4%	0.5g, 4 hours, semi-occlusive Vehicle: purified water for injection	No mortality, clinical signs or effect on bodyweight Erythema: 0.0 Oedema: 0.0	CA 5.2.4/003 Report no: 24877 (2009)
OECD 404 (2002) GLP Deviations: Himalayan rabbits, air changes not specified Study acceptable	Rabbit; Himalayan Males 3/dose	Glyphosate TC Batch: 20080801 Purity: 98.8%	0.5g, 4 hours, semi-occlusive Vehicle: purified water for injection	No mortality, clinical signs or effect on bodyweight Erythema: 0.0 Oedema: 0.0	CA 5.2.4/004 Report no: 23913 (2009)
US EPA GLP No significant deviations Study acceptable	Rabbit; New Zealand White 1 male and 2 females	Glyphosate tech grade; Mixed 5-Batch Batch: 080704-1 thru 5 Purity: 96.4%	0.5g, 4 hours, semi-occlusive Vehicle: deionized water	No mortality, clinical signs or effect on bodyweight Erythema: 0.0 Oedema: 0.0	CA 5.2.4/005 Report no: 12173-08 (2009)
OECD 404 (2002) GLP No significant deviations Study acceptable	Rabbit; New Zealand White Females 3/dose	Glyphosate Technical Batch: 20070606 Purity: 98.05%	0.5g, 4 hours, semi-occlusive Vehicle: not specified	No mortality, clinical signs or effect on bodyweight Erythema: 0.0 Oedema: 0.0	CA 5.2.4/006 Report no: [redacted]-3996.311.476.07 (2008)
OECD 404 (2002) GLP	Rabbit; New Zealand White	Glyphosate technical material	0.5g, 4 hours, semi-occlusive	No mortality, clinical signs or effect on bodyweight	CA 5.2.4/007 Report no: B02777 (2007)

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Results - Observations and time point of onset[2] - Mean scores/animal - Reversibility	Reference
No significant deviations Study acceptable	1 male and 2 females	Batch: 0507 Purity: 96.1%	Vehicle: purified water	Erythema: 0.0 Oedema: 0.0	
OECD 404 (2002) GLP Deviations: treated surface >6cm² Study acceptable	Rabbit; New Zealand White 1 male and 2 females	Glyphosate technical (NUP 05068) Batch: 200609062 Purity: 95.1%	0.5g, 4 hours, semi-occlusive Vehicle: purified water	No mortality, clinical signs or effect on bodyweight Erythema: 0.0 Oedema: 0.0	CA 5.2.4/008 Report no: B02294 (2007)
OECD 404 (2002) GLP No significant deviations Study acceptable	Rabbit; New Zealand White Males 3/dose	Glyphosate Acid Technical Batch: 040205 Purity: 97.23%	0.5g, 4 hours, semi-occlusive Vehicle: distilled water	No mortality, clinical signs or effect on bodyweight Erythema: 0.0 Oedema: 0.0 Reversibility very slight erythema one animal by 24 hours	CA 5.2.4/009 Report no: 15278 (2005)
OECD 404 (1992) GLP Deviations: occlusive dressing Study acceptable	Rabbit; New Zealand White Females 6/dose	Glyphosate Acid Batch: P24 Purity: 95.6%	0.5g, 4 hours, occlusive Vehicle: deionized water	No mortality, clinical signs or effect on bodyweight Erythema: 0.0 Oedema: 0.0	CA 5.2.4/010 Report no: [redacted]/P/4695 (1996)
OECD 404 (1992) GLP Deviations: occlusive dressing, clinical signs not reported Study acceptable	Rabbit; New Zealand White Females 6/dose	HR-001 (glyphosate technical) Batch: T-941209 Purity: 97.56%	0.5g, 4 hours, occlusive Vehicle: deionized water	No mortality or effect on bodyweight Erythema: 0.0 Oedema: 0.0	CA 5.2.4/011 Report no: [redacted] 95-0035 (1995)
US EPA GLP Deviations: water solubility and physicochemical properties not reported Study acceptable **Remark RMS:** **For the process under Regulation (EC) No 1107/2009, the**	Rabbit; New Zealand White 1 male and 5 females	Glyphosate Premix Batch: 290-JaK-146-4 Purity: 62.2 % as glyphosate isopropylamine salt (46.1 % as glyphosate)	0.5mL, 4 hours, semi-occlusive	No mortality, clinical signs or effect on bodyweight Erythema: 0.11 Oedema: 0.0 Reversibility very slight erythema 2 animals by 48 hours	CA 5.2.4/012 Report no: 545/40 (1994)

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Results - Observations and time point of onset[2] - Mean scores/animal - Reversibility	Reference
applicant is requested to justify why for the same batch different conclusions are drawn regarding the purity and the acceptability of acute toxicity studies. <u>For the process under the Regulation (EC) No 1272/2008, the applicant is asked to submit the missing information during the public consultation period</u>. **CA 5.2.1/020 acceptable** **CA 5.2.3/016 acceptable** **CA 5.2.4/012 supportive due to low purity** **CA 5.2.5/015 supportive due to low purity** **CA 5.2.6/016 acceptable**					
OECD 404 (1992) GLP Deviations: purity test substance not reported, data on mortality, clinical sings, bodyweight and necropsy missing Study acceptable but with restrictions	Rabbit; New Zealand White 2 males and 1 female	Glyphosate salt Batch: 1056 Purity: -	0.5mL, 4 hours, semi-occlusive	Erythema: 0.0 Oedema: 0.0	CA 5.2.4/013 Report no: 710/29 (1994)
OECD 404 (1981) GLP Deviations: moistening of test item not reported, occlusive dressing, bodyweights not	Rabbit; New Zealand White Sex unknow 4/dose	Glyphosate Batch: 36300892 Purity: 99.6%	0.5g, 4hours, occlusive Vehicle: none	No mortality or clinical signs Erythema: 0.0 Oedema: 0.08 Very slight oedema in one animal at 24 hours	CA 5.2.4/014 Report no: [redacted]-93-404/N (1994)

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Results - Observations and time point of onset[2] - Mean scores/animal - Reversibility	Reference
recorded, limited reporting Study unacceptable					
OECD 404 (1987) GLP Deviations: occlusive dressing, limited reporting Study acceptable but with restrictions	Rabbit; New Zealand White Sex 2 male and 1 female	Glyphosate technical Batch: 60 Purity: 96.8%	0.5g, 4hours, occlusive Vehicle: distilled water	No mortality, clinical signs or effect on bodyweight Erythema: 0.0 Oedema: 0.0	CA 5.2.4/015 Report no: [redacted].878.SKIN (1991)
No guideline followed Not GLP Deviations: limited reporting Study unacceptable	Rabbit; New Zealand White Males 3/dose	Glyphosate acid Batch: not reported Purity: 98%	0.5g, 4hours, semi-occlusive Vehicle: saline	Erythema: 0.33 Oedema: 0.0 Reversibility slight erythema in all animals by 48 hours	CA 5.2.4/016 Report no: 910259 (1991)
OECD 404 (1981) GLP Deviations: limited reporting Study acceptable but with restrictions	Rabbit; New Zealand White Males 3/dose	Glyphosate acid Batch: 0190 A Purity: 98.1%	0.5g, 4hours, semi-occlusive Vehicle: saline	No mortality, clinical signs or effect on bodyweight Erythema: 0.0 Oedema: 0.0	CA 5.2.4/017 Report no: [redacted]-900822A (1990)
US EPA GLP Deviations: limited reporting Study acceptable but with restrictions	Rabbit; New Zealand White 2 males and 4 females	Glyphosate technical Batch: 206-Jak-25-1 Purity: 98.6 (not specified in study report	0.5g, 4hours, semi-occlusive Vehicle: water	Erythema: 0.0 Oedema: 0.0	CA 5.2.4/018 Report no: 5885 (1989)
Study unacceptable	Rabbit; 3 females (intact skin), 3 males (abraded skin)	Glyphosate salt, purity 62 %	4-hour semi-occlusive application on intact and abraded skin	Study report not available, so no conclusion drawn	CA 5.2.4/019 Report no: 238072 (1989)
US EPA GLP Deviations: irritation evaluated at 30 min instead of 1 hour, six instead of 3 animals used and treated simultaneously, age	Rabbit; New Zealand White 3 males and 3 females	Glyphosate Batch: XLI-55 Purity: 97.76%	0.5g, 4hours, semi-occlusive Vehicle: saline	Erythema: 0.0 Oedema: 0.0	CA 5.2.4/020 Report no: 88.2053.010 (1988)

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Results - Observations and time point of onset[2] - Mean scores/animal - Reversibility	Reference
animals not reported, air changes, water solubility and stability not reported, no justification for choice and use vehicle included Study acceptable					
US EPA GLP Deviations: limited reporting Study acceptable but with restrictions	Rabbit; New Zealand White Male 6/dose	MON 8750 Batch: XLG-225 (Assigned FDRL Identification 86-0616) Purity: 90.8%	0.5g, 4hours, semi-occlusive Vehicle: saline	Erythema: 0.0 Oedema: 0.0	CA 5.2.4/021 Report no: [redacted]-86-431/9308A (1987)
No guideline followed Not GLP Deviations: limited reporting Study unacceptable	Rabbit; New Zealand White 3 males and 3 females	Glyphosate technical Batch: R&D Sample Purity: 95%	0.5g, 4hours, occlusion unknown Vehicle: saline	Erythema: 0.0 Oedema: 0.0	CA 5.2.4/022 Report no: 400060 (1983)
No guideline followed Not GLP Deviations: occlusive dressing, limited reporting Study unacceptable	Rabbit; New Zealand White 3 males and 3 females	MON 0139 Batch: SSRT-11012 Purity: -	0.5mL, 4hours, occlusive	No measurement at 48 hours. 24 hours: 1/6 animals erythema grade 1, no signs of oedema 72 hours: no signs of erythema or oedema	CA 5.2.4/023 Report no: 800259 (1981)
No guideline Not GLP Deviations: occlusive dressing, limited reporting Study unacceptable	Rabbit; New Zealand White 3 males and 3 females	Glyphosate technical Batch: XLI-180 Purity: 99%	4 times 0.125 g per test site (0.5 g per animal) 24hours	No measurement at 48 hours. 24 hours: 1/6 animals erythema grade 1, no signs of oedema 72 hours: no signs of erythema or oedema	CA 5.2.4/024 Report no: [redacted]-77-428 (1979)

Table 28: Summary table of human data on skin corrosion/irritation

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference

No cases of skin effects after exposure to non-formulated glyphosate alone were reported. Therefore, no data relevant for classification purposes is available from humans.

Table 29: Summary table of other studies relevant for skin corrosion/irritation

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No information from other studies is considered relevant for classification purposes with respect to skin irritation. Data from repeated dose toxicity studies after dermal application do not indicate dermal irritant properties of glyphosate (refer to Vol 3 CA B.6.3.3) as in rats and rabbits either no or only slight dermal irritating effects were observed (at high doses after repeated exposure).				

2.6.2.4.1 Short summary and overall relevance of the provided information on skin corrosion/irritation

There were 23 skin irritation studies of which 18 studies were concluded to be in accordance with OECD test guidelines and to be acceptable (acceptable or acceptable but with restrictions). In all available studies glyphosate showed either no or only very slight skin irritation potential.

No human data is available on skin exposure to pure glyphosate.

2.6.2.4.2 Comparison with the CLP criteria regarding skin corrosion/irritation

The classification criteria for skin corrosion (Category 1) under Regulation 1272/2008 are as followed:

> Destruction of skin tissue, namely, visible necrosis through the epidermis and into the dermis, in at least one tested animal after exposure ≤ 4 h

The classification criteria for skin irritation (Category 2) under Regulation 1272/2008 are as followed:

> (1) Mean score of ≥ 2,3 - ≤ 4,0 for erythema/eschar or for oedema in at least 2 of 3 tested animals from gradings at 24, 48 and 72 hours after patch removal or, if reactions are delayed, from grades on 3 consecutive days after the onset of skin reactions; or
>
> (2) Inflammation that persists to the end of the observation period normally 14 days in at least 2 animals, particularly taking into account alopecia (limited area), hyperkeratosis, hyperplasia, and scaling; or
>
> (3) In some cases where there is pronounced variability of response among animals, with very definite positive effects related to chemical exposure in a single animal but less than the criteria above.

In the available skin irritation studies, most studies did not show any skin irritation potential with erythema and oedema scores of 0. In the few studies that did show some skin irritation the highest mean irritation score was 0.33 for erythema which is below the criteria for classification. Therefore, based on the results of these studies it can be concluded that glyphosate does not required classification for skin irritation.

2.6.2.4.3 Conclusion on classification and labelling for skin corrosion/irritation

Glyphosate does not need to be classified for skin corrosion or irritation according to the CLP Regulation (EU) No. 1272/2008.

Not classified – Conclusive but not sufficient for classification.

2.6.2.5 Serious eye damage/eye irritation [equivalent to section 10.5 of the CLH report template]

Table 30: Summary table of animal studies on serious eye damage/eye irritation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels duration of exposure	Results - Observations and time point of onset - Mean scores/animal - Reversibility	Reference
OECD 405 (2002) GLP Deviations: pH 1.99, but treatment performed Study acceptable	Rabbit; New Zealand White Males 1 rabbit only	Glyphosate technical Batch: 569753 Purity: 96.3%	0.1g, undiluted solid glyphosate, 24 hours No rinsing of eyes	No mortality, clinical signs or effect on bodyweight Pain reaction score of 3 (0-5 scale), conjunctival discharge, corneal erosion, redness of conjunctiva with pale areas, pink, clean ocular discharge, oedema of the eyelids, a few black points on the conjunctiva and dry surface of the eye at 1 hour. Positive fluorescein staining and conjunctival discharge at 24 hours. Study terminated at 24 hours 1-24 hours scores: 3 corneal opacity, 1 iris lesions, 3 conjunctival redness, 4 conjunctival chemosis.	CA 5.2.5/001 Report no: 10/218-005N (20011)
OECD 405 (2002) GLP Deviations: Himalayan rabbits, air changes not reported Study acceptable	Rabbit; Himalayan Males 3/dose	Glyphosate TC Batch: 20090506 Purity: 97.3%	0.1g, undiluted solid glyphosate Eyes washed with sodium chloride 1 hour post dosing 7 days observation	No mortality, clinical signs or effect on bodyweight Conjunctival redness and chemosis observed from 1 hour post dosing Corneal opacity and lesions of iris observed from 24 hour post dosing for corneal opacity: 1.00, 1.00, 1.00 (reversibility 4-6 days) for iris lesions: 0.67, 0.67, 1.00 (reversibility 72 hours - 4 days) for conjunctival redness: 1.00, 1.33, 2.00 (reversibility 5-7 days) for conjunctival chemosis: 0.33, 0.33, 0.33 (reversibility 48 hours)	CA 5.2.5/002 Report no: 24606 (2010)
OECD 405 (2002) GLP Deviations: Himalayan rabbits, air changes not reported	Rabbit; Himalayan Males 3/dose	Glyphosate TC Batch: 2009051501 Purity: 96.4%	0.1g, undiluted solid glyphosate Eyes washed with saline 1 hour post dosing	No mortality, clinical signs or effect on bodyweight Conjunctival redness and chemosis observed from 1 hour post dosing Corneal opacity and lesions of iris observed from 24 hour post dosing	CA 5.2.5/003 Report no: 24878 (2009)

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels duration of exposure	Results - Observations and time point of onset - Mean scores/animal - Reversibility	Reference
Study acceptable			8 days observation	for corneal opacity: 1.00, 1.00, 1.00 (reversibility 4-8 days) for iris lesions: 1.00, 0.67, 0.33 (reversibility 48 hours - 4 days) for conjunctival redness: 1.00, 1.00, 1.00 (reversibility 4-6 days) for chemosis of the conjunctiva: 0.67, 0.33, 0.00 (reversibility 24-72 hours)	
OECD 405 (2002) GLP Deviations: Himalayan rabbits, air changes not reported Study acceptable	Rabbit; Himalayan Males 3/dose	Glyphosate TC Batch: 20080801 Purity: 98.8%	0.1g, undiluted solid glyphosate Eyes washed with sodium chloride 1 hour post dosing 4 days observation	No mortality, clinical signs or effect on bodyweight Conjunctival redness observed from 1 hour post dosing Corneal opacity observed from 24 hour post dosing for corneal opacity: 1.00, 0,00, 0.67 (reversibility 72 hours - 4 days) for iris lesions: 0.00, 0.00, 0.00 for conjunctival redness: 1.00, 0.67, 0.67 (reversibility 72 hours - 4 days) for conjunctival chemosis: 0.67, 0.00, 0.00	CA 5.2.5/004 Report no: 23914 (2009)
OECD 405 (2002) GLP No significant deviations	-	Glyphosate technical Batch: GI-1045 Purity: 96.66%	-	pH 1.93, no test performed. Based on the very low pH the test substance item has corrosive properties; therefore, no eye irritation study with this batch of glyphosate technical was performed in rabbits.	CA 5.2.5/005 Report no: C22897 (2009)
OECD 405 (2002) GLP Deviations: temperature and humidity outside range, clinical sings not investigated, compound stability not reported	Rabbit; New Zealand White 2 males and 1 female	Glyphosate Tech Grade Mixed 5-Batch Batch: 080704-1 thru 5 Purity: 96.4%	0.1mL (93.2 mg), undiluted solid glyphosate Eyes washed with water after 24 h 17 days observation	No mortality or effect on bodyweight Conjunctival redness and chemosis observed from 1 hour post dosing Corneal opacity and lesions of iris observed from 24 hour post dosing for corneal opacity: 1.00, 0.00, 2.00 (reversibility 4-10 days)	CA 5.2.5/006 Report no: 12172-08 (2009)

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels duration of exposure	Results - Observations and time point of onset - Mean scores/animal - Reversibility	Reference
Study acceptable				for iris lesions: 0.00, 0.00, 1.00 (reversibility 7 days) for conjunctival redness: 2.00, 0.67, 2.00 (reversibility 72 hours - 17 days) for chemosis of the conjunctiva: 1.67, 0.00, 3.00 (reversibility 24 hours - 17 days)	
OECD 405 (2002) GLP No significant deviations Study acceptable	Rabbit; New Zealand White 1 male and 1 female	Glyphosate technical Batch: 20070606 Purity: 98.05%	0.1g, undiluted solid glyphosate 21 day observation No rinsing of eyes	No mortality or effect on bodyweight Conjunctival redness and chemosis, corneal opacity and lesions of iris observed from 1 hour post dosing for corneal opacity: 3.33 and 3.67 (1 animal reversibility 7 days, 1 animal not reversible at 21 days) for iris lesions: 1.00 and 1.00 (reversibility 7 days) for conjunctival redness: 3.00 and 2.67 (1 animal reversibility 14 days, 1 animal not reversible at 21 days) for chemosis of the conjunctiva: 2.00 and 1.33 (reversibility 21 days)	CA 5.2.5/007 Report no: [redacted]-3996.312.599.07 (2008)
OECD 405 (2002) GLP No significant deviations Study acceptable	Rabbit; New Zealand White 2 males and 1 female	Glyphosate technical material Batch: 0507 Purity: 96.1%	0.1g, undiluted solid glyphosate 7 day observation No rinsing of eyes	No mortality, clinical signs or effect on bodyweight Conjunctival redness and chemosis observed from 1 hour post dosing for corneal opacity: 0.00, 0.00, 00.0 for iris lesions: 0.00, 0.00, 0.00 for conjunctival redness: 0.67, 1.67, 1.67 (reversibility 72 hours – 7 days) for conjunctival chemosis: 0.00, 0.33, 1.00 (reversibility 24 – 72 hours)	CA 5.2.5/008 Report no: B02788 (2007)
OECD 405 (2002) GLP No significant deviations	Rabbit; New Zealand White 1 male and 2 females	Glyphosate technical (NUP 05068) Batch: 200609062 Purity: 95.1%	0.1g, undiluted solid glyphosate 14 day observation	No mortality, clinical signs or effect on bodyweight Conjunctival redness and chemosis, corneal opacity and reddening of the sclera	CA 5.2.5/009 Report no: B02305 (2007)

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels duration of exposure	Results - Observations and time point of onset - Mean scores/animal - Reversibility	Reference
Study acceptable			No rinsing of eyes	observed from 1 hour post dosing for corneal opacity: 1.67, 2.00 and 0.67; (reversibility 72 hours – 7 days) for iris lesions: 0.00, 0.00, 0.00 for conjunctival redness: 2.67, 2.00, 2.00 (reversibility 10 days) for chemosis of the conjunctiva: 2.00, 2.00, 1.00. (reversibility 72 hours – 7 days)	
OECD 405 (2002) GLP Deviations: low amount of test substance, humidity and air changes not reported Acceptable but with restrictions	Rabbit; New Zealand White Males 3/dose	Glyphosate acid technical Batch: 040205 Purity: 97.23%	0.06 g powdered glyphosate 10 day observation No rinsing of eyes	No mortality or clinical signs Conjunctival redness and chemosis, corneal opacity and lesions of iris observed from 1 hour post dosing Corneal opacity: 1.00, 1.00 and 1.00 (reversibility 10 days); Iris lesions: 1.00, 1.00 and 1.00 (reversibility 7 days) for conjunctival redness: 2.33, 2.67 and 2.67 (reversibility 10 days) for chemosis of the conjunctiva: 1.67, 2.00 and 2.00. (reversibility 7 days)	CA 5.2.5/010 Report no: 15277 (2005)
OECD 405 (1987) GLP No significant deviations Study acceptable	Rabbit; New Zealand White Females 6/dose	Glyphosate acid Batch: P24 Purity: 95.6%	0.1g, undiluted solid glyphosate 8 day observation No rinsing of eyes	No mortality, clinical signs or effect on bodyweight Initial pain reaction was none to moderate 0-3 (scale 0-5) Conjunctival redness and chemosis observed from 1 hour post dosing Corneal opacity observed from 1- 24 hour post dosing Lesions of iris observed from 24-48 hours post dosing for corneal opacity: 0.67, 1.00, 1.33, 2.00, 1.00, 2.00, (reversibility 72 hours – 7 days)	CA 5.2.5/011 Report no: [redacted]/P/5138 (1997)

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels duration of exposure	Results - Observations and time point of onset - Mean scores/animal - Reversibility	Reference
				for iris lesions: 0.33, 0.67, 0.67, 0.67, 1.00, 1.00, (reversibility 72 hours – 4 days) for conjunctival redness: 1.67, 2.00, 2.00, 2.00, 2.00, 2.00 (reversibility 7-8 days) for conjunctival chemosis: 1.33, 1.33, 1.67, 2.00, 2.00, 2.00. (reversibility 4-7 days)	
US EPA (1984) GLP Deviations: some animals eyes were rinsed 30 sec post dosing Study acceptable	Rabbit; New Zealand White 6 males and 3 females	Glyphosate Technical (WetCake) Batch: 120594 Purity: 98.2%	0.1 mL (equivalent to 0.065 g), undiluted solid glyphosate 21 day observation Eyes rinsed 30 sec after application in 3 male animals	Results of animals with rinsed eyes not reported here No mortality or clinical signs Conjunctival redness and chemosis observed from 1 hour post dosing Corneal opacity observed from 1- 24 hour post dosing for corneal opacity: 1.00, 1.00, 2.00, 1.00 1.00, 1.00 (reversibility day 4-21; not reversible within 21 days for 2 animals) for iris lesions: 0.00, 0.00, 0.00, 0.00, 0.00, 0.00 for conjunctival redness: 2.67, 3.00, 2.67, 3.00, 2.33, 3.00 (reversibility 10-21 days; not reversible within 21 days for 2 animals) for chemosis of the conjunctiva: 1.67, 2.67, 2.33, 2.00, 2.00, 2.33 (reversibility 4-21 days; not reversible within 21 days for 1 animal)	CA 5.2.5/012 Report no: 2981-96 (1996)
OECD 405 (1987) GLP Deviations: eyes of groups B and C were rinsed before 1 hour Study acceptable	Rabbit; New Zealand White Females 12/dose	HR-001 (glyphosate technical) Batch: T941209 Purity: 97.56%	0.1 g, undiluted solid glyphosate 21 day observation Eyes rinsed after 30 seconds in group B and 2 minutes in group C	Results of animals with rinsed eyes not reported here No mortality, clinical signs or effect on bodyweight Effects observed from 1 hour post dosing for corneal opacity: 2.00, 2.67. 2.00, 2.00, 2.00, 1.67 (not reversible in 3 animals; reversibility 7-13 days) for iris lesions: 1.00, 1.00,	CA 5.2.5/013 Report no: [redacted] 95-0034 (1995)

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels duration of exposure	Results - Observations and time point of onset - Mean scores/animal - Reversibility	Reference
				1.00, 1.00, 1.00, 0.67 (reversibility 4-10 days) for conjunctival redness: 2.00 for all animals (reversibility 7-16 days) for conjunctival chemosis: 2.00, 1.67, 2.33, 2.33, 2.00, 1.67 (reversibility 4-7 days)	
US EPA (1984) GLP Deviations: None Study supplementary due to low purity. **Remark RMS: <u>For the process under Regulation (EC) No 1107/2009,</u> the applicant is requested to justify why for the same batch different conclusions are drawn regarding the purity and the acceptability of acute toxicity studies. <u>For the process under the Regulation (EC) No 1272/2008,</u> the applicant is asked to submit the missing information during the public**	Rabbit; New Zealand White 3 males and 3 females	Glyphosate premix (technical concentrate) Batch 290-JaK-146-4 Purity: 62.2% as glyphosate isopropylamine salt, 46.1% as glyphosate	0.1mL undiluted, 72 hours No rinsing of eyes	No mortality or clinical signs Conjunctival redness and chemosis observed at 1 hour post dosing in all animals Lesions of iris observed at 1 hour post dosing in 4 animals for corneal opacity: 0.00, 0.00, 0.00, 0.00, 0.00, 0.00 for iris lesions: 0.00, 0.00, 0.00, 0.00, 0.00, 0.00 for conjunctival redness: 0.00, 0.00, 0.00, 0.00, 0.00, 0.00 for chemosis of the conjunctiva: 0.00, 0.00, 0.00, 0.00, 0.00, 0.00	CA 5.2.5/014 Report no: 545/41 (1994)

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels duration of exposure	Results - Observations and time point of onset - Mean scores/animal - Reversibility	Reference
consultation period. **CA 5.2.1/020 acceptable** **CA 5.2.3/016 acceptable** **CA 5.2.4/012 supportive due to low purity** **CA 5.2.5/015 supportive due to low purity** **CA 5.2.6/016 acceptable**					
OECD 405 (1987) GLP Deviations: low amount of test substance used and limited reporting Study unacceptable	Rabbit; New Zealand White Female 1/dose	Glyphosate technical Batch: not reported Purity: 95%	0.76 mg, 1 hour No rinsing of eyes	Due to the severe eye reactions, test was stopped after 1 hour for humane reasons. Clinical signs: Opaque corneal opacity, iridial inflammation and moderate conjunctival irritation. Additionally, sloughing of the cornea, haemorrhage of the lower conjunctival membrane and blood stained discharge were reported	CA 5.2.5/015 Report no: 710/18 (1994)
OECD 405 (1981) GLP Deviations: limited reporting Study acceptable but with restrictions	Rabbit; New Zealand White Female 4/dose	Glyphosate technical Batch: 36300892 Purity: 99.6%	0.1g, undiluted solid glyphosate 14 day observation No rinsing of eyes	No mortality or clinical signs for corneal opacity: 2.00, 1.00, 1.33, 1.00 (reversibility 7-14 days) for iris lesions: 1.00, 1.00, 0.33, 1.00 (reversibility 48 hours-7 days) for conjunctival redness: 1.00, 1.67, 2.00, 2.00 (reversibility 14 days) for chemosis of the conjunctiva: 2.00, 1.67, 2.00, 3.00 (reversibility 14 days)	CA 5.2.5/016 Report no: [redacted]-93-405/N (1994)
OECD 405 (1987) GLP Deviations: Individual scores not reported Study unacceptable	Rabbit; New Zealand White 2 males and 1 female	Glyphosate technical Batch: 60 Purity: 96.8%	0.1g, 21 days Eyes rinsed 24 hours after application	One female rabbit died on day 2 No clinical signs or effects on bodyweight reported Necropsy findings showed opacity of the treated eye in all animals, involving 3/4 or 1/2 of the area, and one male rabbit showed ulceration. Enteritis was seen in the deceased animal (female).	CA 5.2.5/017 Report no: [redacted].879.EYE (1991)

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels duration of exposure	Results - Observations and time point of onset - Mean scores/animal - Reversibility	Reference
				for corneal opacity: 2.00, 2.00 (score for the third animal not available due to its death after the 48 hour scoring) for iris lesions: 0.00, 0.00 (score for the third animal not available due to its death after the 48 hour scoring) for conjunctival redness: 1.44 (mean for all three animals due to missing information on individual scores) for chemosis of the conjunctiva: 1.11 (mean for all three animals due to missing information on individual scores) Effects were not reversible by day 21.	
EEC guidelines Not GLP Deviations: limited reporting Study unacceptable	Rabbit; New Zealand White Males 3/dose	Glyphosate acid Batch: not reported Purity: 98%	0.1g, 21 days No rinsing of eyes	No mortality or clinical signs. Conjunctival redness and chemosis observed from 1 hour post dosing Corneal opacity and lesions of iris observed from 24 hours post dosing for corneal opacity: 1.00, 1.00, 1.00 (reversibility 72 hours) for iris lesions: 0.67, 0.67, 0.67 (reversibility 72 hours) for conjunctival redness: 1.67, 1.67, 1.67 (reversibility 7 days) for chemosis of the conjunctiva: 1.00, 1.00, 1.00 (reversibility 72 hours)	CA 5.2.5/018 Report no: 910260 (1991)
OECD 405 (1981) GLP Deviations: no data on clinical signs or bodyweight and other limited reporting	Rabbit; New Zealand White Females 3/dose	Glyphosate technical Batch: 0190 A Purity: 98.1%	0.1g, undiluted solid glyphosate 8 day observation No rinsing of eyes	No mortality Corneal opacity and conjunctival chemosis observed from 1 hour post dosing Conjunctival chemosis observed from 24 hours post dosing Lesions of iris observed from 48 hours post dosing	CA 5.2.5/019 Report no: [redacted]-900822 (1990)

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels duration of exposure	Results - Observations and time point of onset - Mean scores/animal - Reversibility	Reference
Study acceptable but with restrictions				for corneal opacity: 1.00, 1.00, 1.67 (reversibility 6-8 days) for iris lesions: 0.00, 0.00, 0.67 (reversibility 7 days) for conjunctival redness: 1.00, 1.00, 1.33 (reversibility 7 days) for chemosis of the conjunctiva: 0.67, 0.67, 1.00 (reversibility 72 hours)	
US EPA GLP Deviations: only one animal tested. No data on clinical signs and bodyweight, compound stability not reported Study acceptable but with restrictions	Rabbit; New Zealand White Male 1/dose	Glyphosate technical Batch: 206-Jak-25-1 Purity: 98.6%	0.1g, undiluted solid glyphosate 4 day observation No rinsing of eyes	No mortality Dullness of the cornea of the whole area observed from 1 - 24 hours post dosing Conjunctival redness and chemosis observed 1 hour post dosing Lesions of iris observed 24 hours post dosing for corneal opacity: 1.00 (not reversible within 4 days) for iris lesions: 1.00 (reversibility 4 days) for conjunctival redness: 2.00 (reversibility 4 days) for chemosis of the conjunctiva: 2.00 (reversibility 4 days)	CA 5.2.5/020 Report no: 5886 (1989)
OECD 405 (1987) GLP Deviations: half of animals with rinsed eyes after 30 seconds Study unacceptable	Rabbit; New Zealand White 3 males and 3 females	Glyphosate IPA salt Batch: 21/39 Purity: 62 % in water equivalent to 46% of N-phos-phono-methyl-glycine acid	0.1mL, 72 hours Eyes of female animals rinsed 30 seconds after treatment.	Results of animals with rinsed eyes not reported here No mortality, clinical signs or effect on bodyweight Conjunctival redness and chemosis observed from 1 hour post dosing For corneal opacity: 0.00, 0.00, 0.00 For iris lesions: 0.00, 0.00, 0.00 For conjunctival redness: 0.33, 0.33, 0.00 For conjunctival redness: 0.00, 0.00, 0.00	CA 5.2.5/021 Report no: 238083 (1989)
US EPA (1984) GLP	Rabbit; New Zealand White	Glyphosate Batch: XLI-55 Purity: 97.76%	0.1g, undiluted solid glyphosate	Mortality: 1 rabbit died following exhibited anorexia, and gross necropsy revealed a clear gel-like substance in	CA 5.2.5/022 Report no: 88.2053.009 (1988)

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels duration of exposure	Results - Observations and time point of onset - Mean scores/animal - Reversibility	Reference
Deviations: Clinical signs, bodyweight and necropsy findings not reported and other limited reporting Study acceptable but with restrictions	Males and females 6/dose Sex distribution unknown		21 day observation Eyes rinsed 24 hours post dosing	the large intestine. These findings are consistent with mucoid enteropathy Conjunctival redness and chemosis observed from 1 hour post dosing Corneal opacity and lesions of iris observed from 1- 24 hour post dosing for corneal opacity: 2.67, 1.67, 2.00, 1.00, 2.33, 2.67 (reversibility 14-21 days; not reversible in 4 animals including the animal dead on day 14) for iris lesions: 0.00, 0.00, 1.00, 0.00, 0.00, 0.00 (reversibility 21 days) for conjunctival redness: 2.00, 2.00, 2.00, 2.00, 2.00, 2.00 (reversibility 14-21 days; not reversible within 21 days in one animal) for conjunctival chemosis: 2.00, 3.33, 3.33, 2.67, 2.00, 2.00 (reversibility 14-21 days)	
US EPA (1982) GLP Deviations: sex of animals not specified and limited reporting Study supplementary due to low purity.	Rabbit; New Zealand White 6/dose Sex unknown	Glyphosate sodium salt (MON 8722) Batch: XLG-256 Purity: 70.7%	0.1g, undiluted solid glyphosate 72 hour observation Eyes rinsed 24 hours post dosing	No mortality or clinical signs Conjunctival redness and chemosis observed from 1 hour post dosing for corneal opacity: 0.00, 0.00, 0.00, 0.00, 0.00, 0.00 for iris lesions: 0.00, 0.00, 0.00, 0.00, 0.00, 0.00 for conjunctival redness: 0.33, 0.00, 0.00, 0.00, 0.00, 0.00 (reversibility 24-48 hours) for chemosis of the conjunctiva: 0.00, 0.00, 0.00, 0.00, 0.00, 0.00 (reversibility 24 hours	CA 5.2.5/023 Report no: 9307A (1987)
US EPA (1982) GLP Deviations: limited reporting. Clinical signs	Rabbit; New Zealand White 6/dose	Glyphosate (MON 8750) Batch: XLG-255 Purity: 90.8%	0.1g, undiluted solid glyphosate 72 hour observation	No mortality Conjunctival redness and chemosis observed from 1 hour post dosing for corneal opacity: 0.00, 0.00, 0.00, 0.00, 0.00, 0.00	CA 5.2.5/024 Report no: [redacted]-86-431/9308A (1987)

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels duration of exposure	Results - Observations and time point of onset - Mean scores/animal - Reversibility	Reference
and bodyweight not investigated Study acceptable but with restrictions	Sex unknown		Eyes rinsed 24 hours post dosing	for iris lesions: 0.00, 0.00, 0.00, 0.00, 0.00, 0.00 for conjunctival redness: 0.33, 0.67, 0.33, 0.33, 0.67, 0.33 (reversibility 48-72 hours) for chemosis of the conjunctiva: 0.00, 0.00, 0.00, 0.00, 0.00, 0.00 (reversibility 24 hours)	
No guideline followed Not GLP Deviations: limited reporting eyes washed after 30 seconds, pdf study report of low quality. No eye observations before 24 hours Study unacceptable	Rabbit; New Zealand White Three males and three females	Glyphosate technical Batch: R&D sample, sample 8.7.83 Purity: 95%	0.1g, 15days Eyes rinsed 30 seconds post dosing	No mortality or clinical signs Conjunctival redness and chemosis, corneal opacity and lesions of iris observed from 24 hours post dosing for corneal opacity: 0.33, 0.33, 0.33, 0.33, 0.00, 0.33 (reversibility 48 hours) for iris lesions: 0.33, 0.33, 0.33, 0.33, 0.33, 0.33 (reversibility 48 hours) for conjunctival redness: 1.00, 1.00, 0.67, 1.00, 0.67, 1.00 (reversibility 72 hours) for chemosis of the conjunctiva: 2.00, 1.33, 1.33, 1.00, 0.67, 1.00 (reversibility 72 hours)	CA 5.2.5/025 Report no: not reported (1983)
No guidelines followed Not GLP Deviations: limited reporting, purity test substance not reported Study unacceptable	Rabbit; New Zealand White 4 males and 5 females	Glyphosate (MON 0139), IPA salt Batch: SSRT-11012 Purity: not reported	0.1mL, 72 hours Eyes of 3 animals were rinsed 20 seconds post dosing	Results of animals with rinsed eyes not reported here for corneal opacity: 0.00, 0.00, 0.00, 0.00, 0.00, 0.00 for iris lesions: 0.00, 0.00, 0.00, 0.00, 0.00, 0.00 for conjunctival redness: 0.00, 0.00, 0.00, 0.00, 0.00, 0.00 for chemosis of the conjunctiva: 0.00, 0.00, 0.00, 0.00, 0.00, 0.00 for conjunctival discharge: 0.00, 0.00, 0.00, 0.00, 0.00, 0.00	CA 5.2.5/026 Report no: 800260 (1981)

Table 31: Summary table of human data on serious eye damage/eye irritation

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No cases of eye effects after exposure to non-formulated glyphosate alone were reported. Therefore, no data relevant for classification purposes is available from humans. Refer to summary below.				

Table 32: Summary table of other studies relevant for serious eye damage/eye irritation

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No information from other studies is considered relevant for classification purposes with respect to eye irritation				

2.6.2.5.1 Short summary and overall relevance of the provided information on serious eye damage/eye irritation

There are 26 eye irritation studies available of which 18 were concluded to be fully acceptable or acceptable but with restrictions. A wide range of results were observed in these 18 studies. One study concluded that glyphosate has corrosive properties based on the low pH of the test material in the study (CA 5.2.5/005, pH 1.93). Six studies (CA 5.2.5/001, CA 5.2.5/007, CA 5.2.5/012, CA 5.2.5/013, CA 5.2.5/020 and CA 5.2.5/022) showed irritation scores which meet the criteria for eye damage. Furthermore, eight studies (CA 5.2.5/002, CA 5.2.5/003, CA 5.2.5/006, CA 5.2.5/009, CA 5.2.5/010, CA 5.2.5/011, CA 5.2.5/016, CA 5.2.5/019) indicated an eye irritation potential for glyphosate. In contrast, there were three studies (CA 5.2.5/004 CA 5.2.5/008 and CA 5.2.5/024) were negative.

Of the unacceptable studies, three were negative (CA 5.2.5/021, CA 5.2.5/025, CA 5.2.5/026), one was positive for eye irritation (CA 5.2.5/018) and two were positive for eye damage (CA 5.2.5/015 and CA 5.2.5/017). A further two supplementary studies were available that were negative. However, the purity in these studies was low and therefore these studies are considered of low relevance for the classification and labelling.

Overall, the majority of the studies showed a potential for eye damage or eye irritation.

No cases of eye effects after exposure to non-formulated glyphosate alone were reported. Therefore, no data relevant for classification purposes is available from humans. On the other hand, several publications are available on potential eye effects after human exposure to glyphosate-based formulations (refer to Vol 1 Section 2.6.9 and Vol 3 CA B.6.9). The available studies described that eye exposures to glyphosate-based formulations have generally resulted in temporary conjunctival irritation, clearing after irrigation or in 1-2 days and that permanent eye damage is considered unlikely (Bradberry *et al.*, 2004 (Vol 3 B.6.9.8.16)). Another review on ocular exposures to glyphosate-surfactant formulations (1513 exposures over a 5-year period) described no permanent eye injury (Acquavella *et al.*, 1999 (Vol 3 B.6.9.8.12)). As the described cases were reported after exposure to glyphosate-based formulations, it is not possible to distinguish whether these eye effects are due to glyphosate alone or the co-formulations or a combination of both. Therefore, no data relevant for classification purposes is available from humans.

2.6.2.5.2 Comparison with the CLP criteria regarding serious eye damage/eye irritation

The classification criteria for eye damage (Category 1) under Regulation 1272/2008 are as follows:

(a) in at least one animal effect on the cornea, iris or conjunctiva that are not expected to reverse or have not fully reversed within an observation period of normally 21 days; and/or

(b) in at least 2 of 3 tested animals, a positive response of:
(i) corneal opacity ≥ 3 and/or
(ii) iritis > 1.5
Calculated as the mean scores following grading at 24, 48 and 72 hours after installation of the test material.

Six studies meet the criteria listed above and therefore it is concluded that glyphosate should be classified for Eye Damage, Category 1 (H318). This conclusion is in line with the current harmonized classification and labelling.

2.6.2.5.3 Conclusion on classification and labelling for serious eye damage/eye irritation

Glyphosate should be classified for Eye Damage, Category 1 (H318).

2.6.2.6 Respiratory sensitisation [equivalent to section 10.6 of the CLH report template]

Table 33: Summary table of animal studies on respiratory sensitisation

Method, guideline, deviations[1] if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Results	Reference
No studies available, no information from other studies is considered relevant for classification purposes with respect to respiratory sensitisation.					

Table 34: Summary table of human data on respiratory sensitisation

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No human data available.				

Table 35: Summary table of other studies relevant for respiratory sensitisation

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No information from other studies is considered relevant for classification purposes with respect to respiratory sensitisation.				

2.6.2.6.1 Short summary and overall relevance of the provided information on respiratory sensitisation

There are no *in vivo* or *in vitro* guideline studies available investigating respiratory sensitisation. However, from the testing data available there is no evidence for skin sensitising potential of glyphosate in rodents (see section 2.6.2.7).

2.6.2.6.2 Comparison with the CLP criteria regarding respiratory sensitisation

There are no data available considering the human evidence. Furthermore, no formally recognised and validated animal or *in vitro* tests currently exist for the testing of respiratory sensitisation. Further, glyphosate is not sensitising to the skin.

2.6.2.6.3 Conclusion on classification and labelling for respiratory sensitisation

No data is available indicating that glyphosate causes respiratory sensitisation and therefore no classification is warranted.

2.6.2.7 Skin sensitisation [equivalent to section 10.7 of the CLH report template]

Table 36: Summary table of animal studies on skin sensitisation

Method, guideline, deviations, if any	Species, strain, sex, no/group	Test substance	Dose levels duration of exposure	Results	Reference
OECD 429 (2010) LLNA assay	Mouse, CBA/J Rj, 4 females/ dose	Glyphosate Technical, Batch 569753, purity 96.3% w/w	0, 10, 25 and 50% (w/v)	No mortality, clinical signs or effect on body weight.	CA 5.2.6/001 Report no. 10/218-037E (2011)

Method, guideline, deviations, if any	Species, strain, sex, no/group	Test substance	Dose levels duration of exposure	Results	Reference
GLP No deviations Study acceptable			Positive control: 25% HCA	Simulation index: - 1.0 in negative control; - 1.0 in 25% and 50% glyphosate groups; - 1.2 in 10% glyphosate group; -12.2 in positive control group Not a skin sensitizer.	
OECD 406 GPMT GLP Deviation in temperature animal housing Study acceptable	Guinea pig, Dunkin-Hartley, Females. 5 negative control; 20 positive control; 10 test group	Glyphosate Technical, Batch 2009051501, purity 96.4% Positive control: benzocaine in 40% ethanolic 0.9% NaCl solution	0.01% intracutaneous induction, 50% topical induction, 25% challenge	No dermal response observed in the treatment group or negative control group.	CA 5.2.6/002 Report no. 24879 (2010)
OECD 406 GPMT GLP Deviation in temperature animal housing Study acceptable	Guinea pig, Dunkin-Hartley, males. 5 negative control; 20 positive control; 10 test group	Glyphosate Technical, Batch 20090506, purity 97.3%	0.5% intracutaneous induction, 50% topical induction, 25% challenge	No dermal response observed in the treatment group or negative control group.	CA 5.2.6/003 Report no. 24607 (2010)
OECD 406 GPMT GLP Deviation in temperature animal housing Study acceptable	Guinea pig, Albino Dunkin-Hartley, males. 5 negative control; 10 test group	Glyphosate Technical, Batch GI-1045, purity 96.66% w/w Positive control: α-hexylcinnamaldehyde at 3% in PEG 300	10% intradermal induction, 50% topical induction, 15% challenge	No dermal response observed in the treatment group or negative control group. Not a skin sensitizer.	CA 5.2.6/004 Report no. C22908 (2009)

Method, guideline, deviations, if any	Species, strain, sex, no/group	Test substance	Dose levels duration of exposure	Results	Reference
OECD 406 GPMT GLP Deviation in temperature animal housing Study acceptable	Guinea pig, Dunkin-Hartley, males. 5 negative control; 20 positive control; 10 test group	Glyphosate Technical, Batch 20080801, purity 98.8%	0.5% intradermal induction, 50% topical induction, 50% challenge	No dermal response observed in the treatment group or negative control group.	CA 5.2.6/005 Report no. 23915 (2009)
OECD 406 Buehler assay GLP Deviations: negative control not treated with the vehicle; evaluation of skin reaction 24 and 48 hours after challenge instead of 30 and 54 hours; deviations in humidity; clinical signs were not reported Study supplementary	Guinea pig, Hartley-Albino, males and females. 5/sex negative control; 10/sex test group	Glyphosate Tech Grade Mixed 5-Batch (Batch: 080704-1 thru 5, Purity: 96.4 %)	400 mg moistened topical inductions (3x) and topical challenge	No dermal response observed in the treatment group or negative control group. Study supplementary since a negative Buehler assay is considered less sensitive compared to an LLNA or GPMT.	CA 5.2.6/006 Report no 12174-08 (2009)
OECD 406 Buehler assay GLP Deviations: no record of weight at completion of study; DMSO as solvent (not preferred); no concurrent positive control or HCD Study not acceptable	Guinea pig, Hartley, males, 10 negative control, 20 test group	Glyphosate Technical (Batch: 20070606, Purity: 98.05 %)	50% topical induction (3x) and topical challenge	No dermal response observed in the treatment group or negative control group. Study not acceptable since no positive controls are available. Besides, a negative Buehler assay is considered less sensitive	CA 5.2.6/007 Report no [redacted]-3996.318.431.07 (2008)

Method, guideline, deviations, if any	Species, strain, sex, no/group	Test substance	Dose levels duration of exposure	Results	Reference
				compared to an LLNA or GPMT.	
OECD 406 GPMT GLP No deviations Study acceptable	Guinea pig (Albino Dunkin Hartley, CRL:(HA)BR, SPF), female, 5 negative control, 10 test group	Glyphosate technical (NUP 05068) (Batch: 200609062, Purity: 95.1%) Positive control: α-hexylcinnamaldehyde at 3% in PEG 300	Intradermal induction: 3%; topical induction: 50%; challenge: 25%	No dermal response observed in the treatment group or negative control group. Not a skin sensitizer.	CA 5.2.6/008 Report no B02316 (2007)
OECD 429 LLNA assay GLP No deviations Study acceptable	Mouse, CBA/Ca/Ola/Hsd, 4 females/dose	Glyphosate Technical Material (Batch: 0507, Purity: 96.1%)	0, 10, 25 and 45% (w/v) Positive control: 0, 5, 10, 25% HCA	No mortality, clinical signs or effect on body weight. Simulation index: <3.0 in all test groups; ≥3.0 in the 25% positive control group. Not a skin sensitizer.	CA 5.2.6/009 Report no. GM8048-REG (2007)
OECD 406 GPMT GLP Deviations: no clinical signs reported; no data on diet; minor deviations regarding the room temperature. Study acceptable	Guinea pig (Albino Dunkin Hartley), female, 10 negative control, 20 test group	Glyphosate Technical (Batch: H05H016A, Purity: 95.7%) Positive control (historical): α-hexylcinnamaldehyde	Intradermal induction: 0.195%; topical induction: 60%; challenge: 30 and 60%	No dermal response observed in the treatment group or negative control group.	CA 5.2.6/010 Report no SMK-PH-05/0218 (2006)
OECD 406 Buehler assay GLP Deviations: no data on humidity or clinical signs; negative control not	Guinea pig (Hartley-Albino), male and female, 10 negative control, 20 test group	Glyphosate Acid Technical (Batch: 040205, Purity: 97.23 %) Positive control (historical): α-hexylcinnamaldehyde	70% topical induction (3x) and topical challenge	Skin reactions observed in 6/20 and 1/20 test animals and 2/10 and 0/10 negative control animals after 14 and 48 hours, respectively.	CA 5.2.6/011 Report no 15279 (2005)

Method, guideline, deviations, if any	Species, strain, sex, no/group	Test substance	Dose levels duration of exposure	Results	Reference
treated with the vehicle Study acceptable.				Study results considered equivocal.	
OECD 406 GPMT GLP No deviations Study acceptable	Guinea pig (Albino Cr1 (HA) BR), female, 10 negative control, 20 test group	Glyphosate acid (Batch: P24, Purity: 95.6% w/w) Positive control (historical): α-hexylcinnamaldehyde	Intradermal induction: 0.1%; topical induction: 75%; challenge: 75 and 30%	Scattered mild redness in 3/20 and 1/10 test and control animals, respectively, when challenged with 75%. No dermal responses observed at 48 h, or in the groups tested at 30%. Test item not considered a skin sensitiser.	CA 5.2.6/012 Report no [redacted]/P/4699 (1996)
OECD 406 GPMT GLP No deviations Study acceptable but with restrictions	Guinea pig (Crj:Hartley), female, 20 negative control, 20 test group, 10 positive control, 10 negative control of the positive control group	HR-001 (Glyphosate Technical, Batch: T 941209; Purity: 97.56 %)	Intradermal induction: 5%; topical induction: 25%; challenge: 25%	No dermal response observed in the test group or negative control group. Positive control showed the expected reactions. Study acceptable but with restriction since the dose at topical challenge is not considered sufficiently high.	CA 5.2.6/013 Report no [redacted] 95-0036 (1995)
OECD 406 GPMT GLP Deviations: batch not reported	Guinea pig (Dunkin-Hartley), female, 5 negative control, 10 test group	Glyphosate technical (batch not reported; purity 95%)	Intradermal induction: 1%; topical induction: 50%; challenge: 25% and 50%	No dermal response observed in the test group or negative control group after challenge.	CA 5.2.6/014 Report no 710/19 (1994)

Method, guideline, deviations, if any	Species, strain, sex, no/group	Test substance	Dose levels duration of exposure	Results	Reference
Study acceptable but with restrictions				Positive control (HCD) demonstrated the reliability of the test system.	
OECD 406 GPMT GLP No deviations Study acceptable **Remark RMS: <u>For the process under Regulation (EC) No 1107/2009, the applicant is requested to justify why for the same batch different conclusions are drawn regarding the purity and the acceptability of acute toxicity studies. For the process under the Regulation (EC) No 1272/2008, the applicant is asked to submit the missing information during the public consultation period.</u>** **CA 5.2.1/020 acceptable** **CA 5.2.3/016 acceptable**	Guinea pig (Albino Dunkin-Hartley), female, 10 negative control, 20 test group	Glyphosate premix (Batch: 290-Jak-146-4; Purity: 62.2% as glyphosate isopropylamine salt; 46.1% as glyphosate)	Intradermal induction: 25%; topical induction: undiluted; challenge: undiluted and 75%	No dermal response observed in the test group or negative control group after challenge. Positive control (HCD) demonstrated the reliability of the test system.	CA 5.2.6/015 Report no 545/42 (1994)

Method, guideline, deviations, if any	Species, strain, sex, no/group	Test substance	Dose levels duration of exposure	Results	Reference
CA 5.2.4/012 supportive due to low purity CA 5.2.5/015 supportive due to low purity CA 5.2.6/016 acceptable					
OECD 406 Buehler method GLP Multiple deviations, e.g. regarding number of animals, exposure time, test concentrations, controls Study not acceptable	Guinea pig (Albino), male, 8 test group, no negative control	Glyphosate (Batch: 36300892; Purity: 97.2 %)	1.2% topical induction (10x) and topical challenge for 30 seconds only	No conclusions are made based on this study due to multiple deviations from the guideline.	CA 5.2.6/016 Report no [redacted]-94-406/G (1994)
OECD 406 GPMT GLP Deviations: batch not reported, no concurrent or historical positive control data Study acceptable but with restrictions	Guinea pig (English), sex unknown, 20 negative control, 20 test group	Glyphosate technical (batch not reported; purity 95% min.)	Intradermal induction: 5%; topical induction: 50%; challenge: 50%	No dermal response observed in the test group or negative control group after challenge. Positive control data are not available to demonstrate the reliability of the test system.	CA 5.2.6/017 Report no [redacted] 1230 (1993)
OECD 406 Buehler method GLP Multiple deviation, e.g. regarding number of animals, unknown purity, control	Guinea pig (Albino Dunkin-Hartley), male and female, 10 negative control, 10 treatment group	MON 8722 (glyphosate sodium salt, Batch: RUD-9108-3241-F; Purity: not reported) Historical control data from animals exposed to dinitrochlorobenzene	100% topical induction (3x) and topical challenge	Dermal reactions (slight patchy erythema; 0.5) in 4/10 treated and 1/10 control animals 24 hours after challenge. Dermal reactions (slight patchy	CA 5.2.6/018 Report no 3044.229 (1992)

Method, guideline, deviations, if any	Species, strain, sex, no/group	Test substance	Dose levels duration of exposure	Results	Reference
animals not treated with vehicle Study not acceptable				erythema; 0.5) in 1/10 treated animals and no control animals 48 hours after challenge. Study results considered equivocal. However, study not acceptable due to multiple deviations.	
OECD 406 GPMT GLP Deviations: e.g. batch and purity not reported; positive HCD slightly older than required by OECD 406 Study acceptable but with restrictions	Guinea pig (Albino Dunkin-Hartley), female, 10 negative control, 20 treatment group	Glyphosate technical (Batch and Purity not reported)	Intradermal induction: 0.1%; topical induction: 50%; challenge: 25%	No dermal response observed in the test group or negative control group after challenge.	CA 5.2.6/019 Report no 349/11 (1991)
OECD 406 GPMT GLP Deviations: e.g. limited data on positive HCD Study acceptable	Guinea pig (Albino Dunkin-Hartley), female, 20 negative control, 20 treatment group	Glyphosate technical (Batch 206-JaK-25-1; Purity not reported but assumed to be 98.6 %, see CA 5.2.1/025)	Intradermal induction: 10%; topical induction: 25%; challenge: 25%	No dermal response observed in the test group or negative control group after challenge.	CA 5.2.6/020 Report no 5887 (1989)
OECD 406 Buehler method GLP Deviations: e.g. number of animals too low, negative	Guinea pig (Albino Hartley), male and female, 10 negative control, 10 treatment group	MON 8750 (Batch: XLH-274; Purity: 95.2% glyphosate as ammonium salt (corresponding to 86.2% glyphosate acid)) Note: it concerns a study with a	100% topical induction (3x) and topical challenge	No dermal response observed in the test group or negative control group after challenge. Positive HCD were reported	CA 5.2.6/021 Report no [redacted]-87-218/4470-87 (1988)

Method, guideline, deviations, if any	Species, strain, sex, no/group	Test substance	Dose levels duration of exposure	Results	Reference
control not treated with vehicle, positive HCD too old Study not acceptable		formulation, not with the active substance		but are too old.	
Similar to OECD 406 Buehler method. Non-GLP Deviations: e.g. number of animals too low, treatment schedule not in line with OECD 406 Study not acceptable	Guinea pig (Albino Hartley), male and female, 10 negative control, 10 positive control, 10 treatment group. In addition 6 per treatment group as irritation control (only treated at challenge)	Glyphosate (Batch: NBP 1782610, Purity: 99.7 %)	100% topical induction (9x) and topical challenge	No dermal response observed in the test group or negative control group after challenge. Positive control group confirmed reliability of the test system.	CA 5.2.6/022 Report no 4235-82 (1983)

Table 37: Summary table of human data on skin sensitisation

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No human data available.				

Table 38: Summary table of other studies relevant for skin sensitisation

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
Public literature *in vitro* study on a transcriptomic- and proteomic-based approach to evaluate the skin sensitization potential of glyphosate Study reliable with restrictions	Glyphosate, batch and purity unknown.	*in vitro* GARD™skin (transcriptome analysis) MUTZ-3-derived cells (human dendritic cells) Exposure to 500 µM glyphosate (non-cytotoxic concentration), DMSO (solvent	The test predicted that glyphosate is not a skin sensitizer. Positive and negative controls were also correctly categorised by the test system. A clear separation between sensitizers (PPD) and non-sensitizers was observed when examining the cellular proteome.	CA 5.2.6/023, Lindberg, 2020

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
		control), water (negative control), or PPD (positive control) for 24 hours		

2.6.2.7.1 Short summary and overall relevance of the provided information on skin sensitisation

There are 22 skin sensitisation studies available of which 16 were concluded to be fully acceptable or acceptable but with restrictions. Of these 16 studies, two were LLNA studies, thirteen were Magnusson & Kligman Guinea Pig Maximisation Tests (GPMT) and one was a Buehler assay. All these studies, except the Buehler assay, concluded that the test substance is not a skin sensitizer. One Buehler study (CA 5.2.6/011; Report no 15279) showed equivocal results which are further discussed in the next paragraphs.

Of the remaining six studies which were either considered supplementary (one study; CA 5.2.6/006) or not acceptable (four studies; CA 5.2.6/007, CA 5.2.6/016, CA 5.2.6/021, CA 5.2.6/022), also one Buehler study showed an equivocal response (CA 5.2.6/018; Report no 3044.229).

Considering the two Buehler assays with an equivocal result (one considered acceptable and one unacceptable), a faint skin reaction was observed in 6/20 and 4/10 animals, respectively, which were scored as 0.5 in all animals. According to OECD 406, animals should be scored with whole numbers only. Therefore, it is debatable whether the severity of the skin reactions should instead be scored as '1'. Therefore, the outcome of both assays is considered equivocal (also in the light of the negative results of the 12 studies which are fully acceptable or acceptable but with restrictions and the negative results in the four supplementary studies).

Overall, the large majority of the studies was negative for skin sensitisation.

2.6.2.7.2 Comparison with the CLP criteria regarding skin sensitisation

According to the CLP Regulation (EU) No. 1272/2008 a substance shall be classified as a skin sensitizer if a significant effect has been obtained in an acceptable *in vivo* test (LLNA, Buehler assay and GPMT). A significant skin sensitising effect is defined as follows (only the relevant assays used for glyphosate are listed):

- LLNA (OECD 429): Stimulation index ≥ 3
- GPMT (OECD 406): Redness (Score ≥ 1) in ≥ 30% of the test animals
- Buehler assay (OECD 406): Redness (Score ≥ 1) in ≥ 15% of the test animals

Glyphosate did not elicit a positive response in any of the skin sensitisation assays, however, in two assays a equivocal result was obtained. Considering that the large majority was negative, no classification for skin sensitisation is warranted.

2.6.2.7.3 Conclusion on classification and labelling for skin sensitisation

No classification according to the CLP Regulation (EU) No. 1272/2008 is warranted.

Not classified – Conclusive but not sufficient for classification.

2.6.2.8 Phototoxicity

No phototoxicity study was performed with glyphosate. The endpoint is not required according to Regulation (EU) No 283/2013 as no UV/VIS maximum was observed at wavelengths of >250 nm and as the ultraviolet/visible molar extinction/absorption coefficient of glyphosate is smaller than 10 L/(mol*cm) at wavelengths of ≥ 290 nm and (refer to Volume 1 section 2.2.1 or Volume 3 CA B.2 (section CA 2.4/002)).

Table 39: Summary table of studies on phototoxicity

Method, guideline, deviations[1] if any	Test substance	Dose levels duration of exposure	Results	Reference
No data available.				

Table 40: Summary table of human data on phototoxicity

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data available.				

Table 41: Summary table of other studies relevant for phototoxicity

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data available.				

2.6.2.9 Aspiration hazard [equivalent to section 10.13 of the CLH report template]

Table 42: Summary table of evidence for aspiration hazard

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data available.				

2.6.2.9.1 Short summary and overall relevance of the provided information on aspiration hazard

No data are available indicating aspiration of glyphosate.

2.6.2.9.2 Comparison with the CLP criteria regarding aspiration hazard

According to the CLP Regulation (EU) No. 1272/2008 classification of aspiration hazard relates to liquids or mixtures only, although the definition of aspiration includes the entry of solids into the respiratory system. Glyphosate as the active ingredient is a solid and therefore aspiration hazard is not applicable in this context. Furthermore, no reliable data are available from humans indicating an aspiration hazard.

2.6.2.9.3 Conclusion on classification and labelling for aspiration hazard

Data lacking; no classification for aspiration hazard is proposed as the substance is a solid.

2.6.2.10 Specific target organ toxicity-single exposure (STOT-SE) [equivalent to section 10.11 of the CLH report template]

Table 43: Summary table of animal studies on STOT SE (specific target organ toxicity-single exposure)

Method, guideline, deviations[1] if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL	Reference
Refer to sections 2.6.2.1 to 2.6.2.6 (acute toxicity studies)			
Refer to section 2.6.7 (acute neurotoxicity study)			

Table 44: Summary table of human data on STOT SE (specific target organ toxicity-single exposure)

Type of data/report	Test substance	Route of exposure Relevant information about the study (as applicable)	Observations	Reference
No appropriate data is available for the active substance. No evidence of organ-specific non-lethal effects (except eye irritation) can be derived from poisoning incidents with formulations.				

Table 45: Summary table of other studies relevant for STOT SE (specific target organ toxicity-single exposure)

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data available				

2.6.2.10.1 Short summary and overall relevance of the provided information on specific target organ toxicity – single exposure (STOT-SE)

Based on the multitude of acute toxicity studies in rats, rabbits and mice (refer to sections 2.6.2.1 to 2.6.2.6), classification of STOT-SE (categories 1 or 2) is not appropriate because non-lethal effects were confined to very high doses and were rather unspecific. Clinical findings, if any, that were repeatedly observed in a few acute oral and inhalation toxicity studies included salivation, piloerection, diarrhoea, decreased activity, ruffled fur, sedation and ataxia. Further signs observed after acute inhalation were loss of hair, hunched posture, ocular/nasal discharge or material around the eyes/nose, increased respiratory rate, decreased respiratory rate, congested or irregular breathing/breathing effects and a slight decrease in body weight. These observations occurred specifically at limit dose levels or above (i.e. dose levels of 2000 or 5000 mg/kg bw). This assessment is further supported by the acute neurotoxicity study in rats (refer to Vol 1 2.6.7 and Vol. 3, B.6.7, study report no. [redacted]/P/4866) in which no evidence of neurotoxicity was observed at dose levels of 500, 1000, and 2000 mg/kg bw even though unspecific clinical signs occurred and one single female animal was found dead at the top dose level. No clinical evidence of single (i.e., first) dose effects was obtained from the many toxicological studies with repeated administration in which lower doses were applied. Suitable haematological and clinical chemistry data is not available since sampling was not performed during the first days of treatment but, taking into account the toxicological profile of glyphosate, alterations in these parameters are not expected.

In addition, two acute oral toxicity studies in goats are available (refer to Vol 1 2.6.8.2 or Vol 3 CA B.6.8.2; study report nos. 80006 and 80007). Severe treatment-related effects, including mortality only occurred at high doses (>3090 mg/kg bw). However, both studies are considered supportively only due several limitations. The studies were not performed according to any guideline and the goat does not represent a relevant species. Therefore these are of limited value for the evaluation for classification for STOT-SE.

Considering human data and STOT-SE, no relevant data is available for the active substance. No evidence of organ-specific non-lethal effects (except eye irritation or general local effects) can be derived from poisoning incidents with formulations.

With regard to category 3, no evidence of narcotic effects was obtained in any toxicological study.

Respiratory tract irritation might be expected based on the eye irritating potential of glyphosate. In one acute inhalation study, nasal irritation was observed in many rats (refer to Vol 1 2.6.3.2; report no [redacted]877.AIN). However, this study was not considered acceptable due to the too low exposure concentration (0.644 mg/L air) and due the inconsistency of the results compared to the other studies, casting doubt on the validity of this study. In the other acute inhalation toxicity studies (refer to Vol 1 2.6.3.2), no pathological findings were reported in the respiratory

tract. In the current CLP guidance, it is stated that evaluation, in the absence of validated animal tests, will be based primarily on human data.

In humans, there is no evidence for respiratory tract irritation by the active substance. However, it should be acknowledged that such an exposure will seldomly occur. During the previous assessment, it was noted that for formulations, Burger *et al.* (2009, refer to Volume 1 2.6.9) reported cases from Germany that might indicate respiratory irritation but these findings were considered to be likely due to POEA surfactants (tallowamines) present in the formulation. **The RMS notes that this study was not re-submitted for the present evaluation. For the process under Regulation (EC) No 1107/2009, the applicant is requested to submit the study and an evaluation. For the process under the Regulation (EC) No 1272/2008, the applicant is asked to submit the missing information during the public consultation period**.

Overall, there is no sufficient evidence to classify glyphosate for respiratory tract irritation. It should be taken into account that glyphosate is classified and labelled as a substance which causes serious eye damage and, thus, irritating properties are already adequately covered.

2.6.2.10.2 Comparison with the CLP criteria regarding STOT-SE (specific target organ toxicity-single exposure)

Generally, the STOT-SE concerns effects elicited by a substance at non-lethal doses.

A classification for STOT-SE category 1 is warranted if:

- Reliable and good quality evidence from human cases or epidemiological studies; or
- Observations from appropriate studies in experimental animals in which significant and/or severe toxic effects of relevance to human health were produced at generally low exposure concentrations.

A classification for STOT-SE category 2 is warranted if:

- Studies with experimental animals produced significant toxic effects, of relevance to human health, at generally moderate exposure concentrations.

A classification for STOT-SE category 3 is warranted if criteria for narcotic effects and respiratory irritation are fulfilled:

Respiratory irritation:

- Respiratory irritant effects are observed in humans that impair function with symptoms such as cough, pain, choking and breathing difficulties.
- Subjective human observations could be supported by objective measurements of clear respiratory tract irritation (such as biomarkers of inflammation in nasal or broncho-alveolar lavage fluids)

No animal studies are currently available covering respiratory irritation, but animal studies may provide useful information in terms of clinical sign of toxicity (dyspnoea, rhinitis etc.) and histopathology (e.g. hyperaemia, oedema, minimal inflammation, and thickened mucous layer) which are reversible and may be reflective of the characteristic clinical symptoms described above.

Narcotic effects:

- Central nervous system depression including narcotic effects in humans such as drowsiness, narcosis, reduced alertness, loss of reflexes, lack of coordination, and vertigo. Effects can also be manifested as severe headache or nausea and can lead to reduced judgement, dizziness, irritability, fatigue, impaired memory function, deficits in perception and coordination, reaction time or sleepiness.
- Narcotic effects observed in animal studies may include lethargy, lack of coordination, loss of righting reflex, and ataxia. If these effects are not transient in nature, then they shall be considered to support classification for Category 1 or 2 STOT-SE.

For glyphosate, no human information is available to derive a classification for STOT-SE. Observations from animal studies as mentioned above, occurred specifically at limit dose levels or above and were not consistent

among the studies suggesting that the findings were unspecific reactions to glyphosate administration. There is no evidence for specific target organ toxicity following a single exposure to glyphosate.

In addition, no narcotic effects were observed in any of the performed studies and there is no indication for respiratory tract irritation from the acute inhalation studies (no specific studies for respiratory irritation are available).

2.6.2.10.3 Conclusion on classification and labelling for STOT-SE (specific target organ toxicity-single exposure)

Based on the effects observed in the available acute toxicity studies, no classification for STOT-SE category 1 or 2 is warranted as neither significant nor severe toxic effects were observed at non-lethal doses attributed to the acute exposure to glyphosate.
Further, there is no evidence for narcotic effects or respiratory irritation and therefore no classification for STOT-SE category 3 is warranted.
In conclusion, according to the CLP Regulation (EU) No. 1272/2008, no classification for STOT-SE is needed for glyphosate

2.6.3 Summary of repeated dose toxicity (short-term and long-term toxicity) [section 10.12 of the CLH report]

2.6.3.1 Specific target organ toxicity-repeated exposure (STOT RE) [equivalent to section 10.12 of the CLH report template]

Table 46: Summary table of animal studies on repeated dose toxicity (short-term and long-term toxicity) STOT RE (specific target organ toxicity - repeated exposure)

Method, guideline, deviations[1] if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results - adverse effects - NOAEL/LOAEL	Data points Report number (year)
Oral exposure – 28-day rat/mouse/dog			
OECD 407 (1981) GLP Rat, Wistar, male and female, 5/sex/dose Deviations: The organ weight measurements and the histopathological investigation did not include all required organs. Reticulocyte count, platelet count, urea, total cholesterol parameters were not measured. Thyroid hormone levels were not determined. Acceptable but with restrictions	Glyphosate technical, FSG 03090 H/05 March 1990 / Batch No. 60, purity 96.8% 28-day, dietary dose Doses of 0, 200, 2000, or 20000 ppm (equivalent to 0, 17.6, 178.5 or 1894.9 mg/kg bw/day in males and 21.6, 223.3 or 2250.8 mg/kg/day in females)	200, 2000 and 20000 ppm: No adverse effects observed NOAEL: 20000 ppm (highest dose tested)	CA 5.3.1/001 CA 5.3.1/002 CA 5.3.1/003 [redacted].881.28 DDR (1991)

OECD 407 (1981) GLP Rat, Sprague-Dawley, male and female, 5/sex/dose Deviations: Dose levels exceed the 1000 mg/kg bw/day limit dose. Reticulocytes, platelet count, total cholesterol, urea and bile acids not assessed. Urinalysis not performed. Only liver, kidneys, adrenals, testes, epididymides were weighed. Only liver, heart, kidneys (in males), spleen and adrenals from the control and high dose group and kidney from all dose groups in females were examined histopathologically. Thyroid hormone levels were not determined. Acceptable but with restrictions	Glyphosate technical, 161-JRJ-131-2, purity 99.5% 28-day, dietary dose Doses of 0, 50, 250, 1000 and 2500 mg/kg bw/day	50, 250 and 1000 mg/kg bw/day: No adverse effects observed 2500 mg/kg bw/day: Decreased body weight gain in females (-11%), increased alkaline phosphatase in males (+60%) and females (+42%), increased bilirubin in females (+63%) and soft stool in males (3/5) An increased incidence of very mild and mild nephrocalcinosis in females was noted at 250, 1000 and 2500 mg/kg bw/day (2/5; 2/5; 4/5), however, this was considered of unknown toxicological relevance NOAEL: 1000 mg/kg bw/day LOAEL: 2500 mg/kg bw/day	CA 5.3.1/004 5626 (1991)

No guideline reported; in general compliance to OECD 407 (1981) GLP Rat, Sprague-Dawley, male and female, 5/sex/dose Deviations: Designed as a dose-range finding study with limited parameters included (no haematology/clinical chemistry, no organ weights or histopathology). Thyroid hormone levels were not determined. Acceptable but with restrictions	Glyphosate, batch XLI-203, purity 97.67% 28-day dietary dose-range finding study Doses of 0, 30000, 40000 or 50000 ppm (equivalent to 0, 1921.1, 2634.1 or 3278.1 mg/kg bw/day for males and 0, 2310.6, 3256.4 or 4150.2 mg/kg bw/day for females)	<u>30000, 40000 and 50000 ppm</u>: Reduced body weight gain (-22.9% at 30000 ppm, -20.8% at 40000 ppm and -15.4% at 50000 ppm in males and -15.4% at 30000 ppm, -17.8% at 40000 ppm and 12.6% at 50000 ppm in females), reduced food consumption (in males -17% at 40000 ppm and -28% at 50000 ppm from Day 1-8 and in females -14% at 40000 ppm and -9% at 50000 ppm from Day 1-8)), soft stool (5/5 males and 4/5 females at 30000 ppm, 5/5 males and 5/5 females at 40000 ppm and 4/5 males and 5/5 females at 50000 ppm) and diarrhoea (4/5 males and 5/5 females at 40000 ppm and 5/5 males and 5/5 females at 50000 ppm) No NOAEL derived (study with limited parameters; adverse effects at all dose levels)	CA 5.3.1/005 [redacted]-8921 (1989)
Non-guideline Non-GLP Mouse, CD-1, male and female, 5/sex/dose Deviations: No guideline followed, similar to OECD 407 (1981). No sensory reactivity was investigated; no haematology or clinical chemistry was performed; organ weights were not determined; histopathology was not performed. Acceptable but with restrictions (as dose-range finding study)	Glyphosate, XHI-162, purity 83% 28-day dietary dose-range finding study Dose levels of 0, 100, 300 or 1000 mg/kg bw/day (appr. 0, 80, 235 or 800 mg/kg bw/day actual achieved dose, after correction for purity)	No adverse effects at any dose level No NOAEL derived due to limited reporting.	CA 5.3.1/006 77-2110 (1978)

Non-guideline GLP Beagle dog, male and female, 1/sex Deviations: Designed as a non-guideline oral maximum tolerated dose study with only one dog per sex per dosing regime and limited parameters included in the study. No control animals were included in the study. Unacceptable	Glyphosate, 161-JRJ-131-2, purity 99.5% Dose-range finding study; gelatin capsule administration 7-day escalating dose: 1 male and 1 female at 100 (week 1), 300 (week 2), and 1000 mg/kg bw/d (week 3) 14-day: 1 male and 1 female at 1000 mg/kg bw/day	No adverse effects up to 1000 mg/kg bw/day No NOAEL derived as study is not considered acceptable.	CA 5.3.1/001 5660 (1989)
Non-guideline Non-GLP (pre-GLP) Beagle dog, 5 males and 4 females in total Deviations: Study is designed as a non-guideline dose-range finding study with few animals and limited parameters investigated Acceptable but with restrictions (as a dose-range finding study)	MON 0139 (Isopropylamine salt of glyphosate); LURT-12011 (MON 0139); purity 62.49% or Isopropylamine; Luling 2-81; purity 99.7% Dose-range finding study, gavage or gelatin capsule administration Dosages: - 312.5 (five daily doses), 625 (single or as five daily doses), 1250 (single) or 2500 (single dose) mg/kg bw/day for MON 0139 in 1 or 2 dogs/sex. - 72 mg/kg bw/day (single dose, 1 dog/sex) or 19.43 mg/kg bw/day (5 daily doses in 1 male) for isopropylamine	<u>Isopropylamine salt of glyphosate at all dose levels</u>: Mild body weight loss and reduced food consumption, diarrhoea and emesis (not at the lowest dose). <u>Isopropylamine:</u> emesis, bloody emesis and loose stools. A single dose of 72 mg/kg bw/day resulted in severe oedema, haemorrhage, and necrosis of the rugae in the stomachs (both dogs were sacrificed in extremis at 30 min after dosing). Five day treatment at 19.43 mg/kg bw/day resulted in mucosal erosions of the stomach and oesophagus. No NOAEL was derived from this study.	CA 5.3.1/008 [redacted]-2155 (1982)

Non-guideline Non-GLP (literature study) Mouse, ICR, six mice per group Deviations: Investigative study which only included observation of body weight, liver and kidney weight, kidney histology and several other kidney parameters. Reliable with restrictions	Glyphosate, purity 96% *In vivo:* 28-day repeated oral dose by gavage Dose levels: 0 or 400 mg/kg bw/day In vitro: 20 to 100 µM mono-isopropylamine salt	<u>400 mg/kg bw/day</u>: Exfoliation of renal tubular cells, upregulation of apoptosis and NMDAR1 exposure in the proximal tubule epithelium, imbalance of oxidant/antioxidant balance, a transient increase in urine albumin and urinary β2-microglobulin	CA 5.3.1/009 Gao, 2019
Non-guideline Non-GLP (literature study) Rat, Sprague-Dawley, eight males per group Deviations: The study is a non-guideline and non-GLP investigative study. The purity of the test substance was not provided, only 8 instead of 10 animals were treated per dose group and only males were treated. Supportive	Glyphosate, purity not reported 35-day repeated oral exposure by gavage Dose levels: 5, 50, and 500 mg/kg bw/day	<u>5 mg/kg bw/day</u>: No adverse effects <u>50 and 500 mg/kg bw/day</u>: reduced body weight gain at both dose levels (-43% at 50 and 500 mg/kg bw/d); decreased spleen weight (abs/rel) at 500 mg/kg bw/day (-23% abs/ -24% rel); signs of oxidative stress, upregulation of liver inflammatory genes and upregulation of genes related to lipid metabolism were noted at both dose levels, but effects were mainly slight and/or clinical relevance of these findings is lacking.	CA 5.3.1/0011 Tang *et al.*, 2017
Oral exposure – 90-day rat			

OECD 408 (1998) GLP Rat, Alpk:AP (now known as Alpk:APfSD) Wistar-derived, 12/dose/sex Deviations: No pre-dose ophthalmology, no reticulocyte count, T3, T4, TSH, less blood clinical chemistry parameters evaluated (no sodium, potassium, HDL, LDL, blood urea nitrogen and creatinine evaluated) thyroids, epididymis, prostrate, uterus, ovaries, thymus, spleen and pituitary gland not weighed, part of the tissues were stored and no histopathology was performed (eyes, Harderian gland, larynx, nasal cavity, mouth, prostrate, seminal vesicles, skin and voluntary muscle), vaginal smears not taken; sensory reactivity to different stimuli was not evaluated. Deviations mainly due to older version of the OECD test guideline 408 (1998). Acceptable	Glyphosate acid, batch P15, purity 97.4% 90-day repeated dietary oral dose Dose levels: 0, 1000, 5000 or 20000 ppm (equivalent to 0, 81.33, 413.5, or 1612 mg/kg bw/day in males and 0, 90.42, 446.9 or 1821 mg/kg bw/day in females).	1000 and 5000 ppm: No adverse effects observed. 20000 ppm: Decreased body weight gain in males (-11%) and increased alkaline phosphatase in males (+45%) and females (+54%). NOAEL: 5000 ppm LOAEL: 20000 ppm	CA 5.3.2/001 CA 5.3.2/002 [redacted]/P/1599 (1996)

OECD 408 (1998) GLP Rat, Sprague-Dawley (CD), 10/dose/sex Deviations: Reticulocytes not counted; cholesterol not measured, no blood hormones (T3, T4 and TSH) measured; thymus, uterus, epididymis, prostate and seminal vesicles not weighed with testes; epididymis, coagulating glands not examined microscopically and spinal cord only examined at one level; vaginal smears not taken; sensory reactivity to different stimuli was not evaluated. Deviations mainly due to older version of the OECD test guideline 408 (1998). Acceptable	Technical glyphosate, batch H95D 161 A, purity 95.3% 90-day repeated dietary oral dose Dose levels: 0, 1000, 10000 or 50000 ppm (equivalent to 0, 79, 730 or 3706 mg/kg bw/day for males and 0, 90, 844, or 4188 mg/kg bw/day for females)	1000 ppm: No adverse effects observed. 10000 ppm: Increased alkaline phosphatase in females (+77%) Caecum atrophy in 1/10 male and 2/10 females. 50000 ppm: Soft faeces and diarrhoea in both sexes (10/10), decreased body weight in both sexes (Day 90 -26% in males and -11% in females, not significant), decreased food consumption in males, increased alkaline phosphatase (males +60%; females +56%), slight effects in other blood chemistry parameters, increased relative kidney weight in both sexes (+19% and +10% in males and females, respectively), effect on the caecum (enlarged and filled with fluid in 10/10 animals of both sexes and atrophy in 5/10 animals of both sexes). NOAEL: 1000 ppm LOAEL: 10000 ppm	CA 5.3.2/003 434/016 (1996)

OECD 408 (1998) GLP Rat, Sprague-Dawley (Crj:CD), 12/dose/sex Deviations: Reticulocytes not counted, clotting not evaluated, total cholesterol but not HDL and LDL measured, urea not measured, no blood hormones (T3, T4 and TSH) measured; organ weights limited to brain, liver, kidneys, testes, adrenals and cecum; vaginal smears not taken; sensory reactivity to different stimuli was not evaluated. Deviations from the current version of OECD 408 (2018) are mainly due to older version of the OECD test guideline 408. In addition, three different batches of the test compound with a different purity were used. Acceptable	HR-001 (glyphosate technical), Batches: 940908-1 (95.68% purity), 941209 (95.0% purity) and T-941209 (97.56% purity) 941209 T-941209 13-week repeated dietary oral dose Dose levels: 0, 3000, 10000 or 30000 ppm (equivalent to 0, 168.4, 569 or 1735 mg/kg bw/day for males and 0, 195.2, 637 or 1892 mg/kg bw/day for females)	3000 ppm: No adverse effects observed. 10000 ppm: Distension of the caecum in males (3/12) and increased absolute/relative weight of caecum (+20/16% in males and +50/54% in females). 30000 ppm: Decreased body weight in males (up to -10%), decreased food consumption in week 1 in males (-9%) and females (-14%), increased alkaline phosphatase in females (+82%), distension (9/12 in males and 7/12 in females) and increased absolute/relative weight of the caecum (+106/122% in males and +123/143% in females), increased relative liver weight in females (+19%). NOAEL: 3000 ppm LOAEL: 10000 ppm	CA 5.3.2/004 [redacted] 94-0138 (1995)

OECD 408 (1981) GLP Rat, Sprague-Dawley (Crl:CD®BR VAF/Plus®), 10/dose/sex Deviations: Reticulocytes not counted; blood clotting not evaluated; total cholesterol but not HDL and LDL, T3, T4 and TSH evaluated; no blood hormones measured; prostate, uterus, thymus, pituitary, thyroids not weighed; seminal vesicles and coagulating glands were not examined microscopically; vaginal smears not taken; sensory reactivity to different stimuli was not evaluated. Deviations from the current version of OECD 408 (2018) mainly due to older (1981) version of the OECD test guideline 408. Acceptable	Glyphosate acid, Batch 46540992, purity 97.5% 13-week repeated dietary oral dose Dose levels: 0, 2000, 6000 or 20000 ppm (equivalent to 0, 125.2, 371.9 or 1262.1 mg/kg bw/day for males and 0, 156.3, 481.2 or 1686.5 mg/kg bw/day for females)	2000 and 6000 ppm: No adverse effects observed. 20000 ppm: Diarrhoea in both sexes (10/10 and 9/10 in males and females, respectively), blood in urine (10.5 and 3 times higher in males and females, respectively, compared to the control), decreased body weight gain in both sexes at day 50 and 85 (-51% and -85% in males; -71% and -54% in females), decreased absolute and relative adrenal weights in males (-26% and -21%), increased absolute and relative spleen weight in females (+18% and +24%). NOAEL: 6000 ppm LOAEL: 20000 ppm	CA 5.3.2/005 CA 5.3.2/006 CA 5.3.2/007 011-0001 (1993)

OECD 408 (1981) GLP Rat, Wistar, 10/dose/sex Deviations: The following organs were not noted in the gross pathology and histopathological evaluation: aorta, cervix, epididymides, mammary gland, peripheral nerve, prostate, skeletal muscle and bone, skin, spinal cord, thymus, vagina. The following organs were not weighed: testes, epididymides, prostate and seminal vesicles with coagulating glands, thymus, heart, brain, and spleen. Thyroid hormone levels (i.e., T4, T3, and TSH) were not measured. No ophthalmological examination and urinary analysis were conducted. Vaginal smears not taken. Sensory reactivity to different stimuli was not evaluated. The rationale for dose selection was not provided. Acceptable but with restrictions	Glyphosate technical, Batch FSG 03090 H/05 March 1990, purity 96.8% 90-day repeated dietary oral dose Dose levels: 0, 200, 2000, or 20000 ppm (equivalent to 0, 14.0, 147.3, or 1358.6 mg/kg bw/day for males and 0, 18.6, 195.7, or 2012.4 mg/kg bw/day for females)	200 and 2000 ppm: No adverse effects observed. 20000 ppm: Decreased body weight in females (up to -13% (week 10)), increased alkaline phosphatase in males (+50%) and increased blood glucose in females (+24%). NOAEL: 2000 ppm LOAEL: 20000 ppm	CA 5.3.2/008 CA 5.3.2/009 CA 5.3.2/010 [redacted].882.90 OR (1992)

OECD 408 (1981) GLP Rat, Sprague-Dawley, 10/dose/sex Deviations: Haematology was performed without determining reticulocyte count; clinical chemistry was performed without determining HDL, LDL, T4, T3 and TSH; organ weights of the thyroid gland was not determined; histopathology was performed without bone/bone marrow, cervix, coagulating glands, spinal cord and vagina. Vaginal smears not taken. Sensory reactivity to different stimuli was not evaluated. Deviations from the current version of OECD 408 (2018) mainly due to older version of the OECD test guideline 408. Acceptable	Glyphosate technical, Batch 206-Jak-25-1, purity 98.6% 13-week repeated dietary oral dose Dose levels: 0, 30, 300 or 1000 mg/kg bw/day.	<u>30 mg/kg bw/day:</u> The increased incidence of parotid cellular alteration was statistically significant, but only for females. The incidence was 70% in males (compared to 30% in control) and 80% in females (compared to 20% in control) and the severity grade of findings was minor (mostly very mild). <u>300 mg/kg bw/day:</u> Statistically significant increased incidence of parotid cellular alteration was observed in both sexes. The incidence was the same as for the high dose animals, but the severity grade was lower when compared to the high dose group. At 300 mg/kg bw/day the severity grade of finding in animals was very mild to mild (except for a single male animal which showed moderate cellular alteration). <u>1000 mg/kg bw/day:</u> Increased blood glucose in females (+11%), decreased urinary pH in both sexes (-12%), statistically significant increased incidence of parotid cellular alterations in the salivary gland of both sexes at 1000 mg/kg bw/day. The finding was described by study author as deep basophilic staining and enlargement of cytoplasm. The incidence of this finding was 100% for males (compared to 30% in control) and 90% for females (compared to 20% in control). The severity grade of finding was minimal to severe in males, and minimal to moderate in females. LOAEL: 30 mg/kg bw/day NOAEL: < 30 mg/kg bw/day	CA 5.3.2/011 7136 (1991)

OECD 408 (1981) GLP Rat, CD, 10/dose/sex Deviations: No sensory reactivity was examined; haematology was performed without determining reticulocyte count; clinical chemistry was performed without determining cholesterol, HDL, LDL, blood urea nitrogen, T4, T3 and TSH; organ weights of the brain, epididymides, heart, ovaries, pituitary gland, prostate (seminal vesicles and coagulating glands), spleen, thyroid gland, thymus and the uterus were not determined; histopathology was performed without coagulating glands and vagina. No rationale for target dose selection is provided. Deviations from the current version of OECD 408 (2018) mainly due to an older version of the OECD test guideline 408. Uncertainties regarding the exact achieved dose levels in the study. Unacceptable	Glyphosate technical, Batch 0190 A, purity 98.1% 90-day repeated dietary oral dose Dose levels: 0, 2000, 5000 and 7500 ppm (equivalent to 0, 129.1, 320.7 or 482.1 mg/kg bw/day for males and 0, 174.3, 441.6 or 647.3 mg/kg bw/day for females)	2000 and 5000 ppm: No adverse effects observed. 7500 ppm: Decreased food consumption in males and females, increased blood glucose in males. No NOAEL proposed as study is not considered acceptable.	CA 5.3.2/012 [redacted]-900914 (1990)

OECD 408 (1981) GLP Rat, CD, 10/dose/sex in low, intermediate low, intermediate high and high dose group, 20/sex in the control group Deviations: Haematology was performed without determining reticulocyte count; clinical chemistry was performed without determining cholesterol, HDL, LDL, T4, T3 and TSH. Organ weight of the brain, epididymides, heart, ovaries, prostate with seminal vesicles, spleen, thyroid, thymus, pituitary gland and uterus was not determined; histopathology was performed without bone/bone marrow, coagulating glands, gross lesions, lymph nodes, male mammary glands, seminal vesicles and vagina. No rationale for target dose selection is provided. Deviations from the current version of OECD 408 (2018) mainly due to an older version of the OECD test guideline 408 (1981). Acceptable but with restrictions	Glyphosate technical, Batch L1656, purity 97.1% 90-day repeated dietary oral dose Dose levels: 0, 2000, 3000, 5000, or 7500 ppm (equivalent to 0, 100, 150, 250 or 375 mg/kg bw/day for males and females)	<u>All dose levels</u>: No adverse effects observed. NOAEL: 7500 ppm (highest dose tested)	CA 5.3.2/013 [redacted]891002 (1989)

OECD 408 (1981) GLP Rat, Sprague-Dawley, 12/dose/sex Deviations: Clinical signs were not recorded daily; no sensory reactivity was examined; haematology was performed without determining prothrombin time; clinical chemistry was performed without determining HDL, LDL, T4, T3 and TSH; organ weights of the adrenals, brain, heart, ovaries, pituitary gland, prostate (seminal vesicles and coagulating glands), spleen, thyroid gland, thymus and the uterus were not determined; histopathology was performed without coagulating glands and vagina. Deviations from the current version of OECD 408 (2018) mainly due to an older version of the OECD test guideline 408. Acceptable	Glyphosate, Batch Lot XLG 161, purity 95.21% 90-day repeated dietary oral dose Dose levels: achieved dose levels of 0, 950, 4600 and 19000 ppm (equivalent to 0, 63, 317 or 1267 mg/kg bw/day for males and 0, 84, 404 or 1623 mg/kg bw/day for females)	<u>All dose levels</u>: No adverse effects observed. NOAEL: 19000 ppm (highest dose tested)	CA 5.3.2/014 [redacted]-7375 (1987)

Similar to OECD 408 (1981) GLP status not reported Rat, Wistar, 10/dose/sex Deviations: The study is considered unacceptable due to serious reporting deficiencies, e.g. the year when the study was performed not stated, no information on the guideline followed and on GLP status, statistical analysis of the results was not reported and purity and batch number of the test substance was not reported. Unacceptable	Glyphosate (batch and purity not reported) 90-day repeated oral dose (by gavage) Dose levels: 0 (control group receiving the vehicle, i.e. 0.1% Tween 80 in water), 300, 1200 or 2400 mg/kg bw/day	300 and 1200 mg/kg bw/day: No adverse effects observed 2400 mg/kg bw/day: Lower body weight gain and food intake No NOAEL is proposed as the study is not considered acceptable.	CA 5.3.2/015 Report number not reported, 1985 (refer to CA 5.3.2/015)
Similar to OECD 408 (1981) GLP not compulsory at time of study Rat, Wistar, 10/dose/sex Deviations: The study is considered unacceptable due to serious reporting deficiencies, measurement of mean daily intake of the test substance was not performed, when the study was performed GLP was not compulsory. Unacceptable	Glyphosate, purity 96.8% 90-day repeated dietary oral dose Dose levels: 0, 1000, 3000 or 10000 ppm (equivalent to 0, 102.0, 284.0 and 1103.7 mg/kg bw/day in males and 105.4, 376.8 and 1310.8 mg/kg bw/day in females).	1000 ppm: No adverse effects observed 3000 ppm: Reduced red blood cell count, increase in leucocyte and platelet count. 10000 ppm: Increased blood glucose in females, increased alkaline phosphatase, AST and ALT activity in both sexes, increased liver weights in both sexes No NOAEL is proposed as the study is not considered acceptable.	CA 5.3.2/016 Report number not reported, 1981 (refer to CA 5.3.2/016)
Oral exposure – 90-day mouse			

OECD 408 (1981) GLP Mouse, SPF ICR (Crj:CD-1), 12/dose/sex Deviations: Reticulocytes not counted, clotting not evaluated, total cholesterol but not HDL and LDL measured, urea not measured, no blood hormones (T3, T4 and TSH) measured; organ weights limited to brain, liver, kidneys, testes, adrenals and caecum; vaginal smears not taken; sensory reactivity to different stimuli was not evaluated. Deviations from the current version of OECD 408 (2018) mainly due to an older version of the OECD test guideline 408. Further, it should be noted that the highest dose tested (~6000-7000 mg/kg bw/day) is far above the limit dose of 1000 mg/kg bw/day according to OECD 408. Acceptable	HR-001 (glyphosate technical), Batch T-941209, purity 97.56% 90-day repeated dietary oral dose Dose levels: 0, 5000, 10000 or 50000 ppm (equivalent to 0, 600.2, 1221 or 6295 mg/kg bw/day for males and 0, 765.0, 1486 or 7435 mg/kg bw/day for females).	<u>5000 and 10000 ppm:</u> No adverse effects observed <u>50000 ppm:</u> Decreased food consumption in first week in males (-28%), increased alkaline phosphatase in both sexes (+84% in males, +50% in females), increased blood phosphorus in females (+28%), increased creatinine phosphokinase in females (~9.4 times higher), decreased urinary pH (not considered adverse), distension of the caecum (12/12 and 10/12 in males and females; 0/12 in males and females from the control group) and increased absolute/relative caecum weight in both sexes (+138/163% in males and +87/95% in females), and an increased incidence of cystitis in the urinary bladder in males (4/12 cf. 0/12 in control group). NOAEL: 10000 ppm LOAEL: 50000 ppm	CA 5.3.2/017 [redacted] 94-0136 (1995)

OECD 408 (1981) GLP Mouse, CD-1, 10/dose/sex Deviations: Ophthalmoscopy and detailed clinical observations were not performed prior to dosing. Sensory reactivity to stimuli was not performed towards the end of exposure period. Reticulocyte count, platelet count and a measure of blood clotting time/potential was not measured during the haematological examinations. Clinical biochemistry determination did not include the following parameters: HDL, LDL and urea. Serum total T4, T3 and TSH were not measured at study termination. At necropsy, the oestrus cycle of all females was not determined. Organ weight of the thyroid gland was not determined; histopathology was performed without bone/bone marrow, cervix, coagulating glands, spinal cord and vagina. Deviations from the current version of OECD 408 (2018) mainly due to an older version of the OECD test guideline 408. Further, it should be noted that the highest dose tested (~4500 mg/kg bw/day) is far above the limit dose of 1000 mg/kg bw/day according to OECD 408. Acceptable but with restrictions	Glyphosate, Batch 161-JRJ-131-2 (purity 99.5%) and 003-89-A (purity 98.0%) 13-week repeated dietary oral dose Dose levels: 0, 200, 1000 or 4500 mg/kg bw/day.	<u>All dose levels</u>: No adverse effects observed. However, evaluation of clinical chemistry parameters was of limited value since a number of parameters could not be analysed or only be performed on a low number of animals due to low sample volumes. NOAEL: 4500 mg/kg bw/day (of limited value)	CA 5.3.2/018 7024 (1991)

Similar to OECD 408 (1981) Non-GLP (pre-GLP) Mouse, CD-1, 15/dose/sex (10/dose/sex for histopathology) Deviations: No sensory reactivity was investigated; no ophthalmoscopy was performed; no haematology or clinical chemistry was performed; organ weights of the adrenals, epididymides, prostate and seminal vesicles and coagulating glands, pituitary gland, thyroid, thymus and uterus were not determined; histopathology was performed without aorta, coagulating glands, mammary glands, seminal vesicles, skin, trachea and vagina. Acceptable but with restrictions	Glyphosate, batch XHJ-64, purity 98.7% 13-week repeated dietary oral dose Dose levels: 0, 5000, 10000 or 50000 ppm (equivalent to 0, 944.1, 1867.2 or 9707.0 mg/kg bw/day for males and 0, 1527.7, 2734.7 or 14858.2 mg/kg bw/day for females)	<u>5000 and 10000 ppm</u>: No adverse effects. <u>50000 ppm:</u> Decreased body weight (up to -10% in both sexes) and body weight gain (week 0-13: -24% in males and -18% in females; not significant). NOAEL: 10000 ppm LOAEL: 50000 ppm NOAEL and LOAEL of limited value as no haematology or clinical chemistry investigations was performed.	CA 5.3.2/019 77-2111 (1979)
Oral exposure – 90-day / 1-year dog			

OECD 409 (1998) GLP Dog, Beagle, 4/dose/sex Acceptable, no deviations	Glyphosate technical, Batch H05H016A, purity 95.7% 13-week repeated oral dose, gelatine capsule Dose levels: 0, 30, 300 or 1000 mg/kg bw/day	<u>30 and 300 mg/kg bw/day</u>: No adverse effects <u>1000 mg/kg bw/day</u>: Clinical signs (liquid/soft faeces, dehydration, vomiting; incidence varying between one animal and all animals); early sacrifice of two moribund animals and termination of high dose groups after 11 weeks for humane reason; decreased final body weight (-22% in males and -19% in females after 11 weeks), decreased body weight gain in males (+0.5 kg vs. +2.3 kg in controls), body weight loss in females (-0.5 kg vs +1.0 kg in controls); reduced food consumption in both sexes (25-75% reduced); clinical chemistry alterations (between -17% and +321% regarding blood chemistry and depending on the effect, see Volume 3) and urine parameters alterations (decrease in mean specific gravity in 1/3 males and 3/3 females; increase in mean urinary volume accompanied by less marked colour of urine in 3/3 females), prostate atrophy (2/3 males vs 0/4 in the control group) and uterus atrophy (3/3 females vs. 0/4 in the control group); histological lesions in many organs (such as kidney liver, bone marrow). NOAEL: 300 mg/kg bw/day LOAEL: 1000 mg/kg bw/day	CA 5.3.2/020 29646 [redacted] (2007)

OECD 409 (1981) GLP Dog, Beagle, 4/dose/sex Deviations: Detailed clinical observations only performed monthly, not weekly. Urinalysis only performed at study termination. Several organ weights missing: epididymides, ovaries, uterus, thymus, spleen, brain, heart; several organs were not sampled: gross lesions, spinal cord, eyes with optic nerve, trachea, skin, mammary gland, prostate or other accessory sex organs. Deviations from the current version of OECD 409 (1998) mainly due an older version of the OECD test guideline 409. Acceptable	Glyphosate technical, Batch (expiry dates) 01.12.1997 & 01.06.1997, purity > 95% 13-week repeated dietary oral dose Dose levels: 0, 200, 2000 or 10000 ppm (equivalent to 5.2, 54.2 or 252.4 mg/kg bw/day in males and 5.4, 52.8 and 252.7 mg/kg bw/day in females)	<u>200 and 2000 ppm</u>: No adverse effects <u>10000 ppm:</u> Decreased food consumption in week 2 in both sexes (-47% in males and -37% in females), increased GGT (+171% in males and +91% in females after 45 days), alkaline phosphatase (+129% in males after 45 days), and bilirubin (+98% in males and +79% in females after 90 days). NOAEL: 2000 ppm LOAEL: 10000 ppm	CA 5.3.2/021 CA 5.3.2/022 CA 5.3.2/023 CA 5.3.2/024 1816 (1999)
OECD 409 (1981) GLP Dog, Beagle, 4/dose/sex Heart, thymus, spleen and uterus were not weighed; microscopic examination of spinal cord was performed only at lumbar level. Deviations from the current version of OECD 409 (1998) are mainly due to an older version of the OECD test guideline 409. Acceptable	Glyphosate acid, Batch D4490/1, P18, purity 99.1% 13-week repeated dietary oral dose Dose levels: 0, 2000, 10000 or 50000 ppm (equivalent to 0, 68, 323 or 1680 mg/kg bw/day for males and 0, 68, 334 or 1750 mg/kg bw/day for females)	<u>2000 ppm and 10000 ppm</u>: No adverse effects. <u>50000 ppm</u>: Decreased body weight gain in males (between -18% and -35%) and females (between -8% and -41%), decreased plasma calcium in males (-4% to -7%). NOAEL: 10000 ppm LOAEL: 50000 ppm	CA 5.3.2/025 CA 5.3.2/026 [redacted]/P/1802 (1996)

OECD 409 (1981) GLP Dog, Beagle, 4/dose/sex Deviations: Reticulocytes not counted, clotting not evaluated; blood chloride, sodium and potassium not measured; uterus and thymus not weighed. Deviations from the current version of OECD 409 (1998) mainly due to an older version of the OECD test guideline 409. Acceptable	HR-001 (glyphosate technical), Batch T-950308, purity 94.61% 13-week repeated dietary oral dose Dose levels: 0, 1600, 8000 or 40000 ppm (equivalent to 0, 39.7, 198 or 1015 mg/kg bw/day for males and 0, 39.8, 201 or 1014 mg/kg bw/day for females)	1600, 8000 or 40000 ppm: No adverse effects. NOAEL: 40000 ppm (highest dose tested)	CA 5.3.2/027 [redacted] 94-0158 (1996)
Guideline not stated GLP status not reported Dog, Mongrel, 3/sex/dose Deviations: The study was considered invalid due to serious reporting deficiencies, e.g., absence of information on batch and purity of the test material. Unacceptable	Glyphosate (batch and purity not reported) 90-day repeated oral dietary dose Dose levels: 0, 100, 250 or 500 mg/kg bw/day	100 and 250 mg/kg bw/day: No adverse effects 500 mg/kg bw/day: Reduced body weight gain and food consumption in both sexes. Increased absolute and relative liver weight in males. No NOAEL is proposed as the study is not considered acceptable.	CA 5.3.2/028 Report number not reported, 1985 (refer to CA 5.3.2/028)

OECD 409 (1981) GLP Dog, Beagle, 6/dose/sex Deviations: Blood chloride and urine volume were not measured, unclear if a middle section of the spinal cord was observed microscopically. Deviations from the current version of OECD 409 (1998) mainly due to an older version of the OECD test guideline 409. Acceptable	MON 0139 (Isopropylamine salt of glyphosate); LUTR-12011 (MON 0139); purity 62.49% Six months, oral administration by gelatin capsule at daily doses of 0, 10, 60 or 300 mg/kg bw/day	<u>10 and 60 mg/kg bw/day:</u> No adverse effects <u>300 mg/kg bw/day:</u> Decreased body weight in males (-13%) NOAEL: 60 mg/kg bw/day LOAEL: 300 mg/kg bw/day	CA 5.3.2/029 810166 (1983)
Design similar to OECD 409 (1981) Non-GLP (pre-GLP) Dog, Beagle, 6/dose/sex Deviations: Not all required haematological, clinical chemistry, urinalysis parameters were evaluated; some organs were not weighed or microscopically examined. Formulated diets were not analysed for concentration, homogeneity or stability. Purity of the test substance not stated in the revised report since the respective supplement to the original report was missing to the author of the revised report. Individual and group data not reported for body weight, food consumption, haematology, clinical chemistry and organ weights. Unacceptable	Glyphosate (source, batch and purity not reported 3-month repeated oral dietary dose Dose levels: 0, 200, 600 and 2000 ppm (equivalent to 0, 9.08, 24.92 or 77.43 mg/kg bw/day)	<u>200 ppm:</u> No findings. <u>600 ppm:</u> Histopathological finding in liver (2 M and 1 F) characterized by round shaped and enlarged hepatocytes, hepatocytic trabeculae narrowing and slight dissociation of the liver structure. <u>2000 ppm:</u> Congestion of the liver (3 M and all F). Histopathological finding in liver (2 M and all F) characterized by round shaped and enlarged hepatocytes, hepatocytic trabeculae narrowing and slight dissociation of the liver structure. No NOAEL proposed as study is not considered acceptable.	CA 5.3.2/030 8011 (1981)

OECD 452 (1981) GLP Acceptable, no deviations Dog, Beagle, 4/dose/sex	Glyphosate technical, Batch H05H016A, purity 95.7% 1-year repeated oral dose, gelatine capsule Dose levels: 0, 30, 125 or 500 mg/kg bw/day	<u>30 and 125 mg/kg bw/day:</u> No adverse findings <u>500 mg/kg bw/day:</u> Decreased body weight gain in males (-29%) NOAEL: 125 mg/kg bw/day LOAEL: 500 mg/kg bw/day	CA 5.3.2/031 29647[redacted] (2007)
OECD 452 (1981) and OECD 409 (1981) GLP Dog, Beagle, 4/dose/sex Deviations: Blood clotting time parameters not evaluated; epididymis and uterus weights not reported. Deviations from the current versions of OECD 409 (1998) and OECD 452 (2018) are mainly due older versions of the OECD test guidelines. Acceptable	HR-001 (glyphosate technical), Batch T-940308, purity 94.61% 52-week repeated dietary oral dose Dose levels: 0, 1600, 8000 or 50000 ppm (equivalent to 0, 34.1, 182 or 1203 mg/kg bw/day for males and 0, 37.1, 184 or 1259 mg/kg bw/day for females)	<u>1600 and 8000 ppm:</u> No adverse findings. <u>50000 ppm:</u> Loose stool in males and females (3/4 males, 4/4 females). Decreased body weight gain in males (-19%) and females (-35%), decreased final body weight in females (-11%). Lower urinary pH in both sexes (not considered adverse) Slight anaemia in females (-14%, -14%, and -18% in Ht, Hb, and RBC count). Changes in blood electrolytes in females (up to -28% in inorganic phosphorus). Increased frequency of slight focal pneumonia in females (1/4, 1/4, 1/4, and 4/4 at 0, 1600, 8000, and 50000 ppm). In addition, a higher thyroid weight was noted in males (+36%), accompanied by c-cell hyperplasia in the thyroid. NOAEL: 8000 ppm LOAEL: 50000 ppm	CA 5.3.2/032 [redacted] 94-0157 (1997)

OECD 452 (1981) and OECD 409 (1981) GLP Acceptable, no deviations Dog, Beagle, 4/dose/sex	Glyphosate acid, batch P24, purity reported as 95.6% 52-week repeated dietary oral dose Dose levels: 0, 3000, 15000 or 30000 ppm glyphosate acid (equivalent to 0, 90.9, 440.3 or 906.5 mg/kg bw/day for males and 0, 92.1, 447.8 or 926.2 mg/kg bw/day for females)	<u>3000 and 15000 ppm:</u> No adverse findings. <u>30000 ppm:</u> Decreased body weight in females (-10%) NOAEL: 15000 ppm LOAEL: 30000 ppm	CA 5.3.2/033 CA 5.3.2/034 [redacted]/P/5079 (1996)
OECD 452 (1981) and OECD 409 (1981) GLP Dog, Beagle, 4/dose/sex Deviations: Activated partial thromboplastin time not measured. Clinical signs poorly reported in the report. Deviations from the current versions of OECD 409 (1998) and OECD 452 (2018) mainly due to older versions of the OECD test guidelines. Acceptable	Glyphosate technical, three batches (206-Jak-25-1, purity: 98.6%; 206-Jak-59-5, purity: 99.5% and 229-Jak-5-1, purity: 98.9%) 1-year repeated oral dose, gelatine capsule Dose levels: 0, 30, 300 or 1000 mg/kg bw/day	<u>30 and 300 mg/kg bw/day:</u> No adverse findings <u>1000 mg/kg bw/day:</u> Changes in faecal consistency (incidence not provided in the study report) Decreased body weight gain in males (-25%) and females (-19%) NOAEL: 300 mg/kg bw/day LOAEL: 1000 mg/kg bw/day	CA 5.3.2/035 7502 (1990)

OECD 452 (1981) GLP Dog, Beagle, 6/dose/sex Deviations: Urine volume not measured, spleen and uterus not weighed, unclear of number and location of brain sections observed microscopically. Deviations from the current version of OECD 452 (2018) are mainly due to an older version of the OECD test guideline 452. Acceptable	Glyphosate, NBP 2472136, purity 96.17% 1-year repeated oral dose, gelatine capsule Dose levels: 0, 20, 100 or 500 mg/kg bw/day	<u>20, 100 and 500 mg/kg bw/day</u>: No adverse findings. NOAEL: 500 mg/kg bw/day (highest dose tested)	CA 5.3.2/036 [redacted]-4965 (1985)
Design similar to OECD 452 (1981) Non-GLP (pre-GLP) Dog, Beagle, 4/dose/sex Deviations: The study was considered unacceptable due to serious reporting deficiencies, e.g., absence of information on batch and purity of the test material. Unacceptable	Glyphosate (source, batch and purity not reported 12-month repeated oral dietary dose Dose levels: 0, 30, 100 and 3000 ppm (equivalent to 0, 0.75, 2.5 or 7.5 mg/kg bw/day)	Rounded hepatocytes and narrower sinusoids observed in the livers of some (2/4) high dose male dogs and mid (2/4) and high dose (3/4) females but not in the low dose and in the control groups. There was no further evidence of morphological or functional liver alterations and therefore the reported findings while possibly treatment-related were not considered adverse effects. No NOAEL proposed as study is not considered acceptable.	CA 5.3.2/037 8012 (1982)
For other sub-chronic oral repeated dose toxicity studies refer to sections 2.6.6 (reproductive and developmental toxicity) and 2.6.7 (neurotoxicity).			
Dermal exposure			

OECD 410 (1981) GLP Rat, Wistar-derived, 5/dose/sex Acceptable, no deviations	Glyphosate acid, batch P24, purity 95.6% Vehicle: deionised water 21-day dermal toxicity study, 6h exposure, five times per week Dose levels: 0, 250, 500 or 1000 mg/kg bw/day	<u>250, 500 and 1000 mg/kg bw/day:</u> No adverse findings. $NOAEL_{local\ and\ systemic}$: 1000 mg/kg bw/day (highest dose tested)	CA 5.3.3/001 CA 5.3.3/002 [redacted]/P/4985 (1996)
OECD 410 (1981) GLP Rat, Sprague-Dawley, 5/dose/sex Deviations: Mean weight of the female rats were slightly lighter than requested (195 g instead of 200 – 300 g), organ weights of the adrenals were not determined. Acceptable	Glyphosate, batch 229-Jak-142-6, purity 101.5% Vehicle: diethylphthalate 21-day dermal toxicity study, 6h exposure, five times per week Dose levels: 0 and 1000 mg/kg bw/day (limit test)	<u>1000 mg/kg bw/day:</u> No systemic effects. Mild skin irritation (3/5 males and 5/5 females). $NOAEL_{systemic}$: 1000 mg/kg bw/day (highest dose tested) $NOAEL_{local}$: < 1000 mg/kg bw/day $LOAEL_{local}$: 1000 mg/kg bw/day	CA 5.3.3/003 7839 (1993)
OECD 410 (1981) GLP Rabbit, New Zealand White, 5/dose/sex Acceptable, no deviations	Glyphosate, batch 39730494, purity 99.6% Vehicle: water (50% w/v solution) 28-day dermal toxicity study, 6h exposure, five times per week Dose levels: 0, 500, 1000 or 2000 mg/kg bw/day	<u>500, 1000 and 2000 mg/kg bw/day</u>: No adverse systemic findings. Slight skin irritation in 1/5 top-dose males and 1/5 low-dose females. $NOAEL_{systemic}$: 2000 mg/kg bw/day (highest dose tested) $NOAEL_{local}$: 1000 mg/kg bw/day	CA 5.3.3/004 CA 5.3.3/005 CA 5.3.3/006 [redacted] 214/94 (1994)

Comparable to OECD 410 (1981) No GLP (pre-GLP) Rabbit, New Zealand White, 3/dose/sex Deviations: Test substance purity and batch number or study details like study number were not reported. Additionally, a statistical analysis of the results was not reported. The study design is comparable to OECD 410 (1985), however, sacrifice was after a 14-day recovery period for all animals, which is not in agreement with the OECD guideline. Unacceptable	Glyphosate, purity and batch not reported 21-day dermal toxicity study, 6h exposure, five times per week Dose levels: 0, 500, 1000 or 2000 mg/kg bw/day	500, 1000 and 2000 mg/kg bw/day: No adverse findings. No NOAEL proposed as study is not considered acceptable.	CA 5.3.3/007 Report number not reported, 1985 (refer to CA 5.3.3/007)
Comparable to OECD 410 (1981) GLP Rabbit, New Zealand White, 5/dose/sex The purity of the test substance is not reported. The application area in the high-dose group was about 1.5–2 times higher than the recommended 10% of the body surface area. With the highest test dose of 5000 mg/kg bw/day, the limit dose of 1000 mg/kg bw/day is exceeded. Unacceptable	Glyphosate, batch NBP 1992026, purity not reported 21-day dermal toxicity study, 6h exposure, five times per week on intact and abraded skin Vehicle: water Dose levels: 0, 100, 1000 or 5000 mg/kg bw/day	100 and 1000 mg/kg bw/day: No adverse findings. 5000 mg/kg bw/day: Increased kidney weight (abs/rel) in females. Increased sodium in males. No NOAEL proposed as study is not considered acceptable.	CA 5.3.3/008 [redacted]-81-195 (1982)
Inhalation exposure			

No guideline No GLP (pre-GLP) Rat, Wistar, 5/dose/sex Deviations: The study is not considered acceptable due to serious reporting deficiencies, e.g. absence of statistical analysis, and purity and batch number of the test substance not reported. Unacceptable	Glyphosate (purity and batch not stated) 14-day repeated inhalation toxicity study; by nose and mouth route Target dose levels: 0, 0.25, 1 and 4.0 mg/L air	0.28, 0.93 and 3.8 mg/L air (mean measured concentration): No local (respiratory) or systemic toxic effects. Mass median aerodynamic diameters of all atmospheres were within the respirable range of 0 - 7 µm on all occasions. No NOAEL proposed as study is not considered acceptable.	CA 5.3.3/009 Report number not reported, 1985 (refer to CA 5.3.3/009)
Publications on repeated dose-toxicity via other routes			
Public literature study *In vitro* investigative study HepaRG cell culture Reliable with restrictions (Klimisch score 2)	Glyphosate (purity ≥ 96 %) was purchased from Sigma-Aldrich Test concentrations: 0.06, 6 and 600 µM No positive controls	Aim of the study was to investigate the effect of glyphosate on the transcriptome and metabolome profile of differentiated HepaRG cells. Glyphosate was found to be only weakly toxic inducing little change in transcriptome profiles when compared with the other herbicides tested (quizalofop-p-ethyl, isoxaflutole and mesotrione). A follow-up metabolomics analysis of HepaRG cells exposed to glyphosate at 0.06 µM revealed a significant decrease in the levels of long chain fatty acids and polyunsaturated fatty acids. At the higher glyphosate concentrations of 6 and 600 µM, lower lipid levels were also observed but these did not reach statistical significance. While the study gives some indication of a slight potential effect of glyphosate on transcriptome profile alterations in HepaRG human liver cells *in vitro*, it does not provide information on a potential adverse effect *in vivo*. Therefore, the study is considered to provide no information that will directly impact the risk assessment of glyphosate.	CA 5.3.3/010 Mesnage, R. *et al*., 2018 (refer to CA 5.3.3/010)

Public literature study *in vivo* investigative study Female mice; C57BL/6 background wild type and TLR4-/- mice BALB/c background wild type and IL-13-/- mice Unacceptable (experiments with farm air) / Acceptable but with restrictions (Klimisch score 2) for other parts of the study	Animals were intranasally exposed to filter extracts from 'farm air' samples obtained during and after field spraying with glyphosate and/or to reagent-grade glyphosate at 100 ng, 1 µg or 100 µg The method for the collection and analysis of the farm air samples was not validated.	Glyphosate-rich farm air samples as well as glyphosate alone were found to induce pulmonary IL-13-dependent inflammation and promote Th2 type cytokines. However, no negative control was included (i.e. farm air without glyphosate), therefore the effects found with glyphosate-rich farm air cannot be attributed to glyphosate alone. Glyphosate exposure at 1 µg or 100 µg resulted in increased total cell count, eosinophils, neutrophils, and IgG1 and IgG2a levels in treated mice compared to controls. No effect was seen at 100 ng. The inflammation was confirmed by histological examination. Serum levels of MCPT-1 were higher after glyphosate treatment at 1 µg and comparable to OVA-treated mice, indicating increased mast cell degranulation. Further, IL-33 and TSLP were increased in the respiratory epithelium of glyphosate-treated mice. It should be noted that the air samples and glyphosate were delivered (in 30 ml) to the nose of anesthetized mice in order to aspirate the solution. It is, however, unclear how aspiration relates to exposure to glyphosate by inhalation.	CA 5.3.3/011 Kumar *et al.*, 2014 (refer to CA 5.3.3/011)

Table 47: Summary table of human data on repeated dose toxicity STOT-RE (specific target organ toxicity-repeated exposure)

Type of data/report	Test substance	Route of exposure Relevant information about the study (as applicable)	Observations	Reference
No human data available.				

Table 48: Summary table of other studies relevant for repeated dose toxicity STOT-RE (specific target organ toxicity-repeated exposure)

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No specific data available.				

2.6.3.1.1 Short summary and overall relevance of the provided information on specific target organ toxicity – repeated exposure (short-term and long-term toxicity)

28-day oral studies

A 28-day dietary repeated dose toxicity study was performed in Wistar rats (report number [redacted].881.28 DDR, 1991). The study was performed according to OECD 407 (1981) and conducted under GLP. The study was considered acceptable but with restrictions as there were indications of lung infections among both control and treated animals. Four groups of five male and five females were administered technical glyphosate (FSG 03090 H/05 March 1990 / Batch No. 60, purity 96.8%) for 28 days via the diet at concentrations of 0, 200, 2000, or 20000 ppm (equivalent to 0, 17.6, 178.5, 1894.9 or 1987.5 mg/kg bw/day in males and 21.6, 223.3, 2250.8 or 2129.7 mg/kg/day in females). There was no mortality in any of the study groups during treatment. In general, there were no clinical signs of toxicity observed in any of the treatment groups. However, there were a few incidences of urinary incontinence in the mid- and high-dose groups. There were no notable intergroup differences in body weights, food consumption, or haematological parameters. With regards to clinical chemistry parameters, there was a statistically significant increase in the activity of glutamic pyruvic transaminase (SGPT/ALAT) at the high-dose level, however, this was not considered adverse as levels were increased by 40-45% compared with controls, which is below the cut-off of 50% as proposed by JMPR as a starting point of adversity. There were no notable intergroup differences in organ weights. No gross pathology or histopathology findings attributed to administration of glyphosate were recorded. The NOAEL of the study was 20000 ppm (1895 mg/kg bw/day in males and 2251 mg/kg bw/day in females), the highest dose tested.

A second 28-day dietary repeated dose study was performed in Sprague-Dawley rats (report number 5626, 1991). The study was performed according to OECD 407 (1981) and conducted under GLP. The study was considered acceptable but with restrictions due to several deviations as amongst others only liver, kidneys, adrenals, testes, epididymides were weighed and histopathology was limited to liver, heart, kidneys, spleen and adrenals. In addition, the limit dose of 1000 mg/kg bw/day was exceeded. There was one premature death, an intermediate dose group male that died during the week 4 blood sampling. This was not considered treatment-related. Effects were restricted to the top dose and comprised soft stool in males, decreased body weight gain in females, increased alkaline phosphatase levels in males, increased bilirubin levels in females. Histopathological changes were limited to nephrocalcinosis in 250, 1000 and 2500 mg/kg bw/day females (grade very mild to mild) which was considered to be of unclear toxicological relevance. The NOAEL of the study was 1000 mg/kg bw/day based on a decreased body weight gain in females (-11%), increased alkaline phosphatase in males (+60%) and females (+42%), increased bilirubin in females (+63%) and soft stool in males (3/5) at the LOAEL of 2500 mg/kg bw/day. These findings occurred at a dose above the oral guidance value for classification for specific target organ toxicity (repeated exposure).

A third 28-day dietary study was a dose-range finding study of glyphosate in male and female Sprague-Dawley rats (report number [redacted]-8921, 1989). No guideline was reported for this study, however, the study was generally in compliance with OECD 407 (1981) and was conducted under GLP. The study is considered to be acceptable but with restrictions as this is designed as a dose-range finding study with limited parameters included. All dose levels exceeded the 1000 mg/kg bw/day limit dose. Four groups of five male and five female Sprague-Dawley rats received glyphosate (batch XLI-203, purity 97.67%) in their diet at target concentrations of 0, 30000,

40000 or 50000 ppm (equivalent to 0, 1921.1, 2634.1 or 3278.1 mg/kg bw/day for males and 0, 2310.6, 3256.4 or 4150.2 mg/kg bw/day for females) for four weeks. No animals died during the course of the study. Reduced body weight gains were noted in both sexes at all three dose levels. Food consumption (g/day) was reduced for mid and high dose males during the first week of the study. The only clinical signs of toxicity were soft stool (at all dose levels) and/or diarrhoea (predominantly at the high dose). Gross and microscopic pathology examinations revealed no treatment-related lesions. Based on the results of this 28–day range-finding study, no robust NOAEL can be derived.

In mice, one 28-day dose range-finding study is available (77-2110, 1978). In this study, glyphosate (XHI-162, purity 83%) was administered orally via the diet to mice at target dose levels of 0 (control), 100, 300 or 1000 mg/kg bw/day (approximately 80, 235 or 800 mg/kg bw/day actual achieved dose). The study is considered acceptable but with restrictions as this study is designed as a non-guideline dose-range finding study with very few parameters included. The study not performed under GLP as it was performed before GLP was introduced. All in-life data (physical observations, body weight and food consumption) and gross necropsy observations indicated no adverse effects of glyphosate at any dose level. As the study is considered a dose-range finding study with limited reporting, no NOAEL has been derived.

In Beagle dogs, a dose-range finding study (5660, 1989) was performed in which glyphosate (161-JRJ-131-2, purity 99.5%) was administered by gelatin capsules to one male and one female dog for 7 day periods at escalating dose levels of 100, 300 and 1000 mg/kg bw daily (Part A) and to one dog of each sex for 14 days at 1000 mg/kg bw/day (Part B). The study conducted in compliance with GLP regulations. No adverse effects were observed in this study, however, the study was not considered acceptable as only one dog/sex was included for the 7-day escalating dosing regime and for the 14-day repeated dosing, only few parameters were included in the study and no control animals were used. Therefore, no NOAEL is derived based on this study.

Another dose-range finding study was performed in Beagle dogs ([redacted]-2155, 1982) using either the isopropylamine salt of glyphosate (LURT-12011 (MON 0139); purity 62.49%) or isopropylamine (purity 99.7%) in a single or daily dose for 5 days. The study was not performed under GLP (performed before GLP was introduced). This study was considered as acceptable but with restrictions (as a dose range finding study) as this study is designed as a non-guideline dose-range finding study with few animals and limited parameters investigated. Dosing was by gavage or gelatine capsules with varying regimens of fasting and feeding before and after dosing to try to control emesis. Dosages of the isopropylamine salt of glyphosate ranged from 312.5 to 2500 mg/kg bw/day in single doses or daily doses for 5 days. No animals died during the study. Mild body weight loss and reduced food consumption occurred on and shortly after treatment days at all dose levels. Further, diarrhoea was seen at all dose levels and emesis at all but the lowest dose. Two dose levels of isopropylamine were given: 72 mg/kg bw as a single treatment to a pair of dogs, and 19.43 mg/kg bw/day for five days to a single dog. Emesis, bloody emesis and loose stools were observed. Isopropylamine treatment resulted in severe oedema, haemorrhage, and necrosis of the rugae in the stomachs of the higher dose level dogs (these dogs were sacrificed in extremis on humane grounds 30 minutes after dosing). Mucosal erosions of the stomach and oesophagus were observed in the lower isopropylamine dose level dog. A NOAEL for the isopropylamine salt of glyphosate was not established.

Gao *et al*. (2019) investigated the effects of glyphosate on renal proximal tubule cell *in vitro* and *in vivo*. This is a non-GLP and non-guideline public literature study. The *in vivo* part of the study is considered as reliable with restrictions, as this was an investigative study which only included observation of body weight, liver and kidney weight, kidney histology and several other kidney parameters. The *in vitro* part is also considered as acceptable but with restrictions. In the *in vitro* part of the study, glyphosate (as monoisopropylamine salt solution (40% w/w in water)) was found to reduce cell viability, to increase the incidence of apoptotic cells with an increase in the expression of apoptosis-related proteins, to increase of oxidative stress in a concentration-related manner, to increase of the expression of the NMDA receptor and to increase Ca2+ influx. In the *in vivo* part of the study, kidney histopathology revealed the exfoliation of renal tubular cells in the animals treated with glyphosate at 400 mg/kg bw/day during 28 days. Also, upregulation of apoptosis and NMDAR1 exposure in the proximal tubule epithelium and an imbalance of oxidant/antioxidant balance were observed. Further, a transient increase in urine albumin was observed after 7 and 14 days of treatment (1.8- to 2.0-fold increase compared with controls) and urinary β2-microglobulin levels were statistically significantly increased after 7, 21 and 28 days of treatment (1.7- to 3.5-fold increase compared with controls). Based on this mechanistical study, the authors postulated that glyphosate could affect renal tubule epithelial cells via the NMDAR1/[Ca2+]i/ROS pathway.

Tang *et al.* (2017) investigated the effects of glyphosate on rats' liver function and induction of pathological changes in ion levels and oxidative stress in hepatic tissue. Sprague-Dawley rats were treated orally by gavage with 0, 5, 50, or 500 mg/kg body weight of the glyphosate (purity not reported) daily for 35 days. This public literature study was not performed under GLP and was considered supportive as the purity of the test substance was not

provided, only 8 instead of 10 animals were treated per dose group and only males were treated. Adverse effected were noted at 50 and 500 mg/kg bw/day and comprised reduced body weight gain at both dose levels, decreased absolute and relative spleen weight at 500 mg/kg bw/day. Further, signs of oxidative stress, upregulation of liver inflammatory genes and upregulation of genes related to lipid metabolism were noted at 50 mg/kg bw and above, but effects were mainly slight and/or clinical relevance of these findings is lacking. As the study is not considered acceptable, no NOAEL has been derived.

90-day oral studies in rats

In the first study (report number [redacted]/P/1599, 1996) groups of twelve male and twelve female Wistar-derived rats were fed diets containing 0 (control), 1000, 5000 or 20000 ppm glyphosate acid (batch P15, purity 97.4%) for 90 consecutive days (equivalent to 0, 81.33, 413.5, or 1612 mg/kg bw/day in males and 0, 90.42, 446.9 or 1821 mg/kg bw/day in females). The study was considered acceptable and was in compliance with GLP and with OECD 408 (1998). The were some minor deviations which were mainly due to the fact that the study was aligned to an older version of OECD TG 408. At the low and mid dose (1000 and 5000 ppm, respectively), no adverse effects were observed. At the top dose of 20000 ppm, considered the LOAEL, body weight gain was reduced in males (-11%) and alkaline phosphatase levels were increased in both males (+45%) and females (+54%). The NOAEL of this study is 5000 ppm glyphosate acid (equivalent to 413.5 mg/kg bw/day for males and 446.9 mg/kg bw/day for females).

In the second study (report number 434/016, 1996), glyphosate technical (batch H95D 161 A, purity 95.3%) was administered to four groups, each of ten male and ten female Sprague Dawley (CD) strain rats, for ninety consecutive days, at dietary concentrations of 0 (control), 1000, 10000 or 50000 ppm (equivalent to 79, 730 or 3706 mg/kg bw/day for males and 90, 844 or 4188 mg/kg bw/day for females). The study was considered acceptable and was in compliance with GLP and with OECD 408 (1998). The were some minor deviations which were mainly due to the fact that the study was aligned to an older version of OECD TG 408. In addition, the limit dose of 1000 mg/kg bw/day was exceeded with top dose levels equivalent to 3706 and 4188 mg/kg bw/day, for males and females, respectively. At the top dose of 50000 ppm soft faeces and diarrhoea was noted in all animals of both sexes. Also a decreased body weight was noted in males and females and food consumption was reduced in males only. Further, relative kidney weight was increased in both sexes. In addition, treatment-related changes were observed in the caecum which was and enlarged and filled with fluid in all animals of both sexes and atrophy of the caecum characterised by flattening of the intestinal mucosa in five out of ten rats of both sexes. This atrophy in the caecum was also seen in one male and two females at the mid dose of 10000 ppm. Due to this finding and the increased alkaline phosphatase levels in females (+77%), this mid dose level of 10000 ppm is considered the LOAEL. The NOAEL of the study is 1000 ppm (equivalent to 79 mg/kg bw/day for males, and 90 mg/kg bw/day for females). It should be noted that this is relatively low compared to NOAELs of other rat studies, however, this is mainly due to the large dose spacing in the study (factor 10 between low and mid dose).

The third study (report number [redacted] 94-0138, 1995) describes a 13-week (91 day) sub-chronic oral toxicity study of glyphosate technical was conducted in Sprague-Dawley (Crj:CD) rats. The test substance glyphosate (HR-001, batches: 940908-1 (95.68% purity), 941209 (95.0% purity) and T-941209 (97.56% purity)) was administered to rats of both sexes (12 animals/group/sex) at dose levels of 0, 3000, 10000 or 30000 ppm (equivalent to 0, 168.4, 569 or 1735 mg/kg bw/day for males and 0, 195.2, 637 or 1892 mg/kg bw/day for females) for a period of 13 weeks (91 days). The study was considered acceptable and was in compliance with GLP and with OECD 408 (1998). The were some minor deviations which were mainly due to the fact that the study was aligned to an older version of OECD TG 408. In males, treated at 30000 ppm a decreased body weight was observed. No effects on body weight were observed in top dose females. In both sexes, food consumption was decreased in the first week at the 30000 ppm dose level in both sexes. In females, blood alkaline phosphatase was increased at the 30000 ppm dose level. Distention of the caecum was seen in the majority of the males and females at the top dose, which was also reflected in an increased caecum weight at this dose level. Also at the mid dose, which is considered the LOAEL, 3/12 males showed distension of the caecum and caecum weight was increased in both males and females (+20/16% in males and +50/54% in females). The effects on the caecum are considered treatment-related and adverse. Based on these findings, the NOAEL of the study is 3000 ppm (equivalent to 168.4 and 195.2 mg/kg bw/day for males and females, respectively).

The fourth study (report number 011-0001, 1993) is a 13-week repeated dose dietary toxicity study in which groups of ten male and ten female Sprague-Dawley rats were fed diets containing 0 (control), 2000, 6000 or 20000 ppm glyphosate acid (batch 46540992, purity 97.5%; dosages equivalent to 0, 125.2, 371.9 or 1262.1 mg/kg bw/day for males and 0, 156.3, 481.2 or 1686.5 mg/kg bw/day for females). The study was considered acceptable and was in compliance with GLP and with OECD 408 (1998). The were some minor deviations which were mainly due to the fact that the study was aligned to an older version of OECD TG 408. Signs of toxicity were only noted at the

highest dose of 20000 ppm, which is considered the LOAEL and comprised the occurrence of diarrhoea in both sexes, (10/10 and 9/10 in males and females, respectively), decreased body weight gain in both sexes at day 50 and 85 (-51% and -85% in males; -71% and -54% in females), decreased absolute and relative adrenal weights in males (-26% and -21%), increased absolute and relative spleen weight in females (+18% and +24%) and the occurrence of blood in urine (10.5 and 3 times higher in males and females, respectively, compared to the control). Based on these findings, the NOAEL of this study is 6000 ppm (equivalent to 371.9 mg/kg bw/day for males and 481.2 mg/kg bw/day for females).

In the fifth dietary 90-day toxicity study (report number [redacted].882.90 OR, 1992) groups of 10 male and 10 female Wistar rats were administered technical glyphosate (Batch FSG 03090 H/05 March 1990, purity 96.8%) at concentrations of 0, 200, 2000, or 20000 ppm. These dose levels were equivalent to 0, 14.0, 147.3, or 1358.6 mg/kg bw/day for males and 0, 18.6, 195.7, or 2012.4 mg/kg bw/day for females, respectively. The study was GLP-compliant and broadly complies with OECD 408 (1981). The study was however considered acceptable but with restrictions due to limited histopathology and organ weight reporting. There were also some other minor deviations which were mainly due to the fact that the study was aligned to an older version of OECD TG 408. The rationale for dose selection was not provided. There were no treatment-related effects at any dose level with regards to mortality, clinical signs of toxicity, haematology, organ weight and gross and histopathological findings. At the highest dose level of 20000 ppm, which is considered the LOAEL, decreased body weight in females (up to -13% (week 10)), increased alkaline phosphatase in males (+50%) and increased blood glucose in females (+24%) was observed. Based on these findings, the NOAEL of this study is 2000 ppm glyphosate (corresponding to 147.3 mg/kg bw/day for males and 195.7 mg/kg bw/day for females). It should be noted that this is relatively low compared to NOAELs of other rat studies, however, this is mainly due to the large dose spacing in the study (factor 10 between low and mid dose).

In the sixth study (report number 7136, 1991), glyphosate technical (batch 206-Jak-25-1, purity 98.6%) was administered to rats via the diet over a period of 13 weeks. The concentrations of the diet were adjusted weekly to achieve dose levels of 0, 30, 300 or 1000 mg/kg bw/day. The group size was 10 animals per sex and dose group. The study was considered acceptable and was in compliance with GLP and with OECD 408 (1998). The were some minor deviations which were mainly due to the fact that the study was aligned to an older version of OECD TG 408. There were no mortalities or clinical signs. Body weight, food intake, water consumption, ophthalmoscopy and haematology were unaffected by treatment. Blood glucose was increased in high dose females. A reduction of urinary pH was noted in high dose males.

Histopathology revealed a statistically significant increased incidence of parotid cellular alterations in the salivary gland of both sexes at 1000 mg/kg bw/day. The finding was described by study author as deep basophilic staining and enlargement of cytoplasm. The incidence of this finding was 100% for males (compared to 30% in control) and 90% for females (compared to 20% in control). The severity grade of finding was minimal to severe in males, and minimal to moderate in females.

Also at 300 mg/kg bw/day a statistically significant increased incidence of parotid cellular alteration was observed in both sexes. The incidence was the same as for the high dose animals, but the severity grade was lower when compared to the high dose group. At 300 mg/kg bw/day the severity grade of finding in animals was very mild to mild (except for a single male animal which showed moderate cellular alteration).

At the lowest dose of 30 mg/kg bw/day, which is considered the LOAEL, the increased incidence of parotid cellular alteration was statistically significant but only for females. The incidence was 70% in males (compared to 30% in control) and 80% in females (compared to 20% in control) and the severity grade of findings was minor (mostly very mild).

This effect on the salivary gland is considered a treatment-related effect for which human relevance cannot be excluded (refer to Vol 1 section 2.6.8.2). However, for the interpretation of effects on salivary glands several aspects are considered in order to decide if the effect is adverse or not. The RMS considers salivary gland weight changes and histopathology (severity grade and incidence) along with dose-response and statistical significance. A histopathological finding which is statistically significant is not considered adverse if the severity grade of the finding is minor and there are no salivary gland weight changes. In the event that there are no data for salivary gland weights, the effect on histopathology might be considered as potentially adverse in the absence of such data as a precautionary approach.

In this 90-day study, no increase of salivary gland weights was reported. However, salivary gland weight was determined for the parotid, submaxillary and sublingual glands together. In this case, the conclusion that no effect was seen on salivary gland weight might be blurred as three different glands are weighed together whereas the effect only occurs in one of the glands (parotid mostly, submaxillary in some other studies). As for this study no data is available on the parotid gland weight, the **RMS proposes to set the LOAEL at the lowest dose level of 30 mg/kg bw/day as a precautionary approach although the severity grade of findings observed at this dose level was minimal (very mild).**

The seventh 90-day oral repeated dose toxicity study ([redacted]-900914, 1990) is a study in which groups of 10 animals per sex were exposed to glyphosate (batch 0190 A, purity 98.1%) at 0 (control), 2000, 5000 and 7500 ppm (equivalent to 0, 129.1, 320.7 or 482.1 mg/kg bw/day for males and 0, 174.3, 441.6 or 647.3 mg/kg bw/day for females). However, the study was not considered acceptable due to poor homogeneity of some batches of the test diet and uncertainties regarding the achieved dose levels in the study. Based on the dietary analysis the achieved concentrations were much lower than the target concentrations, however, these were measured 8-16 weeks after administration. Therefore, it is unclear which dose level was achieved during the study. Treatment-related and adverse effects observed during the study were a decreased food consumption in both sexes and an increase in blood glucose in males at 7500 ppm. However, as the study is not considered acceptable, no NOAEL is proposed by the RMS.

In the eighth sub-chronic oral repeated dose toxicity study ([redacted]-891002, 1989) male and female rats were dosed with glyphosate technical (batch L1656, purity 97.1%) over a 90 – 92 day period. The test substance was administered in the diet at levels of 0, 2000, 3000, 5000, or 7500 ppm (equivalent to 0, 100, 150, 250 or 375 mg/kg bw/day for males and females). The study was GLP-compliant and broadly complies with OECD 408 (1981). The study was however considered acceptable but with restrictions due several deviations with comprised limited haematology, clinical chemistry, organ weighting and histopathology. The deviations from the current version of OECD 408 (2018) are mainly due to the fact that the study was aligned to an older version of the OECD test guideline 408. No rationale for target dose selection is provided (highest dose lower than recommended 1000 mg/kg bw/day). There were no treatment-related adverse effects on survival, clinical signs, body weight, haematology, clinical chemistry and histopathology. Therefore, the NOAEL of this study is ≥ 7500 ppm in the diet (equivalent to 375 mg/kg bw/day in males and females).

In the ninth study ([redacted]-7375 (1987), glyphosate was administered to groups of 12 male and female Sprague-Dawley rats at target levels of 0, 1000, 5000 or 20000 ppm in the feed (equivalent to actual doses of approx. 0, 63, 317 or 1267 mg/kg bw/day for males and 84, 404 or 1623 mg/kg bw/day for females) for 90-days. The study was GLP-compliant and broadly complies with OECD 408 (1981). The study was considered acceptable as there were some minor deviations from the current version of OECD 408 (2018) which are mainly due to the fact that the study was aligned to an older version of the OECD test guideline 408. There was no evidence of toxicological effects observed in any parameter examined at any dose level. Therefore, the NOAEL for glyphosate, as administered in this study, is ≥ 19000 ppm (actual dose; equivalent to 1267 mg/kg bw/day for males and 1623 mg/kg bw/day for females).

The tenth 90-day oral repeated dose toxicity study (reported in the RAR as CA 5.3.2/015, 1985), in which Wistar rats were dosed by gavage at 0 (control), 300, 1200 or 2400 mg glyphosate/kg bw/day, was not considered acceptable due to serious reporting deficiencies, e.g. absence of statistical analysis, report identification and dates of experimental work were not given, and purity and batch number of the test substance was not reported. The only effect seen in the study was a decrease in both mean body weight and food consumption in both sexes at termination. There were no treatment-related effects at the mid and low dose. However, no NOAEL is proposed as the study is not considered acceptable.

Also the 11th study investigating the sub-chronic oral toxicity of glyphosate in rats (reported in the RAR as CA 5.3.2/015, 1981) was not considered acceptable due to missing information on the actual concentration of the test substance in the diet and on the homogeneity and the stability of the test substance. At the lowest dose level of 1000 ppm (with a mean calculated compound intake of 102.0 or 105.4 mg/kg in males and females, respectively), no adverse effects were observed. At the mid dose of 3000 ppm, a reduced red blood cell count and an increase in leucocyte and platelet count was observed. At the top dose of 10000 ppm, increased blood glucose in females, increased alkaline phosphatase, AST and ALT activity in both sexes and increased liver weights in both sexes were reported. However, as the study was not considered acceptable, no NOAEL is proposed.

NTP study in rats (1992): For the process under Regulation (EC) No 1107/2009, the applicant is requested to provide the study and an assessment. For the process under the Regulation (EC) No 1272/2008, the applicant is asked to submit the missing information during the public consultation period.

Overall, most of the above studies demonstrated a low toxicity of glyphosate in different rat strains upon sub-chronic repeated oral administration. Several studies showed no adverse effects up and above the limit dose of 1000 mg/kg bw/day. Toxicological effects attributed to glyphosate exposure were soft stool, diarrhoea, decreased body weight gain and food consumption, which might suggest some irritation of the gastrointestinal tract by glyphosate. Further, a decrease in urinary pH was frequently reported. Other effects reported in rats are increased liver weight and changes in blood chemistry (increase in alkaline phosphatase, AST and ALT, increase in blood glucose). At dose levels above the limit dose of 1000 mg/kg bw/day, one study reported increased kidney weights.

Further, the caecum was identified as a target organ because of certain findings (distention, elevated weight of this part of the intestines and its contents, mucosal atrophy). At much lower dose levels, one study reported histopathological changes in the parotid salivary gland which comprised deep basophilic staining and enlargement of cytoplasm at the lowest dose level (30 mg/kg b/day) and above. The RMS considers this a treatment-related effect for which human relevance cannot be excluded (refer to Vol 1 section 2.6.8.2). However, for the interpretation of effects on salivary glands several aspects are considered in order to decide if the effect is adverse or not. The RMS considers salivary gland weight changes and histopathology (severity grade and incidence) along with dose-response and statistical significance. A histopathological finding which is statistically significant is not considered adverse if the severity grade of the finding is minor and there are no salivary gland weight changes. In the event that there are no data for salivary gland weights, the effect on histopathology might be considered as potentially adverse in the absence of such data as a precautionary approach. **As for this 90-day study no data is available on the parotid gland weight, the RMS proposes to set the LOAEL at the lowest dose level of 30 mg/kg bw/day as a precautionary approach although the severity grade of findings observed at this dose level was minimal (very mild).**

90-day oral studies in mice

In the first sub-chronic toxicity study in mice (report number [redacted] 94-0136, 1995), glyphosate (HR-001, batch T-941209, purity 97.56%) was administered through diet to each dose group of 12 males and 12 females of SPF ICR mice (Crj:CD-1) at a dose level of 0, 5000, 10000 or 50000 ppm (equivalent to 0, 600.2, 1221 or 6295 mg/kg bw/day for males and 0, 765.0, 1486 or 7435 mg/kg bw/day for females) for a period of 13 weeks. The study was GLP-compliant and complies with OECD 408 (1981). The study was considered acceptable as the deviations from the current version of OECD 408 (2018) are mainly due to the fact that the study was aligned to an older version of the OECD test guideline. It should be noted however that the highest dose tested (~6000-7000 mg/kg bw/day) is far above the limit dose of 1000 mg/kg bw/day according to OECD 408. Treatment-related and adverse effects were only observed at the top dose of 50000 ppm, which is therefore considered the LOAEL. At this dose level, a reduced food consumption in the first week was observed in males (-28%), increased alkaline phosphatase in both sexes (+84% in males, +50% in females), increased blood phosphorus in females (+28%), increased creatinine phosphokinase in females (~9.4 times higher), distension of the caecum (12/12 and 10/12 in males and females; 0/12 in males and females from the control group) and increased absolute and relative caecum weight in both sexes (+138/163% in males and +87/95% in females), and an increased incidence of cystitis in the urinary bladder in males (4/12 cf. 0/12 in control group). In addition, a shift towards lower urinary pH was observed in all dose groups (significant in males, not significant in females), however, this effect was not considered adverse as this is due to acidic properties of the test substance and is therefore not considered a toxic effect. The effect on caecum distension in one female and slight caecum weight increases observed at the mid dose were not considered adverse as these were not accompanied by histopathological changes. The NOAEL of this study is 3000 ppm (equivalent to 1221 mg/kg bw/day for males and 1486 mg/kg bw/day for females).

The second study (report number 7024, 1991) was designed to give toxicity information over 13 weeks on glyphosate (batch 161-JRJ-131-2 (purity 99.5%) and 003-89-A (purity 98.0%)) administered to CD-1 mice via the diet at concentrations calculated to achieve dose levels of 0, 200, 1000 or 4500 mg/kg bw/day. The group size was 10 animals per sex and dose group. The study was GLP-compliant and complies with OECD 408 (1981). The deviations from the current version of OECD 408 (2018) are mainly due to the fact that the study was aligned to an older version of the OECD test guideline and were considered minor. It should be noted that the highest dose tested (~4500 mg/kg bw/day) is far above the limit dose of 1000 mg/kg bw/day according to OECD 408. The study was considered acceptable but with restrictions as only a limited number of samples could be analysed for clinical chemistry due to low sample volumes. Dosing CD-1 mice via the diet for 13 weeks with up to and including 4500 mg/kg bw/day glyphosate produced no findings which could be directly attributed to administration of the test material. The NOAEL is 4500 mg/kg bw/day, the highest dose tested. However, it should be noted that evaluation of clinical chemistry parameters was of limited scientific value only.

In the third sub-chronic mouse study (77-2111, 1979), the test material glyphosate (batch XHJ-64, purity 98.7%) was administered to groups of CD-1 mice at dose levels of 0, 5000, 10000 or 50000 ppm (equivalent to 0, 944.1, 1867.2 or 9707.0 mg/kg bw/day for males and 0, 1527.7, 2734.7 or 14858.2 mg/kg bw/day for females) via the diet for three months. The study was not performed under GLP as it was performed before GLP was introduced. The study was performed according to a testing regime similar to OECD 408 (1981). Although the main deficiency was that haematological and clinical chemistry parameters were not included, overall the study was well performed. Therefore, this study is considered as acceptable but with restrictions. It should be noted however that the highest dose tested (~10000-15000 mg/kg bw/day) is far above the limit dose of 1000 mg/kg bw/day according to OECD 408. No treatment-related and adverse effects were observed at the low and mid dose. At the top dose of 50000 ppm, which is considered the LOAEL, body weight was decreased in both males and females at several time points during

the study (week 0-13: -24% in males and -18% in females; not significant), which was reflected in a decreased body weight gain in both sexes (up to -10% in both sexes). There were no other adverse findings at this dose level. The NOAEL of the study is 10000 ppm (equivalent to 1867.2 and 2734.7 mg/kg bw/day in males and females, respectively). However, it should be noted that no haematological and clinical chemistry parameters were included in this study and therefore this NOAEL is of limited value.

NTP study in mice (1992): For the process under Regulation (EC) No 1107/2009, the applicant is requested to provide the study and an assessment. For the process under the Regulation (EC) No 1272/2008, the applicant is asked to submit the missing information during the public consultation period.

Toxicity of glyphosate to mice was investigated in a relatively small number of sub-chronic studies. At very high doses (>6000 mg/kg bw/day) a reduction in body weight (gain), food consumption and alterations in some haematological and clinical chemistry parameters with the latter findings pointing to liver toxicity. Gross necropsy revealed caecum distention that was supported by a higher organ weight but not accompanied by histological lesions. Cystitis of urinary bladder became histologically apparent in some high dose males. Low urinary pH (most likely due to acidic properties of the test substance) was noted in all treated male groups, but this was not considered adverse as this was attributed to the acidity of the test substance. The first study (Report no [redacted] 94-0136, 1995) is considered the only study relevant for "overall" NOAEL setting in mice as the NOAELs of the other studies were of limited value due to missing or only partial haematology and clinical chemistry investigation. Therefore the NOAEL for sub-chronic exposure to glyphosate is considered 600 mg/kg bw/day. However, it should be noted that in the previous assessment a NOAEL of 500 mg/kg bw/day was proposed based on salivary gland findings in the NTP study in mice. **However, this study has not been submitted (data requirement) and therefore the NOAEL of 600 mg/kg bw/day should be considered a provisional NOAEL. As already indicated above, for the process under Regulation (EC) No 1107/2009, the applicant is requested to provide the study and an assessment. For the process under the Regulation (EC) No 1272/2008, the applicant is asked to submit the missing information during the public consultation period.** It is however noted that the LOAEL is clearly above the guidance values for classification.

90-day and 1-year oral studies in dogs

In the first study (29646 [redacted] 2007), groups of four Beagle dogs per sex received glyphosate technical (batch H05H016A, purity 95.7%) by daily administration by gelatine capsule at dose levels of 0, 30, 300 or 1000 mg/kg bw/day for 11/13 weeks. The duration of the treatment period for the high-dose group was shortened to 11 weeks for ethical reasons due to marked toxic effects. The study was GLP-compliant and was performed according to OECD 409 (1998). There were no deviations from the guideline. The study is considered acceptable. In the low- and mid-dose groups no treatment-related signs were noted. However, at the top dose level of 1000 mg/kg bw/day, the maximum tolerable dose (MTD) was clearly exceeded. At this dose level which is considered the LOAEL, clinical signs were observed (liquid/soft faeces, dehydration, vomiting; incidence varying between one animal and all animals) which led to early sacrifice of two moribund animals and making termination of high dose groups after 11 weeks necessary. Further, a decreased body weight (-22% in males and -19% in females after 11 weeks), body weight gain (males: +0.5 kg vs. +2.3 kg in controls and in females: 0.5 kg vs +1.0 kg in controls) and food consumption was observed in both sexes (25-75% reduced), clinical chemistry (between -17% and +321% regarding blood chemistry and depending on the effect, see Volume 3) and urine parameters were altered (decrease in mean specific gravity in 1/3 males and 3/3 females; increase in mean urinary volume accompanied by less marked colour of urine in 3/3 females), prostate (2/3 males vs 0/4 in the control group) and uterus atrophy (3/3 females vs. 0/4 in the control group) was seen and histological lesions in many organs (such as kidney liver, bone marrow) related to the moribund state of the dogs. The NOAEL is set at 300 mg/kg bw/day.

In the second study (1816, 1999), three treated groups of four male and four female Beagle dogs received glyphosate technical (batch (expiry dates) 01.12.1997 & 01.06.1997, purity > 95%) at dietary dose-levels of 0, 200, 2000 or 10000 ppm (corresponding to 5.2, 54.2 or 252.4 mg/kg bw/day in males and 5.4, 52.8 and 252.7 mg/kg bw/day in females) for 90 days. The study is a GLP-study and is in compliance with OECD 409 (1981). The study was considered acceptable as deviations from the current version of OECD 409 (1998) are mainly due to the fact that the study was aligned to an older version of the OECD test guideline. Deviations were that detailed clinical observations were only performed monthly, not weekly and urinalysis was only performed at study termination. Several organ weights were missing: epididymides, ovaries, uterus, thymus, spleen, brain, heart; several organs were not sampled: gross lesions, spinal cord, eyes with optic nerve, trachea, skin, mammary gland, prostate or other accessory sex organs. In the low- and mid-dose groups no treatment-related signs were noted. At the top dose of 10000 ppm which is considered the LOAEL, a decreased food consumption was observed in both sexes in the second week of treatment (-47% in males and -37% in females). Further, increased levels of GGT (+171% in males and +91% in females after 45 days) and alkaline phosphatase (+129% in males after 45 days) were also observed in

high-dose animals. In addition, higher levels of total bilirubin were seen at all dose levels (+98% in males and +79% in females after 90 days), however, as no effects were seen on the liver, only the increased levels at the top dose were considered adverse as these were accompanied by increased GGT and ALP levels. Based on these findings, the NOAEL of the study is 2000 ppm (corresponding to 54.2 mg/kg bw/day in males and 52.8 mg/kg bw/day in females).

In the third sub-chronic oral toxicity study ([redacted]/P/1802, 1996), glyphosate acid (batch D4490/1, P18, purity 99.1%) was administered to groups of four male and four female Beagle dogs at dose levels of 0 (control), 2000, 10000 or 50000 ppm (equivalent to 0, 68, 323 or 1680 mg/kg bw/day for males and 0, 68, 334 or 1750 mg/kg bw/day for females) a period of 90 days. The study is in compliance with GLP and performed according to OECD 409 (1981). The study was considered acceptable as deviations from the current version of OECD 409 (1998) are mainly due to the fact that the study was aligned to an older version of the OECD test guideline. Deviations were missing organ weights (heart, thymus, spleen and uterus) and microscopic examination of spinal cord was performed only at lumbar level. No adverse effects were reported at the low dose of 2000 ppm. At the top dose of 50000 ppm, which is considered the LOAEL, an adverse decrease in body weight gain in males (between -18% and -35%) and females (between -8% and -41%) and a decreased in plasma calcium levels in males (-4% to -7%) was seen. Based on these finding, the NOAEL of the study is 10000 ppm (equivalent to 323 mg/kg bw/day in males and 334 mg/kg bw/day in females).

In the fourth sub-chronic oral toxicity study ([redacted] 94-0158, 1996), groups of 4 male and 4 female Beagle dogs were given glyphosate technical (HR-001, batch T-950308, purity 94.61%) by incorporating it into a basal diet at a level of 0, 1600, 8000 or 40000 ppm (equivalent to 0, 39.7, 198 or 1015 mg/kg bw/day for males and 0, 39.8, 201 or 1014 mg/kg bw/day for females) for a period of 13 weeks. The study is in compliance with GLP and performed according to OECD 409 (1981). The study was considered acceptable as deviations from the current version of OECD 409 (1998) are mainly due to the fact that the study was aligned to an older version of the OECD test guideline. Deviations were that reticulocytes were not counted, clotting was not evaluated; blood chloride, sodium and potassium were not measured; and uterus and thymus not weighed. No toxicologically relevant adverse effects were observed in Beagle dogs of both sexes following the dietary treatment up to 40000 ppm for 13 weeks. There was a tendency towards a lower urinary pH in top dose females, which was also seen in other studies, however, this is not considered an adverse effect because it is attributed to the acidic properties of the test substance. As there were no adverse effects in the study, the NOAEL is 40000 ppm (equivalent to 1015 and 1014 mg/kg bw/day for males and females, respectively), the highest dose tested.

The fifth sub-chronic oral toxicity study (report number not reported, refer to CA 5.3.2/028) is not considered an acceptable study due to due to missing information on the batch and purity of the test substance and the amount of test substance in the test diet was not verified (stability, homogeneity, actual concentration). The GLP status of the study was not reported and no guideline was stated in the study report. Groups of three Mongrel dogs per sex and dose were administered glyphosate (batch and purity not reported); orally via their food at target dose levels of 0 (control group receiving 0.2% agar solution mixed in mutton soup), 100, 250 or 500 mg/kg bw/day for 90 days. Treatment-related effects were confined to a decreased body weight and food consumption in both sexes and increased liver weights in males at the top dose of 500 mg/kg bw/day. However, no NOAEL is proposed as the study is not considered acceptable.

The sixth study (810166, 1983) was performed in accordance with GLP and OECD 409 (1981). In this study, the isopropylamine salt of glyphosate was orally administered by gelatine capsule to groups of six male and six female Beagle dogs at daily doses of 0, 10, 60 or 300 mg/kg bw/day for approximately six months. The only deviations from OECD 409 was that blood chloride and urine volume were not measured and it was unclear if a middle section of the spinal cord was observed microscopically. These deviations were mainly due to the fact that the study was aligned to an older version of the OECD test guideline 409. No adverse effects were reported at 10 and 60 mg/kg bw/day. At the highest dose level of 300 mg/kg bw/day, which is considered the LOAEL, a decreased body weight was observed in males (-13%) at the end of the study. Based on this observation, the NOAEL of the study is 60 mg/kg bw/day.

The seventh sub-chronic oral toxicity study (8011, 1981) is not considered an acceptable study due to serious reporting deficiencies as the purity and manufacturer of the test substance is not reported and concentration, homogeneity and stability of the test substance was not verified in the test diet. The submitted report is a revised English version of the original Hungarian report from 1981. In the revised version, reporting tables of body weight, food consumption, haematology, clinical chemistry and organ weights were missing. Only histopathology results were adequately reported. These results showed a histopathological feature called "indistinct structure" in the liver in two high dose males and in all high dose females. This change was also seen at the mid dose (600 ppm) level in a smaller number of dogs (2 males and one female). The histopathological change was characterised by round shaped

and enlarged hepatocytes and occasionally also by the narrowing of some of the hepatocytic trabeculae and slight dissociation of the liver structure. In addition, congestion of the liver was noted in three males and all female dogs in the highest dose group. As already indicated in the first evaluation of this study in the DAR, it should be taken into account that similar liver effects were also seen in the 12-month dog study from the same laboratory (8012, 1982) but were not seen in any other dog study with glyphosate obtained from other manufacturers. As the study is not considered acceptable no NOAEL is being proposed.

In the first one-year dog study (29647 [redacted], 2007), toxicity potential of glyphosate technical (batch H05H016A, purity 95.7%) was assessed in male and female Beagle dogs. Groups of four dogs per sex received daily doses (gelatine capsules) of 0, 30, 125, or 500 mg/kg bw/day for 52 consecutive weeks. The study was performed under GLP and was in compliance with OECD 452 (1981). There were no deviations from the current guideline and the study was considered acceptable. In the study, no treatment-related effects were reported except a reduced body weight gain in males (-29%) treated at 500 mg/kg bw/day, which is considered the LOAEL. Based on these effects, the NOAEL of the study is 125 mg/kg bw/day.

The second one-year dog study ([redacted] 94-0157, 1997) was conducted in Beagle dogs of both sexes. Groups of 4 dogs/sex each were given glyphosate technical (HR-001, batch T-940308, purity 94.61%) by incorporating it into basal diet at a level of 0, 1600, 8000 or 50000 ppm (equivalent to 0, 34.1, 182 or 1203 mg/kg bw/day for males and 0, 37.1, 184 or 1259 mg/kg bw/day for females) for a period of 12 months. The study was GLP-compliant and performed in compliance with OECD 409 (1981) and OECD 452 (1981). There were few deviations (blood clotting time parameters were not evaluated and epididymis and uterus weights were not reported), which were due to the fact that the study was aligned to older versions of the OECD test guidelines. The study was considered acceptable. At the low and mid dose groups of 1600 and 8000 ppm, respectively, no treatment-related effects were observed in either sex. At the top dose, which is considered the LOAEL, loose stool was reported in all animals except one female. A lower bodyweight at termination was seen in top dose females (-11%) only, whereas body weight gain was decreased in both sexes (-19% in males and -35% in females). A lower urinary pH was noted in both sexes, however, this is not considered adverse as this effect may be attributed by the acidity of the test substance. Females treated at the highest dose level were slightly anaemic (-14%, -14%, and -18% in Ht, Hb, and RBC count, respectively) and showed changes in blood electrolytes (up to -28% in inorganic phosphorus). Further, an increased frequency of slight focal pneumonia was noted in top females (1/4, 1/4, 1/4, and 4/4 at 0, 1600, 8000, and 50000 ppm). In addition, a higher thyroid weight was noted in males (+36%), which both showed c-cell hyperplasia in the thyroid. Based on these findings observed at the highest dose level of 50000 ppm, the NOAEL is set at 8000 ppm (equivalent to 182 and 184 mg/kg bw/day for males and females, respectively.

In the third one-year dog study in the RAR ([redacted]/P/5079, 1996) groups of four male and four female Beagle dogs were fed diets containing 0 (control), 3000, 15000 or 30000 ppm glyphosate acid (batch P24, purity 95.6%; equivalent to 0, 90.9, 440.3 or 906.5 mg/kg bw/day for males and 0, 92.1, 447.8 or 926.2 mg/kg bw/day for females) for a period of at least 1 year. The study was GLP-compliant and performed in compliance with OECD 409 (1981) and OECD 452 (1981). There were a few deviations (organ weight of heart, spleen, ovaries and uterus was not determined), which were due to the fact that the study was aligned to older versions of the OECD test guidelines. The study was considered acceptable. Adverse and treatment-related effects were confined to a decreased body weight in females (-10%) during the course of and at the end of the study at 30000 ppm, which is considered the LOAEL. Therefore, the NOAEL of the study is 15000 ppm glyphosate acid (equivalent to an overall mean dose of 447 mg/kg bw/day).

In another one-year dog study, reported as fourth study in the RAR (7502, 1990), groups of four male and four female Beagle dogs were administered glyphosate technical daily via capsule at dose levels of 0, 30, 300 or 1000 mg/kg bw/day. Glyphosate was administered from three batches (206-Jak-25-1, purity: 98.6%; 206-Jak-59-5, purity: 99.5% and 229-Jak-5-1, purity: 98.9%). The study was GLP-compliant and performed in compliance with OECD 409 (1981) and OECD 452 (1981). There were a few deviations (activated partial thromboplastin time not measured and clinical signs poorly reported in the report). As these were not considered to have an impact on the study outcome and partially due to the fact that the study was aligned to older versions of the OECD test guidelines, the study was considered acceptable. In the low and mid dose groups which were treated at 30 and 300 mg/kg bw/day, no treatment-related effects were observed. At the highest dose level, which is considered the LOAEL, changes in faecal consistency (soft/loose/liquid) were recorded more frequently. In addition, a decreased body weight gain was seen in top dose animals of both sexes (-25% in males and -19% in females). Therefore, the NOAEL of this study is 300 mg/kg bw/day.

In the fifth one-year dog study in the RAR ([redacted]-4965, 1985), glyphosate (NBP 2472136, purity 96.17%) was administered orally by gelatine capsule to groups of six male and six female Beagle dogs at daily doses of 0, 20, 100 or 500 mg/kg bw/day for approximately twelve months. The study was GLP-compliant and in general

compliance with OECD 452 (1981). There were a few deviations (urine volume not measured, spleen and uterus not weighed, unclear number and location of brain sections observed microscopically). As these were not considered to have an impact on the study outcome and were mainly due to the fact that the study was aligned to older versions of the OECD test guidelines, the study was considered acceptable. There were no treatment-related and adverse findings observed at any dose level. Therefore, the NOAEL is 500 mg/kg bw/day, the highest dose tested. It is noted that the dose levels tested were quite low compared to other repeated dose studies in dogs.

The sixth one-year dose study in the RAR (8012, 1982) is not considered to be acceptable due to serious reporting deficiencies. The purity and manufacturer of the test substance is not reported. Further, concentration, homogeneity and stability of the test substance was not verified in the test diet and reporting tables of body weight, food consumption, haematology, clinical chemistry and organ weights are missing. The submitted report is a revised English version of the original Hungarian report from 1982. As the study is not considered acceptable, no NOAEL is proposed. It is however noted that rounded hepatocytes and narrower sinusoids were observed in the livers of some (2/4) high dose male dogs and mid (2/4) and high dose (3/4) females, but not in the low dose and in the control groups. There was no further evidence of morphological or functional liver alterations and therefore the reported findings while possibly treatment-related were not considered adverse effects. These histopathological changes were also seen in the 3-month dog study from the same laboratory (8011, 1982) but were not seen in any other dog study with glyphosate obtained from other manufacturers.

Overall, the results show that the dog is of similar sensitivity as the rat when the NOAELs/LOAELs are considered. However, high dose effects may be more severe in dogs than in rats or mice, but appear somehow inconsistent among the studies. In the previous assessment, an overall NOAEL for the dog was set at 300 mg/kg bw/day. This NOAEL is no longer considered valid as in the current assessment for two 90-day dog studies a LOAEL has been set at or around this dose level (LOAELs between 252 to 300 mg/kg bw/day). For these studies a NOAEL was set at 54.2/52.8 and 60 mg/kg bw/day (report numbers 1816 (1999) and 810166 (1983), respectively). Based on these two studies, an **overall NOAEL of 60 mg/kg bw/day (the highest dose level at which no adverse effects were noted) is proposed for sub-chronic toxicity in the dog**. It is noted that this overall NOAEL is below the NOAEL set in the one-year repeated oral exposure studies in dogs. These studies resulted in NOAELs between 125 and 500 mg/kg bw/day.

Short term dermal studies

Repeated exposure to glyphosate through the dermal route was investigated in several 21/28-day studies in rats and rabbits.

In a 21-day dermal toxicity study ([redacted]/P/4985, 1996) groups of five male and five female Wistar-derived rats received 6-hour dermal applications of 0 (control), 250, 500 or 1000 mg glyphosate acid/kg bw/day. Glyphosate acid (P24, purity 95.6%) was prepared as a paste using deionised water as the control substance and vehicle. A total of 15 applications were made over a 21 day period (5 applications per week). The study was in compliance with GLP and OECD 410 (1981) and there were no deviations. The study is considered acceptable. During and at the end of the study no effects indicating systemic toxicity and no dermal irritation occurred at any dose level. Both the systemic and local NOAEL of this study are 1000 mg/kg bw/day, the highest dose tested.

In another 21-day dermal toxicity study in rats (7839, 1993) a group of 5 male and 5 female Sprague-Dawley rats was dosed daily with glyphosate (batch 229-Jak-142-6, purity 101.5%) via the dermal route of application, for a period of ca 6 h per day for 3 weeks. The group was dosed at a the limit dose level of 1000 mg glyphosate/kg bw/day. A further group of 5 males and 5 females served as control and received vehicle only (diethylphthalate) dermally. The study was a GLP study and was in general compliance with OECD 410 (1981). Deviations from OECD 410 were that the mean weight of the female rats was slightly lighter than requested (195 g instead of 200 – 300 g) and that organ weights of the adrenals were not determined. These deviations are not considered to have an impact on the study outcome. The study is considered acceptable. There were no systemic effects observed in animals dermally treated at 1000 mg/kg bw/day for three weeks. However, mild irritant effects (erythema and desquamation) were noted at the dosing site in the animals of the glyphosate-treated group (3/5 males and 5/5 females). The NOAEL for systemic effects is 1000 mg/kg bw/day, which is the limited dose for this type of study. No NOAEL for local effects could be derived. The only dose tested (1000 mg/kg bw/day) is considered the LOAEL for local effects as mild skin irritation (erythema and desquamation) was noted at this dose level.

In rabbits, toxicity potential of glyphosate technical [redacted] 214/94, 1994) was assessed after repeated dermal application to groups of male and female New Zealand whites. Doses of 0, 500, 1000 or 2000 mg/kg bw/day were applied for a 6-hour period on five consecutive days per week over 4 weeks. For application the solid test substance was mixed with water resulting in a 50% (w/v) solution. The study was a GLP study and was in compliance with OECD 410 (1981). There were no deviations and the study is considered acceptable. At all dose levels, treatment-related signs of systemic toxicity were not observed. Local effects were limited to a very slight erythema noted in one high-dose male and one low-dose female. Only the slight dermal irritation in the top dose male is considered for setting a NOAEL for local skin irritation. The local effects in the low-dose female is not further considered as no dose-response was observed. Therefore, the RMS proposed a NOAEL for local effects of 1000 mg/kg bw/day based on the skin irritation observed in high dose males. The NOAEL for systemic toxicity is 2000 mg/kg bw/day, the highest dose tested.

Two additional 21-day dermal toxicity study were performed in rabbits, however, both were considered unacceptable. In the first study (report number not reported, refer to CA 5.3.3/007, 1985) glyphosate (batch and purity not reported) was dermally applied to the intact skin of New Zealand White rabbits for 6 hours per day. The dose levels were 0, 500, 1000, and 2000 mg/kg bw/day and the groups consisted of 3 male and 3 female rabbits per group. Treatment was performed 5 days per week for 3 consecutive weeks, then followed by a 14-day recovery period prior to sacrifice. The study was a pre-GLP study and the design was comparable to OECD 410 with the exception that sacrifice was after a 14-day recovery period for all animals instead, which is not in agreement with the OECD guideline. As also the batch and purity of the test substance is not reported, this study is considered unacceptable. The study concluded that there were no treatment-related effects up to the highest dose level, however, as the study is not considered acceptable, no NOAEL is proposed for this study.

The other study (report number [redacted]-81-195 (1982) investigated the toxicity potential of glyphosate technical (batch NBP 1992026, purity not reported) after repeated dermal application to groups of 5 male and 5 female New Zealand white rabbits on intact and on abraded skin. Doses of 0, 100, 1000 or 5000 mg/kg bw/day were applied five days per week for three consecutive weeks. It has to be noted that the surface areas covered (i.e. 1 – 2%, 5 – 10% and 15 – 20% body surface area for the low, mid- and high-dose group, respectively) were below and above the area of 10% recommended by actual guidelines. Due to the higher exposed surface area in the high dose group, it has to be considered that more test substance can be absorbed through the skin and could be therefore systemically available. The study was a GLP-compliant study and was in general compliance with OECD 410 (1981). Deviations were that the purity and stability of the test substance was not reported, the application area in the high dose group was 1.5 to 2 times larger than the recommended 10% of body surface and the highest test dose of 5000 mg/kg bw/day fairly exceeded the limit dose of 1000 mg/kg bw/day. Mainly due to the fact that the purity and stability of the test substance was not reported, the study is considered unacceptable. Although in the previous assessment it was concluded that no systemic effects were observed up to the highest dose level, in the current assessment the increased absolute and relative kidney weights in females observed at the highest test dose of 5000 mg/kg bw/day were considered treatment-related and adverse. In addition, increased sodium levels were noted in males treated at the top dose. Local effects were confined to a a slight dermal irritation noted at 5000 mg/kg bw/day. However, as the study is not considered acceptable, no NOAEL is proposed.

Short-term inhalation study

Subacute inhalation toxicity of glyphosate technical was studied in a 14-day inhalation study in rats (report number not reported, refer to CA 5.3.3/009, 1985). Four groups of 5 male and 5 females Wistar rats were exposed to an atmosphere containing glyphosate (purity and batch not stated) in propylene glycol for 6 hours per day, 5 days per week for two weeks. There were one low and one high dose group and two intermediate dose groups. One of the latter groups was sacrificed 14 days after the treatment period had been finished (reversal group). Two control groups of the same size were also included, one of them being exposed to filtered air only and the other to an atmosphere containing the vehicle propylene glycol. Glyphosate was mixed with the vehicle and nebulised by using compressed air. The animals were exposed in a dynamic inhalation chamber by mouth and nose route by restraining them in polypropylene tubes. Target dose levels were 0, 0.25, 1 and 4.0 mg/L air and mean measured concentrations were 0, 0.28, 0.93 and 3.8 mg/L air. The study is a pre-GLP study for which no specific guideline is available. The study is not considered acceptable due to serious reporting deficiencies, e.g. absence of statistical analysis, and purity and batch number of the test substance not reported. Up to the highest concentration tested of approx. 3.8 mg/L air (mean measured concentration) repeated inhalation exposure of Wistar rats to an aerosol containing glyphosate did not lead to any local (respiratory) or systemic toxicity. No NOAEC is proposed as the study is not considered acceptable.

2.6.3.1.2 Comparison with the CLP criteria regarding STOT-RE (specific target organ toxicity-repeated exposure)

Identification of toxic effects requiring classification and labelling for specific target organ toxicity - repeated exposure (STOT-RE) is usually based on sub-acute, sub-chronic (28-days, 90-days, in dogs also 1-year) and chronic exposure studies (18 to 24 months in mice, 2 years in rats). In addition, also other study types such a reproductive or developmental toxicity studies and repeated dose neurotoxicity studies also provide relevant information on repeated dose toxicity and may possibly support a need for classification. A multitude of oral short-term studies with glyphosate was conducted mainly in rats and dogs. A smaller number of studies were performed in mice by the oral route or in rats and rabbits by dermal application. In order to identify any toxic effect requiring classification and labelling for specific target organ toxicity - repeated exposure (STOT-RE), all available valid studies were reviewed. There are no human data available relevant for the assessment of specific target organ toxicity after repeated exposure.

The following criteria for classification for specific target organ toxicity – repeated exposure are given in Regulation (EC) No 1272/2008 (CLP Regulation), Annex I, Section 3.9.2.1:

Substances are classified as specific target organ toxicants following repeated exposure by the use of expert judgement (see 1.1.1), on the basis of the weight of all evidence available, including the use of recommended guidance values which take into account the duration of exposure and the dose/concentration which produced the effect(s) (see 3.9.2.9), and are placed in one of two categories, depending upon the nature and severity of the effect(s) observed (Table 3.9.1).

Criteria for classification as STOT-RE according to Table 3.9.1 of CLP Regulation, Annex I

Category 1 (H372):

Substances that have produced significant toxicity in humans or that, on the basis of evidence from studies in experimental animals, can be presumed to have the potential to produce significant toxicity in humans following repeated exposure.

Substances are classified in Category 1 for target organ toxicity (repeat exposure) on the basis of:

- reliable and good quality evidence from human cases or epidemiological studies; or
- observations from appropriate studies in experimental animals in which significant and/or severe toxic effects, of relevance to human health, were produced at generally low exposure concentrations.

Guidance dose/concentration values for different study durations (oral only, since dermal and inhalation studies are not relevant in this case) are provided below (for reference see CLP Regulation, Annex I, Section 3.9.2.9.6):

Rat (oral):

28-days study: $C \leq 30$ mg/kg bw/day[3]

90-days study: $C \leq 10$ mg/kg bw/day

Category 2 (H373):

Substances that, on the basis of evidence from studies in experimental animals, can be presumed to have the potential to be harmful to human health following repeated exposure.

Substances are classified in Category 2 for target organ toxicity (repeat exposure) on the basis of observations from appropriate studies in experimental animals in which significant toxic effects, of relevance to human health, were produced at generally moderate exposure concentrations.

Guidance dose/concentration values for different study durations (oral only, since dermal and inhalation studies are not relevant in this case) are provided below (for reference see CLP Regulation, Annex I, Section 3.9.2.9.7):

Rat (oral):

28-days study: $30 < C \leq 300$ mg/kg bw/day[1]

90-days study: $10 < C \leq 100$ mg/kg bw/day

[3] According to the CLP Regulation, Annex I, Section 3.9.2.9.5, for a 28-day study the guidance values are increased by a factor of three (Haber's rule).

Short-term studies in rodents and non-rodents

The most relevant studies for the assessment of specific target organ toxicity after repeated exposure (STOT-RE) are sub-acute 28-day and sub-chronic 90-day repeated dose studies. For dogs, taking into account the life expectancy of dogs, exposure times of up to one year can still be considered as sub-chronic. In addition, also other study types such a reproductive or developmental toxicity studies and repeated dose neurotoxicity studies provide relevant information on repeated dose toxicity and may possibly support a need for classification.

In general, most reported effects among rat, mice and dogs studies included soft stools and diarrhoea, together with occasionally reduced body weight gain and food consumption, suggesting irritation of the gastrointestinal tract at high dose levels, as well as changes in clinical chemistry, e.g. elevated plasma alanine aminotransferase (ALT) and alkaline phosphatase (ALP) levels possibly indicative of an altered liver metabolism which was not associated with any histopathological change of the liver.

The lowest LOAEL of the acceptable 28-day studies in rats was 2500 mg/kg bw/day. At this dose level, effects of decreased body weight gain, increased ALP, increased bilirubin and soft stool were reported (study report no. 5626 (1991)). In mice, one 28-day dose-range finding study reported no effects up to a dose-level of 800 mg/kg bw/day, however, only a very limited number of parameters was investigated (report no. 77-2110 (1978)). A 28-day non-GLP public-literature study in mice reported exfoliation of renal tubular cells, upregulation of apoptosis and NMDAR1 exposure in the proximal tubule epithelium, imbalance of oxidant/antioxidant balance and a transient increase in urine albumin and urinary β2-microglobulin at a dose level of 400 mg/kg bw/day (Gao, 2019). All above described effects in the acceptable 28-day studies in rats and mice occurred at dose levels above the oral guidance values for classification.

In the 90-day studies in rats, most studies demonstrated a low toxicity of glyphosate in different rat strains upon sub-chronic repeated oral administration. Several studies showed no adverse effects up and above the limit dose of 1000 mg/kg bw/day. Toxicological effects attributed to glyphosate exposure were soft stool, diarrhoea, decreased body weight gain and food consumption, which might suggest some irritation of the gastrointestinal tract by glyphosate. Further, a decrease in urinary pH was frequently reported, which is not considered adverse as it is attributable to the acidity of the test substance. Other effects reported in rats are increased liver weight and changes in blood chemistry (increase in alkaline phosphatase, AST and ALT, increase in blood glucose). The lowest LOAEL for the aforementioned effects in 90-day studies in rats was 844 mg/kg bw/day, which observed an increase in alkaline phosphatase in females (report no. 434/016 (1996)). At dose levels far above the limit dose of 1000 mg/kg bw/day, one study reported increased kidney weights (3706 mg/kg bw/day in males and 4188 mg/kg bw/day in females; study report no. 434/016 (1996)). Further, the caecum was identified as a target organ because of certain findings (distention, elevated weight of this part of the intestines and its contents, mucosal atrophy). In two studies, effects on the caecum were reported at dose levels of 10000 ppm and above (study report no. 434/016 (1996) and study report no. [redacted] 94-0138 (1995)) of which the lowest dose level is equivalent to 569 mg/kg bw/day. All above described effects in the 90-day studies occurred at dose levels above the oral guidance values for classification for STOT-RE. However, the most critical effect observed in the 90-day studies in rats were histopathological changes in the parotid salivary gland which comprised deep basophilic staining and enlargement of cytoplasm observed in one 90-day rat study (7136 (1991)) at the lowest dose level (30 mg/kg b/day) and above. This effect is considered as potentially adverse (refer to study summary above), although the severity grade of findings observed at 30 mg/kg bw/day was minimal (very mild). These effects were observed at a dose level relevant for classification in Category 2 for STOT-RE (90-days study: $10 < C \leq 100$ mg/kg bw/day), however, due to the mild nature of the histopathological changes in the parotid salivary gland, this effect is not considered a significant effect for classification. Therefore, classification for STOT-RE is not warranted. Histopathological changes in the salivary gland with a moderate to severe severity grade were only seen at dose the highest dose level of 1000 mg/kg bw/day in the 90-day study in rats, which is above the oral guidance values for classification.

Toxicity of glyphosate to mice was investigated in a relatively small number of sub-chronic studies. Three 90-day oral repeated dose toxicity studies are available, of which only one is fully acceptable (report no [redacted] 94-0136, 1995). The other two studies are of limited value due to missing or only partial haematology and clinical chemistry investigation (report no. 7024 (1991) and report no. 77-2111 (1979)). At very high doses (>6000 mg/kg bw/day), the main observed effects were a reduction in body weight (gain), food consumption and alterations in some haematological and clinical chemistry parameters with the latter findings pointing to liver toxicity. Gross necropsy revealed caecum distention that was supported by a higher organ weight but not accompanied by histological lesions. Cystitis of urinary bladder became histologically apparent in some high dose males. In mice, no adverse effects were seen at dose levels relevant for classification for STOT-RE.

The available sub-chronic studies in Beagle dogs covering exposures from 90 days up to one year, showed the general signs of toxicity of glyphosate to be similar to that reported in rats (except for effects in salivary glands which were not observed in dogs). However, high dose effects in dogs may be more severe than in rats or mice at

equivalent dose levels but appear somehow inconsistent among the available studies. The effects reported at dose levels from 250 up to 1750 mg/kg bw/day were generally characterized by a reduction in body weight (gain), increase in clinical signs, soft/liquid stool and some effects on clinical pathology parameters. However, in one study (study report no. 29646 (2007)), the high dose group (1000 mg/kg bw/day) was terminated at week 11 due to moribund animals. The lowest LOAELs were reported in two 90-day dog studies with LOAELs of 252 mg/kg bw/day (study report no. 1816 (1999) based on a decreased food consumption and changes in some blood clinical chemistry parameters. As this is above the oral guidance values for classification for STOT-RE, no classification is warranted based on the sub-chronic dog studies.

As no significant or severe toxicity is observed below the oral guidance values, classification for STOT-RE is not warranted.

Short-term studies – other routes

In addition to short-term studies by the oral route (diet, capsule), there are several short-term toxicity studies by the dermal route available in the rat and rabbit. Of these studies, two studies in rats and one in rabbits were considered acceptable and two dermal repeated dose toxicity studies were not considered acceptable. In addition, one study is available for the inhalation route, however, this study was not considered acceptable due to serious reporting deficiencies. In this first 21-day dermal toxicity study in rats (study report no. /P/4985 (1996)), no local or systemic effects were observed up to the highest dose of 1000 mg/kg bw/day. The second 21-day study in rats was a limit dose study (1000 mg/kg bw/day) in which no systemic effects were noted but mild skin irritation was reported in 3/5 males and 5/5 females (7839 (1993)). In the rabbit study (study report no. 214/94 (1994)), also no systemic effects were reported up to the highest dose tested of 2000 mg/kg bw/day. Only a slight skin irritation was reported in males at 2000 mg/kg bw/day. As no significant or severe toxicity is observed below the dermal guidance values for a 28-day dermal toxicity study (≤ 600 mg/kg bw/day for category 2; ≤ 60 mg/kg bw/day for category 1), classification for STOT-RE is not warranted based on these repeated toxicity studies by dermal exposure.

Long-term studies in rodents

Chronic toxicity, i.e. occurrence of non-neoplastic effects in studies of longer duration, might be also relevant for a STOT RE classification. With glyphosate, a large number of long-term studies have been performed in rats and mice. The long-term (2-year) combined chronic toxicity and carcinogenicity studies and one 1-year study in rats and the carcinogenicity studies in mice (18-months or 2-year) are reported in Volume 1 in section 2.6.5.

For the long-term studies in rats, the lowest LOAEL was 100 mg/kg bw/day (Report No. 7867). At this dose level, an increased salivary gland weight and cellular alterations of the salivary gland were reported. However, these effects were observed at dose level above the oral guidance value relevant for classification in Category 2 for STOT-RE observed in long-term studies when correcting for study duration (90-day vs. 2-year). Most reported effects in the other long-term rat studies were reductions in body weight gain, increases in alkaline phosphatase and liver weight changes. Some studies reported increase in incidence of cataracts, inflammation of the gastric mucosa and increased caecum weights. As the aforementioned effects were reported at dose levels of 354 mg/kg bw/day and above, these are not relevant for STOT-RE classification.

In the mouse, non-neoplastic treatment related effects were limited to high dose animals with degenerative changes of the heart at 10000 ppm (1454 mg/kg bw/day for males and 1466.8 mg/kg bw/day for females; report no. Toxi: 1559.CARCI-M), reduced body weight (gain) at 8000 ppm (838.1 mg/kg bw/day in males and 786.8 mg/kg bw/day in females; report no. 94-0151) and urinary bladder epithelium hyperplasia (slight to mild) in males at 5000 ppm (814 mg/kg bw/day in males; Report No. 77-2061). As these effects were reported at dose levels above the oral guidance values for STOT-RE classification, these are not relevant for classification. classification.

Reproductive and developmental studies

The potential of glyphosate to cause effects on sexual function and fertility was examined in several 2-generational studies in the rat, only 6 of which could be considered fully valid or supplementary (refer to Volume 1 section 2.6.6.1). In addition, a one-generation range finding study (Report No.: 42/90619) is available but this study was considered as supplementary data and not valid for NOAEL setting. For the two-generation studies the lowest LOAEL for parental toxicity was 197 mg/kg bw/day (Report No. 47/911129). At this dose level, histopathological changes in the salivary gland were reported. However, this effect was observed at a dose level above the oral guidance value relevant for classification in Category 2 for STOT-RE (10 <C ≤100 mg/kg bw/day). Other effects observed in parental animals were soft stool (at 666 mg/kg bw/day), reduced body weight (at 666 mg/kg bw/day), reduced litter size (at 666 mg/kg bw/day), increase in liver and kidney weights (at limit dose of

1000 mg/kg bw/day), reduced prostate weight (at 2532 mg/kg bw/day), reduced fertility indices (at 2532 mg/kg bw/day), and distended caecum (at 2532 mg/kg bw/day). These effects were reported at dose levels above the oral guidance values for STOT-RE classification and were not considered relevant for STOT-RE classification.

In developmental toxicity studies general toxicity was apparent in rats and rabbits as mortality, gastrointestinal disturbances (loose faeces, diarrhoea) and reduced bodyweight gain (refer to Volume 1 section 2.6.6.2). In rats also observations of noisy respiration and salivation were observed but this occurred at or above the limit dose of 1000 mg/kg bw/day. In rabbits, these symptoms were observed at lower dose levels with LOAELs for mortality at 400 mg/kg bw (2/18; study report 434/020 (KCA 5.6.2/010)), at 300 mg/kg bw/d (1/18, not considered treatment-related; study report [redacted] 94-0153 (KCA 5.6.2/011), at 450 mg/kg bw/d (1/18; study reports [redacted] 45, 39, 40 /901303 (KCA 5.6.2/014)) and at 175 mg/kg w/d (study report 401-056 (KCA 5.6.2/019)). In two other rabbit studies mortality was observed at a dose of 100 mg/kg bw/day (study report [redacted]/P/5009 (KCA 5.6.2/009) and study report TOXI: 884-TER-RB (KCA 5.6.2/012/13). It should be noted that in one of these two studies, not all deaths were considered related to treatment (study report TOXI: 884-TER-RB (KCA 5.6.2/012/13). Two dams in the control group died due to mis-dosing whereas four mid-dose dams (4/16 at 100 mg/kg bw/d) and eight high-dose dams (8/15 at 500 mg/kg bw/d) died apparently as a consequence of treatment. The applicant considers the pathological examination to indicate that two of the deaths among high dose animals and one of the deaths in mid dose could be due to gavage errors (i.e. congestion in lung, trachea, froth in lung). However, the study author has categorised all deaths in mid and high dose animals treatment-related rather than accidental. The deaths observed in the other study were not considered related to treatment since intercurrent deaths occurred in all groups (one in the control and two in each of the 100, 175 and 300 mg/kg/bw/day groups) (study report [redacted]/P/5009 (KCA 5.6.2/009)). Similar to the dam in the control group, the dams in the 100 mg/kg bw/d dose group died after showing slight body weight loss and reduced food consumption. As discussed in Volume 1 section 2.6.6.2.1, rabbits ingest their caecotrophes which may result in an increased exposure to glyphosate as it is excreted unchanged in faeces. Since the substance causes gastrointestinal irritation that results in soft stools and diarrhoea, coprophagy may then be difficult and lead to undernourishment of the rabbits.

Although treatment-related mortality is seen in two rabbit developmental toxicity studies (study report TOXI: 884-TER-RB (KCA 5.6.2/012/13) and study report 401-056 (KCA 5.6.2/019)) at dose levels within the oral guidance value range for classification for STOT-RE category 2 (with the value for a 28-days study of $30 < C \leq 300$ mg/kg bw/day taken as a surrogate), this is not considered relevant for classification as there is uncertainty on the actual exposure of the rabbit due ingestion of caecotrophes which may contain unabsorbed glyphosate (refer to Volume 1 section 2.6.10 for a further explanation). This may lead to an increased exposure to glyphosate as it is excreted unchanged in faeces.
During the previous assessment, in the CLH report (2016) classification for STOT-RE Category 2 was proposed based on the maternal toxicity as observed in the developmental studies in rabbits.
However, RAC concluded that STOT-RE classification is not justified based on a weight of evidence approach (refer to Box 1 below). As no new findings or new evidence was provided in the current assessment, the RMS proposes to align to decision by RAC that classification for STOT-RE is not needed.

Box 1. RAC evaluation of maternal toxicity in developmental studies in rabbits (copied from page 21 et seq. RAC opinion 2017)

According to Annex I: 3.9.2.9.7 of CLP "Classification in Category 2 is applicable, when significant toxic effects observed in a 90-day repeated dose study…are seen to occur within…" a range of $(10 < C \leq 100)$ mg/kg bw/d via oral exposure in the rat. Applying Haber's rule for a study of shorter duration (28 days) allows for extrapolation of the guidance values to a range of $(30 < C \leq 300)$ mg/kg bw/d via the oral route. However, in this case the use of Haber's rule to correct the guidance values includes uncertainties and the results should be used with caution.
The DS described excessive maternal toxicity as a number of unscheduled, treatment-related deaths in 5 out of 7 rabbit developmental studies within a dose range of 100 to 500 mg/kg bw/d. On this basis the DS proposed classification as STOT RE 2. Certainly, large doses of glyphosate are associated with severe maternal toxicity and death in female rabbits. However, the overall weight of evidence for classification is unconvincing due to the following reasons:

1. Strictly, there are only 2 studies with deaths reported below the corrected guidance value, i.e. 4 female rabbits in the [redacted] (1993) study at 100 mg/kg bw/d and 8 female rabbits at 500 mg/kg bw/d, and 2 female rabbits in the [redacted] (1980) study at 175 mg/kg bw/d and 10 female rabbits at 350 mg/kg bw/d where several of the deaths in each study could be related to mal-gavage.

2. In the [redacted] (1993) study, pathological changes in the lungs were noted in one of the dead animals at the 100 mg/kg bw/d and were suggestive of gavage errors. The remaining 3 decedents in the 100 mg/kg bw/d dose-group had no abnormalities and there were no reported clinical signs at this dose level. Five out of 8 mortalities in the high dose group also displayed pathological changes suggestive of gavage errors. The remaining 3 decendents in the 500 mg/kg bw/d group had no abnormalities. Soft stool and diarrhoea was reported, however, a clear association with premature death cannot be established. There were also 2 mis-dosings in the concurrent controls. Overall the frequent reporting of pathological findings in the lung suggestive of gavage errors raises concern regarding the technical skills in dosing via oral gavage and consequently also on the inclusion of this study in the assessment of substance induced mortality.

3. In the [redacted]. (1980) study 1, 1 and 3 premature deaths at 75, 175 and 350 mg/kg bw/d, respectively, out of 1, 2 and 10 premature deaths at these dose levels were reported to be due to pneumonia, respiratory disease, enteritis or gastroenteritis; the remaining death was unexplained.

4. Five of the studies included in the table “Rabbit maternal mortality and toxicity from developmental studies with glyphosate” with dosing over the range 50 to 450 mg/kg bw/d did not reveal signs of an increased mortality as observed in the study by [redacted]. (1993) and [redacted] (1980).

5. The majority of deaths were associated with high doses of glyphosate and the majority of deaths were associated with 2 studies where the cause of death is unclear.

6. The physiology of digestion in the rabbit is in some ways unique. In rabbits, caecotrophy ensures that substances predominantly excreted unchanged in the faeces such as glyphosate are readily available for repeated oral uptake and constitute a potentially significant oral dose relative to other species including humans. This possible recycling of glyphosate and increased exposure in rabbits might explain the particular sensitivity of this species while at the same time casting doubt over the relevance of oral dosing in rabbit studies for humans. However, there is a lack of information regarding whether the rabbits were able to eat their caecotrophes or not, and therefore it is not possible to have a clear picture of a possible recycling of glyphosate and consequently the actual dose absorbed from the GI tract, leading to uncertainties with using Haber's rule to correct the guidance value for a STOT RE classification in these studies.

7. Signs of digestive disturbances (soft/liquid stool and diarrhoea) were consistently reported in the rabbit studies (but also in rats at much higher doses). However, a clear association with premature maternal death cannot be established. The fact that the female rabbits appear to be uniquely sensitive compared to rodent dams further support the the caecotrophy hypothesis and weakens the argument for classification in this case.

Furthermore, an in-depth analysis of all the data from both the short-term and long-term toxicity studies only shows effects at high dose levels exceeding the extrapolated guidance values relevant for a classification with STOT RE.
Mortality in female rabbits has been used to justify the proposal for classification of glyphosate for STOT RE 2 by the DS. According to CLP, Annex I, section 3.9.2.7.3, morbidity or death resulting from repeated or long-term exposure can be taken into account for classification as STOT RE. However, CLP further states that "Morbidity or death may result from repeated exposure, even to relatively low doses/concentrations, due to bioaccumulation of the substance or its metabolites, and/or due to the overwhelming of the de-toxification process by repeated exposure to the substance or its metabolites".
Following exposure to glyphosate, mortality in rabbits is considered to either be related to mis-dosing, infections or diarrhea and the possible mechanism of caecotrophy and recycling of glyphosate. No mortalities were recorded in the rat studies. In addition, bioaccumulation and over-whelming of detoxification mechanisms by repeated exposure as a mechanism of toxicity is not likely for glyphosate.
On the basis of a weight of evidence approach and with due consideration of all data from the short-term, long-term, reproductive and rabbit developmental studies, RAC concludes that **STOT RE classification is not justified** for glyphosate.

Neurotoxicity studies

Two acceptable 90-day sub-chronic neurotoxicity studies are available (refer to Vol 1 section 2.6.7). In the first study, a decreased body weight (gain) and reduced food consumption was observed at the highest dose level of 20000 ppm in males only (dose level equivalent to 1499 mg/kg bw/day; study report no. 2060-0010, 2006). In the second study ([redacted]/P/4867, 1996) the findings were comparable with a decreased body weight gain in males at

20000 ppm (equivalent to 1547 mg/kg bw/day). As no significant or severe toxicity is observed below the oral guidance values, classification for STOT-RE is not warranted based on these studies.

2.6.3.1.3 Conclusion on classification and labelling for STOT-RE (specific target organ toxicity-repeated exposure)

No classification is proposed for glyphosate for STOT-RE (specific target organ toxicity – repeated exposure).

2.6.4 Summary of genotoxicity / germ cell mutagenicity [equivalent to section 10.8 of the CLH report template]

Genotoxicity and mutagenicity of glyphosate were examined in several test systems covering all relevant endpoints *in vitro* (in bacterial and mammalian cells) and *in vivo* (in both somatic and germ cells). In addition, several publications from the open literature have been evaluated and included in the tables below.

In the previous CLH report (BAuA, 2016), the following was mentioned: "in addition to the studies with glyphosate, a large number of published studies with formulations containing glyphosate are available which were tested for different mutagenicity and genotoxicity endpoints in a variety of *in vitro* and *in vivo* mammalian and non-mammalian test systems. A part of these studies revealed positive or at least equivocal results in particular when testing was performed in non-standard systems and when so-called "indicator tests" were employed. It is likely that such results were rather due to co-formulants than to glyphosate. Therefore, they cannot be taken into account for classification of glyphosate for mutagenicity. Furthermore, against the background of an extremely large database using standard test systems (bacteria, mammalian cells and mammals), data obtained in non-standard test systems (e.g. plant, insect, worm, fish etc.) was not considered for classification of health related endpoints even if performed with the active ingredient." The current assessment has been carried out on the same grounds.

Table 49: Summary table of genotoxicity/germ cell mutagenicity tests *in vitro*

Gl	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations /Results	Reference
***In vitro* bacterial gene mutation assays**				
OECD 471 (1997) Study acceptable	Glyphosate technical (refer to Vol 4)	Ames test, ±S9, 3-5000 µg/plate (standard plate test), 33-5000 µg/plate (pre-incubation test), Strains: *S. typhimurium* TA 98, TA 100, TA 1535 and TA 1537 and *E. coli* WP2 *uvrA*	No relevant increase in the number of revertants observed in any experiment with the tested concentrations in the presence or absence of metabolic activation. The test substance is considered non-mutagenic under the conditions of this study.	Report no. 1989201 (2020) Submitted as Vol 4 confidential information
OECD 471 (1997) Study acceptable	Glyphosate technical (refer to Vol 4)	Ames test, ±S9, 50-5000 µg/plate (standard plate test), 50-5000 µg/plate (pre-incubation test), Strains: *S. typhimurium* TA 97a, TA 98, TA 100, TA 102 and TA 1535	No relevant increase in the number of revertants observed in any experiment with the tested concentrations in the presence or absence of metabolic activation. The test substance is considered non-mutagenic under the conditions of this study.	Report no. 18925 (2019) Submitted as Vol 4 confidential information
OECD 471, GLP No significant deviations Study acceptable	Glyphosate Batch: 04062014 Purity: 85.79%	Ames test, ±S9, 1.5-5000 µg/plate (standard plate test), 5-5000 µg/plate (pre-incubation test), Strains: *S. typhimurium* TA 98, TA 100, TA 1535 and TA 1537 and *E. coli* WP2 uvrA	No relevant increase in the number of revertants observed in any experiment with the tested concentrations in the presence or absence of metabolic activation. The test substance is considered non-mutagenic under the conditions of this study.	CA 5.4.1/001; Report no. 41401854 (2014)
OECD 471, GLP No significant deviations Study acceptable	Glyphosate Tech. Batch: 20110107-2 Purity: 97%	Ames test, ±S9, 10-5000 µg/plate (standard plate test and pre-incubation test), Strains: *S. typhimurium* TA 98, TA 100, TA 102, TA 1535 and TA 1537	No relevant increase in the number of revertants observed in any experiment with the tested concentrations in the presence or absence of metabolic activation. The test substance is considered non-mutagenic under the conditions of this study.	CA 5.4.1/002; Report no. 126159 (2012)
OECD 471, GLP No HCD for the positive control, limited HCD for the negative control.	Glyphosate technical Batch: 2009051501 Purity: 95.23%	Ames test, ±S9, 31.6-3160 µg/plate (standard plate test and pre-incubation test), Strains: *S. typhimurium* TA 98, TA 100, TA 102, TA 1535 and TA 1537	No relevant increase in the number of revertants observed in any experiment with the tested concentrations in the presence or absence of metabolic activation. The test substance is considered non-mutagenic under the conditions of this study.	CA 5.4.1/003; Report no. 24880 (2010)

Study acceptable but with restrictions				
OECD 471, GLP 2-AA as sole positive control; no impact on study outcome expected Study acceptable	Glyphosate Tech spiked with glyphosine. Batch: 2009051501 (glyphosate); 1438405 (glyphosine) Purity: glyphosate technical grade (purity 97.16% w/w), containing 0.63% (w/w) glyphosine in the technical grade active ingredient	Ames test, ±S9, 3-5000 µg/plate (standard plate test and pre-incubation test), Strains: *S. typhimurium* TA 98, TA 100, TA 1535 and TA 1537 and *E. coli* WP2 uvrA	No relevant increase in the number of revertants observed in any experiment with the tested concentrations in the presence or absence of metabolic activation. The test substance is considered non-mutagenic under the conditions of this study.	CA 5.4.1/004; Report no. 1332300 (2010)
OECD 471, GLP 2-AA as sole positive control; no impact on study outcome expected Study acceptable	Glyphosate Tech. Batch: 200903051 Purity: 98.2%	Ames test, ±S9, 31.6-5000 µg/plate (standard plate test and pre-incubation test), Strains: *S. typhimurium* TA 98, TA 100, TA 102, TA 1535 and TA 1537	No relevant increase in the number of revertants observed in any experiment with the tested concentrations in the presence or absence of metabolic activation. The test substance is considered non-mutagenic under the conditions of this study.	CA 5.4.1/005; Report no. 101268 (2010)
OECD 471, GLP No HCD for the positive control, limited HCD for the negative control. Study acceptable but with restrictions	Glyphosate technical Batch: 20080801 Purity: 98.8%	Ames test, ±S9, 31.6-3160 µg/plate (standard plate test and pre-incubation test), Strains: *S. typhimurium* TA 98, TA 100, TA 102, TA 1535 and TA 1537	No relevant increase in the number of revertants observed in any experiment with the tested concentrations in the presence or absence of metabolic activation. The test substance is considered non-mutagenic under the conditions of this study.	CA 5.4.1/006; Report no. 23916 (2009)
OECD 471, GLP 2-AA as sole positive control; no impact on study outcome expected	Glyphosate technical Batch: 569753 Purity: 96.3%	Ames test, ±S9, 3-5000 µg/plate (standard plate test), 33-5000 µg/plate (pre-incubation test), Strains: *S. typhimurium* TA 98, TA 100, TA 1535 and TA 1537 and *E. coli* WP2 uvrA (pKM101) and WP2 pKM101	No relevant increase in the number of revertants observed in any experiment with the tested concentrations in the presence or absence of metabolic activation for strains TA 98, TA 100, TA 1535 and TA 1537. For strains WP2 uvrA (pKM101) and WP2 pKM101, the number of	CA 5.4.1/007; Report no. 1264500 (2009)

Study acceptable			revertants were above or below the limits of the HCD in the concurrent untreated and vehicle control, as well as multiple test item concentrations, but these observations are not considered to be biologically relevant. In conclusion, the test substance is considered non-mutagenic under the conditions of this study.	
OECD 471, GLP 2-AA as sole positive control (no impact on study outcome expected); test item only tested up to 1000 µg/plate; no repeat experiment; no HCD available Study **not** acceptable	Glyphosate technical Batch: 20070606 Purity: 98.05%	Ames test, ±S9, 1-1000 µg/plate (standard plate test), Strains: *S. typhimurium* TA 98, TA 100, TA 1535, TA 97a, and TA102	The study is not considered acceptable, mainly because the test item was not tested at sufficiently high concentrations (refer to first column for other deviations). Therefore, no final conclusion can be made. Based on this study, however, no indications for mutagenicity were obtained.	CA 5.4.1/008; Report no. RF-3996.401.392.07 (2008)
OECD 471, GLP No reporting of cell density; 2-AA as sole positive control. No impact on study outcome expected. Study acceptable	Glyphosate technical (NUP-05068) Batch: 200609062 Purity: 95.1%	Ames test, ±S9, 3-5000 µg/plate (standard plate test), 33-5000 µg/plate (pre-incubation test), Strains: *S. typhimurium* TA 98, TA 100, TA 1535 and TA 1537 and *E. coli* WP2 uvrA	No relevant increase in the number of revertants observed in any experiment with the tested concentrations in the presence or absence of metabolic activation for strains TA 98, TA 100, TA 1535 and TA 1537, as well as WP2 uvrA in experiment II. In conclusion, the test substance is considered non-mutagenic under the conditions of this study.	CA 5.4.1/009; Report no. 1061401 (2007)
OECD 471, GLP No reporting of cell density; 2-AA as sole positive control. No impact on study outcome expected Study acceptable	Glyphosate technical (NUP-05070) Batch: 20060901 Purity: 97.7%	Ames test, ±S9, 3-5000 µg/plate (standard plate test), 33-5000 µg/plate (pre-incubation test), Strains: *S. typhimurium* TA 98, TA 100, TA 1535 and TA 1537 and *E. coli* WP2 uvrA	No relevant increase in the number of revertants observed in any experiment with the tested concentrations in the presence or absence of metabolic activation. The test substance is considered non-mutagenic under the conditions of this study.	CA 5.4.1/010; Report no. 1061402 (2007)

OECD 471, GLP No reporting of cell density; 2-AA as sole positive control. No impact on study outcome expected Study acceptable	Glyphosate technical (NUP-05067) Batch: 0609-1 Purity: 95.0%	Ames test, ±S9, 3-5000 µg/plate (standard plate test), 33-5000 µg/plate (pre-incubation test), Strains: *S. typhimurium* TA 98, TA 100, TA 1535 and TA 1537 and *E. coli* WP2 uvrA	No relevant increase in the number of revertants observed in any experiment with the tested concentrations in the presence or absence of metabolic activation. The test substance is considered non-mutagenic under the conditions of this study.	CA 5.4.1/011; Report no. 1061403 (2007)
OECD 471, GLP 2-AA as sole positive control and no HCD available for the positive control; no repeat experiment was performed. Study **not** acceptable	Glyphosate technical (Glifosato Téchnico Helm) Batch: 2007091801 Purity: 98.01%	Ames test, ±S9, 648-5000 µg/plate (standard plate test) Strains: *S. typhimurium* TA 98, TA 100, TA 102, TA 1535 and TA 1537	The study is not considered acceptable, mainly because no repeated experiment was performed (refer to first column for other deviations). Therefore, no final conclusion can be made. Based on this study, however, no indications for mutagenicity were obtained.	CA 5.4.1/012; Report no. RL3393/2007-2.0AM-B (2007)
OECD 471, GLP No HCD; cell density, cytotoxicity and precipitation not reported; 2-AA as sole positive control Study acceptable but with restrictions	Glyphosate acid Batch: P24 Purity: 95.6%	Ames test, ±S9, 100-5000 µg/plate (standard plate test and pre-incubation test), Strains: *S. typhimurium* TA 98, TA 100, TA 1535 and TA 1537 and *E. coli* WP2 uvrA and WP2P (WP2 pKM101)	No relevant increase in the number of revertants observed in any experiment with the tested concentrations in the presence or absence of metabolic activation. The test substance is considered non-mutagenic under the conditions of this study.	CA 5.4.1/013; Report no. CTL/P/4874 (1996)
OECD 471, GLP No HCD; Cytotoxicity not reported in detail; 2-AA as sole positive control; Repeat experiment identical to first experiment	Technical glyphosate Batch: H95D161A Purity: 95.3%	Ames test, ±S9, 50-5000 µg/plate (both assays with standard plate test), Strains: *S. typhimurium* TA 98, TA 100, TA 1535 and TA 1537 and *E. coli* WP2 uvrA	No relevant increase in the number of revertants observed in any experiment with the tested concentrations in the presence or absence of metabolic activation. The test substance is considered non-mutagenic under the conditions of this study.	CA 5.4.1/014; Report no. 434/014 (1996)

Study acceptable but with restrictions				
OECD 471 GLP No HCD; In the repeat-experiment, no parameter was changed. Study acceptable but with restrictions	Technical glyphosate Batch: 940908-1 Purity: 95.68%	Ames test, ±S9, 156-5000 µg/plate (standard plate test and pre-incubation test), Strains: *S. typhimurium* TA 98, TA 100, TA 1535 and TA 1537 and *E. coli* WP2 uvrA	No relevant increase in the number of revertants observed in any experiment with the tested concentrations in the presence or absence of metabolic activation. The test substance is considered non-mutagenic under the conditions of this study.	CA 5.4.1/015; Report no. IET 94-0142 (1995)
OECD 471 GLP Purity not reported Study **not** acceptable	Technical glyphosate Batch: NC01 Purity: Not reported	Ames test, ±S9, 50-5000 µg/plate. Strains: *S. typhimurium* TA 98, TA 100, TA 102, TA 1535 and TA 1537.	The study is not considered acceptable as the purity of the test substance was not reported. Therefore no final conclusion can be made. Based on this study, however, no indications for mutagenicity were obtained.	CA 5.4.1/016; Report no. 940724 (1995)
OECD 471 GLP Batch and purity not reported. Conducted in 4 valid strains only. Strains like *S. typhimurium* TA 102 or *E. coli* WP2 enabling the detection of cross-linking mutagens not included. Study **not** acceptable	Technical glyphosate Purity and batch: not reported	Ames test, ±S9, 8-5000 µg/plate. Strains: *S. typhimurium* TA 98, TA 100, TA 1535, TA 1537 and TA 1538.	The study is not considered acceptable as the purity and batch of the test item was not reported and the test was conducted in four valid strains only. Therefore no final conclusion can be made. Based on this study, however, no indications for mutagenicity were obtained.	CA 5.4.1/017; Report no. 710/20 (1995)
OECD 471 GLP	Technical glyphosate Batch: 046 Purity: 96%	Ames test, ±S9, 1-1000 µg/plate (both assays with standard plate incorporation test)	No relevant increase in the number of revertants observed in any experiment with the tested concentrations in the presence or absence of metabolic activation. The test substance is	CA 5.4.1/018; Report no. 887-MUT.AMES (1993)

No HCD; Conducted in four valid strains only. Strains like *S. typhimurium* TA 102 or *E. coli* WP2 enabling the detection of cross-linking mutagens not included. Bacterial cell density and acceptance criteria were not confirmed or specified. Study supportive		Strains: *S. typhimurium* TA 98, TA 100, TA 1535, TA 1537 and TA 1538	considered non-mutagenic under the conditions of this study.	
OECD 471 GLP No HCD; Only up to 100 µg/plate is used. Conducted in two strains; No confirmatory experiment included. Study **not** acceptable	Technical glyphosate Batch: not reported Purity: 64%	Ames test, ±S9, 0.01-100 µg/plate. Strains: *S. typhimurium* TA 98 and TA 100.	The study is not considered acceptable due to the large number of deviations (refer to deviations in the first column). Therefore, no final conclusion can be made. Based on this study, however, no indications for mutagenicity were obtained.	CA 5.4.1/019; Report no. 87BMA012-E (1993)
OECD 471 GLP No HCD; Conducted in four valid strains only. Strains like *S. typhimurium* TA 102 or *E. coli* WP2 enabling the detection of cross-linking mutagens not included.	Technical glyphosate Batch:206-JaK-25-1 Purity: 98.6%	Ames test, ±S9, 160-2500 µg/plate in the absence of S9 mix and 310-5000 µg/plate in the presence of S9 mix (standard plate test and pre-incubation test), Strains: *S. typhimurium* TA 98, TA 100, TA 1535 and TA 1537	No relevant increase in the number of revertants observed in any experiment with the tested concentrations in the presence or absence of metabolic activation. The test substance is considered non-mutagenic under the conditions of this study.	CA 5.4.1/020; Report no. 12323 (1991)

Acceptance and evaluation criteria not specified. 2-aminoanthracene used as sole positive control Study supportive				
OECD 471 GLP No HCD; Strains like *S. typhimurium* TA 102 or *E. coli* WP2 enabling the detection of cross-linking mutagens not included. Purity not reported. 2-aminoanthracene used as sole positive control (+S9 mix) Study **not** acceptable	Technical glyphosate Batch: 0190A Purity: not reported	Ames test, ±S9, 8-5000 µg/plate in the first experiment and 312.5-5000 µg/plate in the second experiment, Strains: *S. typhimurium* TA 98, TA 100, TA 1535, TA 1537 and TA 1538.	The study is not considered acceptable due to the large number of deviations (refer to deviations in the first column). Therefore, no final conclusions can be made. Based on this study, however, no indications for mutagenicity were obtained.	CA 5.4.1/021; Report no. 300/1 (1990)
OECD 471 GLP No HCD; Purity/batch not reported. No correct controls Study **not** acceptable	Glyphosate Batch: not reported Purity: not reported	Ames test, ±S9, 10-1000 µg/plate. Strains: *S. typhimurium* TA 98, TA 100, TA 1535, TA 1537 and *E. coli* strain WP2 uvrA.	The study is not considered acceptable due to the large number of deviations (refer to deviations in the first column). Therefore, no final conclusions can be made. Based on this study, however, no indications for mutagenicity were obtained.	CA 5.4.1/022; Report no. not reported (1986)
No guideline followed No GLP (not compulsory)	Glyphosate Batch: not reported Purity: not reported	Ames test, ±S9, 1-1000 µg/plate. Strains: *S. typhimurium* his-G46, TA 1537 and TA 1538.	The study is not considered acceptable due to the large number of deviations (refer to deviations in the first column). Therefore, no final conclusions can be made. Based on this study, however, no indications for mutagenicity were obtained.	CA 5.4.1/023; Report no. 710/20 (1981)

Only two valid strains used. Purity/batch not reported. Poor description materials &methods Study **not** acceptable				
No guideline followed No GLP (not compulsory) No HCD; Instead *of E. coli* strain WP2 uvrA strain WP2 hcr was used. 2- aminoanthracene used as sole positive control (+S9 mix) Cytotoxicity and precipitation data not reported. Study supportive	Glyphosate Batch: XHJ-46 Purity: 98.4%	Ames test, ±S9, 10-5000 µg/plate. Strains: *S. typhimurium* TA98, TA100, TA 1535, TA 1537, TA 1538 and *E.coli* strain WP2 hcr.	No relevant increase in the number of revertants observed in any experiment with the tested concentrations in the presence or absence of metabolic activation. The test substance is considered non-mutagenic under the conditions of this study.	CA 5.4.1/024; Report no. ET-78-241 (1978)
U.S. EPA FIFRA Guidelines, Subdivision F GLP Rec assay not a standard method for the endpoint (DNA damage and repair). The dose selection not explained and viability data not included in the study report.	Glyphosate Batch: HR-001, 940908-1 Purity:95.68%	DNA repair test (Rec-assay), ±S9, 7.5-240 µg/disk	No relevant DNA-damaging activity in the presence or absence of metabolic activation under the conditions of this study. The test substance is considered non-mutagenic under the conditions of this study.	CA 5.4.1/035; Report no. IET 94-0141 (1995)

Study supportive				
U.S. EPA FIFRA Guidelines, Subdivision F No GLP Rec assay not a standard method for the endpoint (DNA damage and repair). No viability data (actual plate count) provided. Some reporting deficiencies Study **not** acceptable	Glyphosate Batch: XHJ-46 Purity: 98.4%	DNA repair test (Rec- assay), ±S9, 20-2000 µg/disk	The study is not considered acceptable as the study was not conducted under GLP and not according to current testing guidelines. The test was performed in the absence of S9 mix only and no viability data (actual plate count) were provided. In addition, there were some reporting deficiencies. The study is therefore considered not acceptable and no final conclusion can be made. Based on this study, however, no indications for mutagenicity were obtained.	CA 5.4.1/036; Report no. ET-78-241 (1978)
U.S. EPA FIFRA Guidelines, Subdivision F No GLP Growth inhibition observed at the top dose level, the result not confirmed in an independent experiment. Test performed in the absence of metabolic activation only No viability data (actual plate count) provided	Isopropyl-amine salt of glyphosate Batch: SN-75-721 Purity: 64% Given purity refers to the contents of glyphosate in the formulation or the salt300/2	*Escherichia coli.* DNA repair (Pol A^+/A^-) assay, -S9, 0.1-10000 µg/mL	Growth inhibition induced by the test item was observed at one concentration only. The test result did not match the evaluation criteria for a positive result in the absence of metabolic activation under the conditions of this study. However, the study is not considered acceptable as there were many deficiencies (refer to first column).	CA 5.4.1/037; Report no. 87BME014-E (1993)

Study **not** acceptable				
***In vitro* chromosome aberration studies**				
OECD473 GLP No HCD; Only 200 cells in metaphase were evaluated. Only positive control for 20 h sampling point. No short term exposure without metabolic activation. Acceptance criteria not specified. Evaluation criteria inconsistent. Study acceptable but with restrictions.	Glyphosate acid Batch: P24 Purity: 95.6%	Cytogenetic Assay in Human Lymphocytes, ±S9, 100-1250 µg/mL	No relevant increase in percentage of aberrant metaphases observed in any experiment with the tested concentrations in the presence or absence of metabolic activation. The test substance is considered non-clastogenic under the conditions of this study.	CA 5.4.1/025; Report no. CTL/P/6050 (1998)
OECD473 GLP No HCD; Only 200 cells in metaphase were evaluated Acceptance criteria not specified. Evaluation criteria inconsistent Study acceptable but with restrictions	Technical glyphosate Batch: H95D161A Purity: 95.3%	Cytogenetic Assay in Chinese hamster lung cells, ±S9, 39-1250 µg/mL	No relevant increase in percentage of aberrant metaphases observed in any experiment with the tested concentrations in the presence or absence of metabolic activation. The test substance is considered non-clastogenic under the conditions of this study.	CA 5.4.1/026; Report no. 434/015 (1996)
OECD473 GLP	Technical glyphosate Batch: 940908-1 Purity: 95.68%	Cytogenetic Assay in Chinese hamster lung cells, ±S9, 62.5-2000 µg/mL	No relevant increase in percentage of aberrant metaphases observed in any experiment with the tested concentrations in the presence or absence	CA 5.4.1/027; Report no. IET 94-0143

No complete HCD (only for untreated and solvent controls); Only 200 cells in metaphase were evaluated Acceptance criteria not specified. Evaluation criteria inconsistent Study acceptable but with restrictions			of metabolic activation. The test substance is considered non-clastogenic under the conditions of this study.	(1995)
OECD473 GLP No complete HCD (no data for positive control and testing lab); Only 200 cells in metaphase were evaluated Acceptance criteria and. evaluation criteria differed from OECD473 Study acceptable but with restrictions	Glyphosate Batch: 22021 Purity: 96%	Cytogenetic Assay in human peripheral lymphocytes, ±S9, 333-1000 µg/mL.	No relevant increase in percentage of aberrant metaphases observed in any experiment with the tested concentrations in the presence or absence of metabolic activation. The test substance is considered non-clastogenic under the conditions of this study.	CA 5.4.1/028; Report no. 141918 (1995)
OECD473 No GLP (not compulsory No complete HCD (no data for positive control and testing lab);	Glyphosate Batch: 978 Purity: not specified	Cytogenetic Assay in Chinese hamster ovary cells, ±S9, 62.5-1000 µg/mL	The study is not considered acceptable due to the large number of deviations (refer to deviations in the first column). Therefore, no final conclusions can be made. Based on this study, however, no indications for clastogenicity were obtained.	CA 5.4.1/029; Report no. not reported (1989)

Only 100-200 cells in metaphase were evaluated. No cytotoxicity at maximum concentration. Inconsistencies regarding exposure duration and reporting deficiencies Study **not** acceptable				
***In vitro* – micronucleus study**				
OECD487 (2016) GLP Modified treatment schedule Acceptable study	Glyphosate batch AZM30320T0, purity 91.8%	Human peripheral lymphocytes ±S9 Range finding assay: 13.21-1691 µg/mL. Main assay: 105.69-1268.25 µg/mL (highest dose eq. to 10 mM).	Negative for induction of micronuclei in human peripheral lymphocytes *in vitro*, in the presence and absence of metabolic activation.	CA 5.4.1/041 Report no. 8441969 (2021)
***In vitro* – mammalian gene mutation studies**				
OECD476 GLP No complete HCD (no data for positive control); Acceptance criteria and evaluation criteria inconsistent Study acceptable but with restrictions	Glyphosate Batch: P24 Purity: 95.6%	Mouse Lymphoma Gene Mutation Assay , ±S9, Range finding assay: 125-2000 µg/mL. Main asasay: 296-1500 µg/mL.	No relevant increase in mutant frequencies in L5178Y $TG^{+/-}$ cells observed in any experiment with the tested concentrations in the presence or absence of metabolic activation. The test substance is considered non-mutagenic under the conditions of this study.	CA 5.4.1/030; Report no. CTL/P/4991 (1998)
OECD476 GLP	Glyphosate Batch: 206-JaK-25-1	Mouse Lymphoma Gene Mutation Assay, ±S9, 0.52-5000 µg/mL.	No relevant increase in mutant frequencies in L5178Y $TG^{+/-}$ cells observed in any experiment	CA 5.4.1/031; Report no. 12325

No complete HCD (no data for positive and negative control); Acceptance criteria not defined and evaluation criteria not applied Study acceptable but with restrictions	Purity: 98.6%		with the tested concentrations in the presence or absence of metabolic activation. The test substance is considered non-mutagenic under the conditions of this study.	(1991)
OECD476 Non-GLP (not compulsory) No complete HCD (no data for positive and negative control); Acceptance criteria not defined and evaluation criteria not specified Study acceptable but with restrictions	Glyphosate Batch: XHJ-64 Purity: 98.7%	CHO/HGPRT Gene Mutation Assay, ±S9, 2-25 mg/mL.	No relevant increase in gene mutations in the HGPRT locus observed in any experiment with the tested concentrations in the presence or absence of metabolic activation. The test substance is considered non-mutagenic under the conditions of this study.	CA 5.4.1/032; Report no. ML-83-155 (1983)
OECD482 (1986) GLP Only one culture per condition was tested. Study **not** acceptable (OECD 482 was deleted in 2014 and the UDS assay is no longer a standard method)	Glyphosate Batch: F/93/032 Purity: >98%	DNA repair test with primary rat hepatocytes (UDS assay), 0.2-111.69 mM.	The study is considered to be not acceptable due to the noted deviations and since the UDS assay is no longer a standard method. Therefore, no final conclusion can be made. However, no relevant increase in tritiated cytidine incorporations more than 10% when compared to control values. The test substance is considered not to induce DNA damage leading to unscheduled DNA synthesis under the conditions of this study.	CA 5.4.1/033; Report no. 931564 (1994)

			The test substance is considered non-mutagenic under the conditions of this study.	
OECD482(1986) No GLP Selection of test concentration not justified. High concentrations cause no cytotoxicity. No raw data. Study **not** acceptable (OECD 482 was deleted in 2014 and the UDS assay is no longer a standard method)	Glyphosate Batch: XHJ-64 Purity: 98.7%	The hepatocyte primary culture / DNA repair assay (UDS assay), 0.0125-125 µg/mL	The study is considered to be not acceptable due to the noted deviations and since the UDS assay is no longer a standard method. Therefore, no final conclusion can be made. Based on this study, however, no indications for DNA damage were obtained.	CA 5.4.1/034; Report no. M-645649-01-1 (1983)
OECD479 (1986) No GLP Cytotoxicity and solubility/ precipitation not investigated; purity not known; no duplicate experiment Study **not** acceptable	Isopropyl-amine salt of glyphosate Batch: SN-75-721 Purity: 64% Given purity refers to the contents of glyphosate in the formulation or the salt	Sister Chromatid Exchange Assay (SCE Test), ±S9, 0.1-100 µg/mL	The study is not considered acceptable as there were many deficiencies (refer to first column). Therefore, no final conclusion can be made. However, treatment with glyphosate isopropylamine salt did not induce a statistically significant increase in the frequency of SCEs per chromosome up to the highest tested concentration in the presences or absence of metabolic activation under the conditions of this study.	CA 5.4.1/038; Report no. 87BMS013-E (1993)
OECD479 (1986) GLP Only a single experiment performed Negative test result not confirmed in	Glyphosate active Batch: 0190A Purity: not reported	Sister Chromatid Exchange Assay (SCE Test), ±S9, 78.125- 2500 µg/mL	The study is not considered acceptable as there were many deficiencies (refer to first column). Therefore, no final conclusion can be made. However, treatment with glyphosate did not induce a statistically significant increase in the frequency of SCEs per chromosome up to the	CA 5.4.1/039; Report no. 300/2 (1990)

independent experiment. Some reporting deficiencies Study **not** acceptable			highest tested concentration under the conditions of this study.	
OECD 476 (2016) GLP No deviations Study acceptable	Glyphosate Lot/Batch nr AZM30320T0, purity 91.8%	HPRT study in Chinese hamster V79 cells ±S9 Preliminary assay: 13.21-1691 µg/mL Main assay: 105.69-1691 µg/mL	No relevant increase in gene mutations in the HGPRT locus observed in any experiment with the tested concentrations in the presence or absence of metabolic activation. The test substance is considered non-mutagenic under the conditions of this study.	CA 5.4.1/040; Report no. 8441968 (2021)
OECD 476 (1997) GLP Study acceptable	Glyphosate technical	Chinese hamster V79/HGPRT Gene Mutation Assay, ±S9	No relevant increase in gene mutations in the HGPRT locus observed in any experiment with the tested concentrations in the presence or absence of metabolic activation. The test substance is considered non-mutagenic under the conditions of this study.	Report no. 31405 (2014) Study submitted in Vol 4 as confidential information
Other *in vitro* studies (literature studies)				
Non-guideline (although comet assay similar to OECD GL 489) Non-GLP (literature study) Study supportive	Glyphosate (analytical grade; purity not reported); Formulations: Roundup Mega, Fozat 480, Glyfos	*In vitro* comet assay in human mononuclear white blood cells with glyphosate and three formulations Exposure to 1-1000 µM (± S9 mix) for 4 h	Glyphosate did not induce cytotoxicity or genotoxicity, whereas cytotoxicity and genotoxicity was observed when cells were treated with the formulations. Possibly, genotoxicity is the result of the observed cytotoxicity or due to co-formulants present in the formulations. Limitations: No HCD, results of positive control not shown, details about the test substance are missing, number of scored slides not in line with OECD GL 489, stability and conc of tested concentrations not analytically verified	Nagy *et al*., 2019 (KCA 5.4/003)
Non-guideline (although comet assay	Glyphosate (purity 99.5%);	*In vitro* cytotoxicity test in whole blood samples and breast cancer (MCF7 and MDA-	Cytotoxicity (whole blood): cytotoxicity observed, with a dose-response relationship for	De Almeida *et al.*, 2018 (KCA 5.4/004)

similar to OECD GL 489) Non-GLP (literature study) Study supportive	Formulations: Roundup and Wipeout	MB-231) and endometrial cancer (HEC1A) cell lines. Exposure at 0.1-500 µg/mL for 18 hours (whole blood) or at 75-500 µg/mL for 24 hours (cell lines). No information regarding metabolic activation. *In vitro* comet assay in breast cancer cells (MCF7 and MDA-MB-231) and endometrial cancer cells (HEC1A) at 500 and 1000 µg/mL for 4 hours. No information regarding metabolic activation.	Wipeout, but bell-shaped dose-response for glyphosate and Roundup. Cytotoxicity (cell lines): at ≥ 75 µg/mL in HEC1A cells, no cytotoxicity in MCF7 and MDA-MB-231 cells upon treatment with glyphosate. No cytotoxicity upon treatment with Roundup in any of the three cell lines. No cytotoxicity upon treatment with Wipeout in MCF-7 and MDA-MB-231 cell lines, however, a significant increase in cell viability observed in the HEC1A cell line. Comet assay (HEC1A; 500 and 1000 µg/ml): positive for glyphosate, Roundup, and Wipeout. Comet assay (MCF7; 500 and 1000 µg/ml): negative for glyphosate, Roundup, and Wipeout. Comet assay (MDA-MB-231; 500 and 100 µg/ml): positive for glyphosate, positive for Roundup except at 800 µg/mL with regard to tail moment, positive for Wipeout at 500 µg/mL, but not at 800 µg/mL. Limitations: At the highest concentration (800-1000 µg/ml) the cytotoxicity has not been assessed. Therefore this study is difficult to interpret since the cytogenicity assay does not indicate any dose-response relationship. Details regarding the tested formulations missing, no HCD, no information regarding metabolic activation, number of scored slides not in line with OECD GL 489. Stability and concentration of test item not analytically verified.	

Similar to OECD GL 473 and 487 Non-GLP (literature study) Study supportive	Glyphosate (purity not reported)	*In vitro* chromosome aberration (CA) assay and micronucleus (MN) assay in human lymphocytes Exposure to 0.0125 – 0.5 µg/mL for 52 h (CA assay) or 72 h (MN assay)	CA assay: positive MN assay: positive Limitations: only continuous treatments without metabolic activation, no HCD, proficiency of the lab not demonstrated, highest dose not in line with the guidelines; treatment started at 24 h after stimulation instead of 48 h; exposure duration exceeded 1.5 cell cycles; purity of test substance not stated, stability and conc of tested concentrations not analytically verified.	Santovito *et al.*, 2018 (KCA 5.4/006)
Non-guideline (although comet assay similar to OECD GL 489) Non-GLP (literature study) Study supportive	Glyphosate (purity not reported)	*In vitro*: cell proliferation, Comet assay, cytokinesis-block micronucleus (CBMN) cytome assay, determination of oxidative stress parameters Exposure of HepG2 cells to 0.5-3.5 µg/mL for 4 and 24 h (Comet assay and MN assay) Assays without metabolic activation only.	Cell proliferation: No statistically significant change. Comet assay: a statistically significant decrease in tail intensity after 4 hours, but not after 24 h. A decrease in tail intensity might indicate DNA cross-links, however, according to OECD TG 489 this cannot be reliably detected with standard experimental conditions. MN assay: equivocal. Oxidative stress: no substance related effect. Reliability of the study is doubted due to several limitations regarding the publication, a.o. lack of statistical significance; no reproducible effects as well as the fact that the control values in the Comet assay and micronucleus assay seem to be highly variable; assay only without metabolitic activation; HCD not reported; proficiency of lab not demonstrated; purity not reported, test substance stability and test concentration not analytically verified.	Kasuba *et al.*, 2017 (KCA 5.4/007)
Non-guideline (although comet assay similar to OECD GL 489)	Glyphosate (95 % purity)	*In vitro*: comet assay, DNA repair, methylation of global DNA, as well as p16 and p53 promotor regions.	Comet assay: positive at ≥ 0.5 mM. Significant DNA repair was observed after 120 min of recovery. Global DNA methylation statistically significant	Kwiatkowska *et al.*, 2017 (KCA 5.4/008)

Non-GLP (literature study) Study supportive		Exposure of human peripheral blood mononuclear cells to 0.25-10 mM glyphosate for 24 h. Assays without metabolic activation only.	decreased at 0.25 mM, but not at 0.5 mM. Methylation p53 promotor regions statistically significant increased at 0.25 and 0.5 mM. No statistically significant change in the methylation of the p16 promotor region. Limitations: The study indicates statistically significant DNA damage but this effect seems only to occur at concentrations above that found *in vivo* in rats given 2000 mg/kg bw (i.e., 0.3 mM) and can, thus, be considered an irrelevant effect. Poor description of donors, low number of donors, HCD not available; proficiency of lab not demonstrated, test substance stability and test concentration not analytically verified.	
Non-guideline study Non-GLP (literature study) Study supportive	Glyphosate and AMPA (purities not reported)	*In vitro*: induction of DNA double strand breaks (immunofluorescence of phosphorylated H2AX foci); induction of proteins involved in DNA recombination (Western blot; glyphosate only) Exposure of human peripheral blood lymphocytes to 0.4-50 µM glyphosate for 1.5 h. Assays without metabolic activation only.	Induction of DNA double strand breaks (DSBs): positive for glyphosate, but no clear dose-response relationship. Positive results of DSBs based on the surrogate marker γ-H2AX foci, however, are difficult to interpret since there is no guideline for this type of study and no information on validation of this assay in available. Negative for AMPA. Induction of proteins involved in DNA recombination: statistically significant increase of p-Ku80, but not of Rad51. Limitations: Purity not reported, HCD not reported, test substance stability and test concentration not analytically verified. In addition, as no guideline or validation of this assay is available the results are difficult to interpret.	Suárez-Larios, K. *et al.*, 2017 (KCA 5.4/009)
Non-guideline (although comet assay similar to OECD GL 489)	Glyphosate (95 % purity)	*In vitro* comet assay. Exposure of human Burkitt's Lymphoma (Raji) cells to 0.1 µM-15 mM glyphosate for	Comet assay: positive at ≥ 1 mM (i.e. doses that are not physiologically relevant) Cytotoxicity: ≥ 10 mM Main deviations from OECD GL 489:	Townsend *et al.*, 2017 (KCA 5.4/010)

Non-GLP (literature study) Study supportive		10-120 min. Assay without metabolic activation only.	description of lysis conditions incomplete, number of scored cells too low, study only performed without metabolic activation, no HCD are available (lab proficiency not proven), test substance stability and test concentration not analytically verified.	
Non-guideline (although MN assay similar to OECD GL 487) Non-GLP (literature study) Study supportive	Glyphosate and AMPA (purities not reported)	*In vitro* micronucleus assay (±S9 and after photoactivation); intracellular ROS determination Exposure of CHO-K1 cells to 5-100 µg/mL glyphosate (±S9, +irradiation) and 0.005-0.01 µg/mL (-S9), 0.1-5 µg/mL (+S9), and 0.00005-0.001 µg/mL (+irradiation) AMPA for 3 h.	Micronucleus assay (glyphosate): negative (-S9); positive (+S9; ≥ 10 µg/mL); positive (+irradiation at 100 µg/mL) ROS formation (glyphosate): negative Micronucleus assay (AMPA): positive (-S9; ≥ 0.01 µg/mL); positive (+S9; ≥ 1 µg/mL); positive (+irradiation ≥ 0.0005 µg/mL) ROS formation (AMPA): elevated Main deviations from OECD GL 487: no continuous treatment schedule, test chemicals not characterized, no positive control or HCD (lab proficiency not proven), test substance stability and test concentration not analytically verified.	Roustan *et al*, 2014 (KCA 5.4/011)
Non-guideline (although comet assay and MN assay similar to OECD GL 489 and 487, respectively) Non-GLP (literature study) Study supportive	Glyphosate (purity: 95%); Roundup Ultramax (450 g/L glyphosate acid)	*In vitro* comet assay, MN assay, and determination of different cytotoxicity assays (LDHe, XTT, SRB) 20 min exposure of human-derived buccal epithelium cells (TR146 cell line) to 10-2000 mg/L (comet assay), 10-20 mg/L (MN assay), and 10-200 mg/L (cytotoxicity assays) glyphosate and Roundup Ultramax. Assay without metabolic activation only.	Comet assay: equivocal for glyphosate and positive Roundup Ultramax (both ≥ 20 mg/L) CBMN assay: positive for glyphosate and Roundup Ultramax (both ≥ 10 mg/L) Cytotoxicity: Glyphosate at ≥ 80 mg/L; Roundup Ultramax at ≥ 10 mg/L Apoptosis seems to be pronounced already at 10 mg/L compared to the medium control (and the positive control). It is therefore not unlikely that the positive effects observed at 10 mg/L and above are due to cytotoxicity. Limitations: Description of the methods very limited, treatment schedule, only conditions	Koller *et al.*, 2013 (KCA 5.4/013)

			without metabolic activation, no positive control in the Comet assay, HCD of limited extent, test substance stability and test concentration not analytically verified.	

Table 50: Summary table of genotoxicity/mutagenicity tests in mammalian somatic or germ cells *in vivo*

Method, guideline, deviations[a] if any	Test substance	Relevant information about the study (as applicable)	Observations/Results	Reference
Genotoxicity/mutagenicity tests in mammalian somatic cells *in vivo*				
OECD474 (2014) GLP Study acceptable	Glyphosate technical with one impurity	*In vivo* micronucleus test in mice, 2000 mg/kg bw/day Oral gavage, vehicle corn oil	The test substance is considered non-clastogenic and non-aneugenic under the conditions of this study. Bone marrow exposure of glyphosate proven by measurement of plasma levels of glyphosate at 24 h after application. In addition, clinical signs (bristling, tachypnoea and motor incoordination) were noted.	Report no. 14613.402.078.14, 2015 Submitted as Vol 4 confidential information
OECD474 (1997) GLP Only 2000 polychromatic erythrocytes evaluated The percentage of polychromatic erythrocytes among total erythrocytes determined for 200 erythrocytes. Bone marrow exposure not confirmed. Historical control data of solvent controls not considered. Study acceptable	Glyphosate TGAI Batch: 20061109 Purity: 98.9%	Micronucleus test of glyphosate TGAI in mice, 2000 mg/kg bw/day Oral gavage, vehicle vegetable oil	Treatment with glyphosate did not induce a statistically significant increase of micronuclei in the bone marrow of Swiss albino mice *in vivo* under the conditions of this study. The test substance is considered non-clastogenic and non-aneugenic under the conditions of this study.	Report no. 485-1-06-4696, 2012 (CA 5.4.2/001)
OECD474 (1997) GLP Only 2000 polychromatic erythrocytes evaluated	Glyphosate Technical Batch: 569753 Purity: 96.3%	Micronucleus Assay in Bone Marrow Cells of the Mouse, 2000 mg/kg bw/day Oral gavage, vehicle 1% carboxymethyl cellulose (CMC)	Treatment with glyphosate did not induce a statistically significant increase of micronuclei in the bone marrow of male NMRI mice i*n vivo* under the conditions of this study.	Report no. 1479200, 2012 (CA 5.4.2/002)

Method, guideline, deviations[a] if any	Test substance	Relevant information about the study (as applicable)	Observations/Results	Reference
Bone marrow exposure not confirmed. Historical control data not considered. Study acceptable.			The test substance is considered non-clastogenic and non-aneugenic under the conditions of this study.	
OECD474 (1997) GLP Only 2000 polychromatic erythrocytes evaluated Acceptance criteria not reported and evaluation criteria specified in the study report inconsistent with the historical control data. No standard deviation given for HCD. Two different batches of the test material were used in the original study and in the amendment to the study. In addition, the treatment of the animals used in the amendment was not described and the amendment does not replace a full study report. Study **not** acceptable	Glyphosate Technical Batch: 20070606 Purity: 98.0%	Micronucleus Assay in bone marrow cells in mice; 15.62 - 375 mg/kg bw/day. Administration by intraperitoneal injection In the original report, dose selection for the main test was justified with the outcome of a preliminary test. In this range finding experiment, 3 males and 3 females per dose level received by intraperitoneal injection glyphosate doses of 62.5, 125, 250, 500, or 1000 mg/kg bw. The top dose level resulted in 100% mortality and at the next lower dose level of 500 mg/kg bw, one male and two female mice died. Based on a clear decrease in the PCE/NCE ratios in both sexes, the intermediate dose of 250 mg/kg bw was found to be cytotoxic. A dose of 125 mg/kg bw was considered an appropriate high dose but, 62.5 mg/kg bw was actually the highest dose used. In 2014, an amendment to this study was submitted. In this document, some results of testing glyphosate at dose level of 125, 250, and 375 mg/kg bw are reported. Clinical signs but no mortality were seen at all dose levels. It is not clear in which way this data is linked to the preliminary test that was performed as part of the original study since the dose levels were not exactly the same and the number of animals was different	The study is not considered acceptable as there were many deficiencies (refer to first and third column). Treatment with glyphosate did not induce a statistically significant increase of micronuclei in the bone marrow of Swiss mice *in vivo* under the conditions of this study.	Report no. [redacted]-3996.402.395.07, 2008 (CA 5.4.2/003) And Report no. [redacted]-3996.402.395.07, 2010 (CA 5.4.2/004)

Method, guideline, deviations[a] if any	Test substance	Relevant information about the study (as applicable)	Observations/Results	Reference
		(this time 5 per sex and dose). Furthermore, in the amendment, more data on micronucleus incidences and PCE/NCE ratios at the dose levels of 15.62, 31.25, and 62.5 mg/kg bw was given, apparently based on 10 animals per sex and dose.		
OECD474 (1997) GLP Only 2000 polychromatic erythrocytes evaluated. Bone marrow exposure not confirmed. Acceptance criteria not specified in the study report. Historical controls not considered in the evaluation criteria Study acceptable	Glyphosate Technical Batch: 20070545 Purity: 99.1%	Micronucleus Assay in bone marrow cells in mice; 500, 1000 and 2000 mg/kg bw Oral gavage, vehicle 0.5% carboxymethyl cellulose (CMC)	Treatment with glyphosate did not induce a statistically significant increase of micronuclei in the bone marrow of NMRI male mice *in vivo* under the conditions of this study. The test substance is considered non-clastogenic and non-aneugenic under the conditions of this study.	Report no. 1158500, 2008 (CA 5.4.2/005)
OECD474 (1997) GLP Low dose tested The mortality observed in the range finding experiment was inconsistent with observations in other studies Study **not** acceptable	Glyphosate Technical Batch: 2007091801 Purity: 98.01%	Mammalian Erythrocyte Micronucleus Test in mice; 8-30 mg/kg bw, dosed twice with a 24-h interval Oral gavage; vehicle deionized water	The study is not considered acceptable as there were many deficiencies (refer to Volume 3, B.6.4.2.6 for details). Oral treatment with glyphosate technical did not induce any mortality in the animals and signs of systemic toxicity were as well not reported. In addition, the ratio of PCE to NCE was not affected upon treatment with the test item up to the highest dose level. When compared to solvent control animals, treatment with glyphosate doses of 8.0 and 15.0 mg/kg bw/day did not lead to a statistically significant increase the frequency of micronucleated PCE. At 30	Report no. RL33393/2007-3.0MN-B, 2007 (CA 5.4.2/006)

Method, guideline, deviations[a] if any	Test substance	Relevant information about the study (as applicable)	Observations/Results	Reference
			mg/kg bw/day, a statistically significant increase in micronucleated PCE was observed, but the values remained within the range of historical control data and were therefore considered to be without biological relevance. Under the conditions of the test, glyphosate technical was considered negative for clastogenic/aneugenic activity in male mice *in vivo*	
OECD474 (1997) GLP Only 2000 polychromatic erythrocytes evaluated. Acceptance criteria not specified in the study report. Historical controls not provided. Individual animal data for clinical signs were detailed in the study report. Study acceptable but with restrictions	Glyphosate Technical Batch: H05H016A Purity: 95.7%	Micronucleus Assay in bone marrow cells in CD-1 male mice; 150, 300 and 600 mg/kg bw Intraperitoneal injection	A small but statistically significant increase in mnPCE after 24 h in the 600 mg/kg bw group, which was within HCD. No increase was seen after 48 hours. The response was interpreted as a haematopoietic effect due to bone marrow toxicity by the study authors. The test substance is considered non-clastogenic and non-aneugenic under the conditions of this study.	Report no. 2060/014, 2006 (CA 5.4.2/007)
OECD474 (1997) GLP Only 1000 polychromatic erythrocytes evaluated. Bone marrow exposure not confirmed.	Glyphosate Technical Nufarm Batch: 037-919-113 Purity: 95%	Micronucleus Assay in bone marrow cells in mice; 187.5, 375 and 562.5 mg/kg bw/day Intraperitoneal injection	Treatment with glyphosate did not induce a statistically significant increase of micronuclei in the bone marrow of male and female Swiss albino mice *in vivo* under the conditions of this study. The test substance is considered non-clastogenic and non-aneugenic under the conditions of this study.	Report no. [redacted]-G12.79/99, 1999 (CA 5.4.2/008)

Method, guideline, deviations[a] if any	Test substance	Relevant information about the study (as applicable)	Observations/Results	Reference
Acceptance and evaluation criteria specified in the study report inconsistent with those specified by the guideline. Historical controls for the positive or vehicle controls not provided. Study acceptable but with restrictions				
OECD474 (1997) GLP Body weights and clinical signs have not been described in the study report. Highest dose 5000 mg/kg bw/day. Only 2000 polychromatic erythrocytes evaluated. Percentage of polychromatic erythrocytes among total erythrocytes was determined for 200 erythrocytes only. Bone marrow exposure not confirmed. Evaluation criteria not specified in the study report. Acceptance criteria inconsistent with those specified by the guideline.	Glyphosate Acid Batch: P24 Purity: 95.6%	Micronucleus Assay in bone marrow cells in mice; 5000 mg/kg bw/day Oral gavage, vehicle saline	Treatment with glyphosate did not induce a statistically significant increase of micronuclei in the bone marrow of male and female CD-1 mice i*n vivo* under the conditions of this study. The test substance is considered non-clastogenic and non-aneugenic under the conditions of this study.	Report no. [redacted]/P/4954, 1996 (CA 5.4.2/009)

Method, guideline, deviations[a] if any	Test substance	Relevant information about the study (as applicable)	Observations/Results	Reference
Historical controls not provided. Study acceptable but with restrictions				
OECD474 (1983) GLP Highest dose 5000 mg/kg bw/day. Only 2000 polychromatic erythrocytes evaluated. Acceptance and evaluation criteria not specified in the study report. Historical controls not provided. Study acceptable but with restrictions	Glyphosate (N-(Phosphonomethyl) glycine) Batch: FSG 03090 H/05 Purity: 96.8%	Micronucleus Assay in bone marrow cells in mice; 50-5000 mg/kg bw/day Oral gavage, vehicle refined groundnut (peanut) oil	Treatment with glyphosate did not induce a statistically significant increase of micronuclei in the bone marrow. For females, at the high dose (above the OECD limit) the test result was considered equivocal. The test substance is considered equivocal for clastogenicity and aneugenicity under the conditions of this study.	Report no. 889-MUT.MN, 1993 (CA 5.4.2/010)
OECD474 (1997) GLP Highest dose 4000 mg/kg bw/day. Only 1000 polychromatic erythrocytes evaluated. Bone marrow exposure not confirmed. No data on proficiency and/or historical control data were provided and the purity of the test material was not reported. Evaluation criteria inconsistent with those specified in the current guideline. The	Glyphosate technical Batch: 0190A Purity: not reported	Micronucleus Assay in bone marrow cells in mice; 4000 mg/kg bw/day Oral gavage	The study is not considered acceptable as there were many deficiencies (see first column and Volume 3, B.6.4.2.11). Treatment with glyphosate did not induce a statistically significant increase of micronuclei in the bone marrow of BKW mice *in vivo* under the conditions of this study.	Report no. 300/3, 1990 (CA 5.4.2/011)

Method, guideline, deviations[a] if any	Test substance	Relevant information about the study (as applicable)	Observations/Results	Reference
mortality observed was inconsistent with observations in other studies Study **not** acceptable				
OECD474 (1983) GLP Highest dose 5000 mg/kg bw/day. Only 2000 polychromatic erythrocytes evaluated. Bone marrow exposure not confirmed. Historical controls not provided. Acceptance and evaluation criteria not specified in the study report. Study acceptable but with restrictions	Glyphosate technical Batch: 206-JaK-25-1 Purity: 98.6%	Micronucleus Assay in bone marrow cells in mice; 5000 mg/kg bw/day Oral gavage, vehicle 0.5% carboxymethyl cellulose in distilled water	Treatment with glyphosate did not induce a statistically significant increase of micronuclei in the bone marrow of NMRI SPF mice *in vivo* under the conditions of this study. The test substance is considered non-clastogenic and non-aneugenic under the conditions of this study.	Report no. 12324, 1991 (CA 5.4.2/012)
OECD474 (1983) No GLP Batch and purity not reported. Only 1000 polychromatic erythrocytes evaluated. Bone marrow exposure not confirmed. Historical controls not provided. Mortality not reported. Evaluation criteria not specified in the study report.	Glyphosate technical Batch: not reported Purity: not reported	Micronucleus Assay in bone marrow cells in NMRI mice; 2000 mg/kg bw/day Oral gavage	The study is not considered acceptable as there were many deficiencies (refer to first column and Volume 3 CA B.6.4.2.13) Treatment with glyphosate did not induce a statistically significant increase of micronuclei in the bone marrow of NMRI mice *in vivo* under the conditions of this study. .	Report no. not reported, 1989 (CA 5.4.2/013)

Method, guideline, deviations[a] if any	Test substance	Relevant information about the study (as applicable)	Observations/Results	Reference
Study **not** acceptable				
OECD474 (1997) GLP Only 2000 polychromatic erythrocytes evaluated. Bone marrow exposure not confirmed. Historical control data for positive controls not provided. Study acceptable buth with restrictions	Glyphosate technical Batch: 20080801 Purity: 98.8%	Micronucleus Assay in bone marrow cells in CD rats; 500-2000 mg/kg bw/day Oral gavage, vehicle 0.8% aqueous hydroxypropylmethyl cellulose	Treatment with glyphosate did not induce a statistically significant increase of chromosome aberrations in the bone marrow of CD rats *in vivo* under the conditions of this study. The test substance is considered non-clastogenic and non-aneugenic under the conditions of this study.	Report no. 23917, 2009 (CA 5.4.2/014)
OECD475 (1984) GLP Only 50 metaphases/ mouse investigated (instead of 200). Mitotic index for 100 cells instead of 1000 cells. Only scoring for CA in high dose group (where toxicity was observed). No justification for second dose. The cell cycle arrest time was insufficient and the sampling time after the second dose was later (24 hours plus additional 1.5 hours cell cycle arrest) than specified in the current guideline	Glyphosate Batch: 046 Purity: 96.8% Positive control cyclophosphamide	*In vivo* mammalian bone marrow chromosome aberration test in mice (5/sex/dose group); 50, 500 or 5000 mg/kg bw/day, dose volume 10 mL/kg bw, 2 days, oral gavage Vehicle refined groundnut (peanut) oil	Bone marrow exposure was indicated by a reduction in the mitotic index and clinical signs of toxicity. Treatment with glyphosate did not induce a statistically significant increase of chromosome aberrations in the bone marrow of Swiss Albino mice *in vivo* under the conditions of this study. The test substance is considered non-cytogenic under the conditions of this study.	Report no. 890-MUT-CH.AB, 1994 (CA 5.4.2/015)

Method, guideline, deviations[a] if any	Test substance	Relevant information about the study (as applicable)	Observations/Results	Reference
(24 hours after 2nd dose including cell cycle arrest). Acceptance and evaluation criteria not specified in the study report Historical control data not provided. Study supportive				
OECD475 (1984) Non-GLP (not compulsory) Only 50 metaphases/ rat investigated (instead of 200). Mitotic index for 100 cells instead of 1000 cells. Dose level of 1000 mg/kg bw (instead of 2000 mg/kg bw); no evidence of bone marrow toxicity. Only sampling at 6, 12 and 24 h, but not at 48 h. Acceptance and evaluation criteria not specified in the study report Historical control data not provided. Study supportive	Glyphosate technical Batch: T830044 Purity: 98.7% Positive control Cyclophosphamide	*In vivo* mammalian bone marrow chromosome aberration test in rats (6/sex/treatment group); 1000 mg/kg bw/day, intraperitoneal administration, colchicine 4, 10 or 22 h after start treatment sacrifice 6, 12 or 24 h after start treatment	Treatment with glyphosate did not induce a statistically significant increase of chromosome aberrations in the bone marrow of Sprague-Dawley rats *in vivo* under the conditions of this study. The test substance is considered non-cytogenic under the conditions of this study.	CA 5.4.2/016; Report no. 830083, 1983

Method, guideline, deviations[a] if any	Test substance	Relevant information about the study (as applicable)	Observations/Results	Reference
Similar to OECD 474 Non-GLP (literature study) Study supportive	Four batches of glyphosate; Purities: 95.7, 98.3, 95.1 and 95.8%	Micronucleus test *in vivo* in CD1 mice, 5/sex/group Oral gavage for two days (24 h interval); 2000 mg/kg bw Sampling: 22 h after 2nd dose	Negative in the *in vivo* MN test for all four batches. No evidence of bone marrow exposure provided. Limitations: Source of glyphosate batches not reported; limited detail on methodology	Ilyushina, N. *et al*., 2018a (KCA 5.4/002)
OECD GL 474 Non-GLP (literature study) Study supportive	Three batches of glyphosate technical (purities: 96.6, 95.8 and 95.7%)	Micronucleus test *in vivo* in CD1 mice, 5 animals/group Oral gavage for two days (24 h interval); 500, 1000, 2000 mg/kg bw; vehicle 1% starch Sampling: 22 h after 2nd dose	Micronucleus test: Positive for batch I Negative for batches II and III. No evidence of bone marrow exposure provided. Limitations: Positive control animals were included but the data are not reported. No ratio of PCE to NCE was reported. Data have been presented per group rather than per animal. HCD data not included. Note: Positive result for batch I postulated as due to the presence of 0.13% formaldehyde as an impurity. However, no data is submitted to support this hypothesis. It is noted that no evidence for induced **systemic** mutations is available for formaldehyde (CLH opinion on formaldehyde, 2012) and it is questionable whether 0.13% of an impurity would induce micronuclei *in vivo*. Due to the several limitations, however, the study is considered supportive in any case.	Ilyushina, N.A. *et al.*, 2018b (KCA 5.4/005)

Method, guideline, deviations[a] if any	Test substance	Relevant information about the study (as applicable)	Observations/Results	Reference
Non-guideline (although the alkaline comet assay largely follows OECD GD 489) Non-GLP (literature study) Study not acceptable for the Comet assay; study reliable with restrictions for other parts of the study. Deviations: - No justification is provided for the harvesting timepoint at 24 h after the last dosing. According to the OECD GD sampling time should be determined from kinetic data (e.g. at Tmax or at the steady state for multiple administrations). - Slides were left 10 minutes for unwinding of the DNA, whereas the OECD GD states for at least 20 minutes. - The frequency of hedgehogs was determined based on visual scoring of 100 nucleoids per sample instead of 150. Further, the data on frequency of hedgehogs was not reported in the publication. - No data on the proficiency of the lab for performing the alkaline comet assay has been provided in the publication (e.g. no historical control data on the	Glyphosate of analytical standard purity grade (purity of ≤100%)	28-day repeated oral dose by gavage Dose levels: 0.1, 0.5, 1.75 and 10 mg/kg bw/day Rat, Wistar, six males per group	Based on the Comet assay, no conclusion could be drawn regarding the genotoxic potential of glyphosate (it cannot be concluded whether the results are positive, negative, or equivocal; see B.6.4.4.14 for details). There was no dose-related effect on oxidative stress markers in plasma and liver and cholinesterase activity in plasma. Body weight gain was lower in the treated animals of all dose groups, however, without a dose-response relationship. No changes in relative liver weight.	Milic *et al*., 2018 (KCA 5.3.1/010) (B.6.4.4.14)

Method, guideline, deviations[a] if any	Test substance	Relevant information about the study (as applicable)	Observations/Results	Reference
positive and negative controls are provided).				
Non-guideline (although comet assay shows some similarity to OECD GL 489) Non-GLP (literature study) Study supportive	Glyphosate and AMPA (Purity 96% and 99%, respectively)	*In vivo* comet assay (blood, liver) and determination of oxidative stress parameters (TBARs, SOD and CAT activity in liver, kidney, lung, and heart) 14-Day exposure of Balb C mice (sex unknown, 6 animals/ group) to 40 or 400 mg/kg bw/day glyphosate and 100 mg/kg bw/day AMPA via drinking water.	Comet assay: positive in blood and liver for glyphosate at 40 and 400 mg/kg bw/day and for AMPA at 100 mg/kg bw. As there is no dose-response observed for glyphosate and as there are no historical control or positive control data provided for this laboratory, the results are difficult to interpret. Together with the limitations reported below, this study does not give clear evidence for positive results for glyphosate or AMPA. Oxidative stress parameters: No statistically significant differences except decrease in SOD activity in heart and increase in CAT activity in kidney for glyphosate at a dose level of 400 mg/kg bw/day. Deviations/limitations: Description of the method very limited (essential details missing), sex of animals unknown, number of doses and scored nucleoids not in line with OECD TG 489, no positive controls used and no HCD provided (therefore lab proficiency is not proven).	Mañas *et al.*, 2013 (KCA 5.4/012) (B.6.4.4.12)
Genotoxicity/mutagenicity tests in mammalian germ cells *in vivo*				
OECD478 (1984) GLP Dose levels were spaced with a factor of 5 (and not 2-4)	Glyphosate Batch: code FSG 03090 H/05, March 1990 Purity: 96.8%	*In vivo* study in germ cells - Dominant lethal test in Wistar rats; 200, 1000 and 5000 mg/kg bw (single dose) Oral gavage	Treatment with glyphosate did not induce a statistically significant increase of dominant lethal effects in Wistar rats under the conditions of this study.	Report no. TOXI-888-DLT, 1992 (CA 5.4.3/001, CA 5.4.3/002 and CA 5.4.3/003)

Method, guideline, deviations[a] if any	Test substance	Relevant information about the study (as applicable)	Observations/Results	Reference
Number of implantations below the recommended number of 400 implants per group Percentages of pre-implantation losses, as well as percentages of post-implantation losses were reported Acceptance and evaluation criteria not specified in the study report Historical control data not provided. Not all raw data reported. Study supportive			The test substance is considered non-genotoxic to germ cells *in vivo* under the conditions of this study.	
OECD478 (2016) Non-GLP Relatively old animals used (12 months at study initiation) Dose levels were relatively low. Number of implantations below the recommended number of 400 implants per group Resorptions, termed as "dead implants" in study report were not distinguished as early or late resorptions.	Glyphosate (N-phosphono-methyl-glycine) Batch: 00260481 Purity: not specified	*In vivo* study in germ cells - Dominant lethal test in male CFY rats; 10, 30 and 100 mg/kg food/day; corresponding to a mean actual achieved test substance intake of 6.8, 20.5 and 70.4 mg/kg bw/day 8-week dietary administration	Treatment with glyphosate did not induce a statistically significant increase of dominant lethal effects in CFY rats under the conditions of this study. The test substance is considered non-genotoxic to germ cells *in vivo* under the conditions of this study.	Report no. not reported, 1982 (CA 5.4.3/004)

Method, guideline, deviations[a] if any	Test substance	Relevant information about the study (as applicable)	Observations/Results	Reference
Acceptance and evaluation criteria not specified in the study report Historical control data not provided. Not all raw data reported (no sd calculated). Study supportive				
OECD478(2016) Non-GLP Number of implantations below the recommended number of 400 implants per group Number of males and females used for mating was too low Acceptance and evaluation criteria not specified in the study report Historical control data not provided. Not all raw data reported. No dominant lethal frequency was calculated in the study report. Study supportive	Technical glyphosate Batch: XHJ-64 Purity: 98.7%	*In vivo* study in germ cells - Dominant lethal test in CD-1 mice; 200, 800 and 2000 mg/kg bw (single dose) Oral gavage	Treatment with glyphosate did not induce a statistically significant increase of dominant lethal effects in CD-1 mice under the conditions of this study. The test substance is considered non-genotoxic to germ cells *in vivo* under the conditions of this study.	Report no. 401-064, 1980 (CA 5.4.3/005)

[a] Deviations from the current guideline.

As mentioned by RAC (RAC 40 opinion, 2017), ″some genotoxicity studies in human populations after occupational exposure to glyphosate-based herbicides (GHB) or exposure of bystanders/area residents exist, but their interpretation in regard to genotoxicity/germ cell mutagenicity of glyphosate is challenging". RAC mentioned that, however, some evidence was suggested in two published studies (described below) which investigated populations believed to be exposed to glyphosate based formulations. Remark RMS: the applicant has not submitted these publications as the publication date is >10 years before submission of the current dossier. RMS has copied a description of these studies from the RAC-opinion in Table 51 and in the summary below and has included the information from the previous RAR for these studies into Volume 3, section B.6.4.4.15. RMS has not re-evaluated these two studies.

Copied from the RAC-opinion:
"Paz-y-Miño and co-workers (2007) examined the consequences of aerial spraying with a glyphosate based herbicide added to a surfactant solution in the northern part of Ecuador. A total of 24 exposed and 21 unexposed control individuals were investigated using the Comet assay 2 weeks to 3 months following intensive aerial spraying. The results showed a higher degree of DNA strand breaks in the exposed group. However, individuals among the exposed group manifested clinical symptoms of toxicity after several exposures to aerial spraying which may by itself have an effect on generation of DNA single strand breaks.
Bolognesi and co-workers (2009) reported on a binucleated micronucleus (MN) biomonitoring study in subjects from five Colombian regions, characterized by different exposures to glyphosate and other pesticides. Blood samples were taken prior to spraying, 5 days and 4 months after spraying and a significant increase in the frequency of MN between first and second sampling was observed in three of the regions. In the post-spray sample, those who reported direct contact with the weedkiller spray showed a higher frequency of MN compared to those without glyphosate exposure. The increase in frequency of MN observed immediately after the glyphosate spraying was not consistent with the rates of application used in the regions and there was no association between self-reported direct contact with eradication sprays and frequency of MN."

Table 51: Summary table of human data relevant for genotoxicity / germ cell mutagenicity

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
Non-guideline study Not reliable	Roundup Ultra (43.9% glyphosate, polyethoxylated tallowamine) combined with adjuvant Cosmoflux 411F	Induction of alkaline SCGE effects in blood lymphocytes of populations living within 3 km of areas sprayed with glyphosate formulation.	Statistically significant increases in damaged cells (comet length 35.5 mu m in exposed group vs. 25.94 mu m in control group). Signs of toxicity reported consistent with severe exposures noted in clinical reports of acute poisoning incidents with formulations; reported application rate was 24.3 l/ha which was 20 times the max. recommended application rate.	Paz-y-Miño et al. (2007)*
Non-guideline Reliability not assessed	Glyphosate formulation and other pesticides	- Lymphocyte cytokinesis block micronucleus (CB MN) in humans living in areas where glyphosate formulation is applied with aerial or manual spraying of glyphosate formulation for illicit crop control and sugar cane maturation. - Buccal cell micronucleus (MN) test in agricultural workers which reported glyphosate formulation use reported (along with numerous other pesticides)	Increase in CB MN but no clear relationship to assumed or reported exposures. Statistically significant increase in buccal cell MN	Bolognesi et al. (2009)*

*Study summaries were copied from the previous RAR; RMS has not re-evaluated these studies.

Table 52: Summary table of other studies relevant for genotoxicity / germ cell mutagenicity

Type of data/report Status study	Test substance	Relevant information about the study (as applicable)	Observations Deviations/limitations	Reference
Non-guideline Non-GLP (literature study) Reliable with restrictions	Glyphosate (IPA salt; MON 0138; dissolved in water at 620 g/L)); RoundUP LB Plus (360 g/L glyphosate eq.)	Cytotoxicity assay in murine (Balb/3T3) and human (hFF) fibroblasts. No information regarding metabolic activation. Also, the difference in cytotoxicity (expressed as the AUC of the % viability vs concentration curve) between glyphosate and RoundUP LB Plus was investigated.	The IC_{50} for glyphosate isopropylamine salt was found to be 954.8 ± 117.1 µg/mL for 3T3 cells and 1211 ± 885.7 µg/mL for hFF cells and the IC_{50} for RoundUP LB Plus was found to be 313.2 ± 29.3 µg/mL for 3T3 cells and 361.6 ± 612 µg/mL for hFF cells. The standard deviations for the IC_{50} in human fibroblasts is relatively high so that solid conclusions cannot be made. No significant difference between the	Adler-Flindt, S., Martin, S., 2019 (KCA 5.4/001)

Type of data/report Status study	Test substance	Relevant information about the study (as applicable)	Observations Deviations/limitations	Reference
			cytotoxicity curves for glyphosate (IPA salt) and RoundUP LB Plus was observed. It is noted that in this study genotoxicity is not investigated, nor is the cytotoxicity discussed in relation to genotoxicity.	
Non-guideline Non-GLP (literature study) Reliable with restrictions	Glyphosate isopropylamine salt (96%), batch 09816 PE	A comet assay was performed in human lymphocytes, erythrocytes of *Oreochromis niloticus* and staminal nuclei of *Tradescantia*.	There was a statistically significant and concentration-dependent increase in the migration (tail length) of human lymphocyte DNA in the comet assay after treatment with glyphosate when compared to untreated control cells. Limitations: negative controls were untreated, no HCD, unclear if slides were scored blinded, only 50 cells or nuclei were scored per slide, unclear if cytotoxicity was assessed after treatment. Acceptability criteria from OECD489 are not met.	Alvarez-Moya C et al., 2014
Non-guideline Non-GLP (literature study) Unreliable	Roundup formulation, unspecified	Human erytrhocytes were exposed to Roundup (5-40 mL per 100mL water) to investigate effect on membrane. Micronucleus test in Swiss mice via i.p. injection of 0.148, 0.754 or 1.28 mg/kg bw Rounup	50% of lysis of human erythrocytes at Roundup concentrations of 31.91 ± 3.86 µL/dL. Above this point the erythrocytes undergo volume contraction, deformation and lysis. In mice, an increase in the occurrence of micronuclei is reported for the two highest doses of the exposed groups. Limitations/deviations from OECD474: test performed with unknown formulation,	Rodrigues, H.G., et al., 2011

Type of data/report Status study	Test substance	Relevant information about the study (as applicable)	Observations Deviations/limitations	Reference
			no info on number of animals used or sex, no HCD, i.p. not a relevant route for humans, no evaluation of toxicity, PCE/NCE ratio not determined, no information on clinical signs, number of cells scored not reported.	

2.6.4.1 Short summary and overall relevance of the provided information on genotoxicity / germ cell mutagenicity

In vitro genotoxicity studies

***In vitro* bacterial gene mutation assays**

A large database is available to assess the mutagenic potential of glyphosate in bacteria based on the reverse mutation ("Ames") test. In total, twenty-six bacterial reverse mutation studies are available for glyphosate, as reported in table 49. These include thirteen studies classified as either fully compliant with OECD GD 471 (acceptable; ten studies) or have only minor deviations that do not affect the validity or integrity of the data (acceptable but with restrictions; five studies). Two fully guideline compliant studies were submitted by the applicant as confidential data in Volume 4. One study was performed using glyphosate technical and one study used glyphosate technical together with one impurity. In addition, three studies were considered as supportive evidence and the remaining eight studies were considered not acceptable when compared with the guideline requirements. The latter studies are not further considered for classification purposes. All available studies were performed with and without metabolic activation to mimic *in vivo* liver metabolism via S9 supplementation. All thirteen studies that were OECD-guideline compliant and thus considered acceptable or acceptable but with restrictions, reported consistently the absence of mutagenicity in bacterial cells *in vitro* with and without metabolic activation in several tester strains. The same was concluded for the four studies that were considered as supportive. Therefore, based on the currently available OECD-compliant studies, there is no evidence that glyphosate causes gene mutations in bacterial gene mutation assays.

***In vitro* gene mutation assay in mammalian cells**

Two mouse lymphoma assays (MLA) and three HPRT gene mutation assays have been conducted with glyphosate (report nos. 434-015, 12325, ML-83-155, 31405 (reported in Vol 4, report no. 8441968). The two mouse lymphoma assays (report nos. 434-015, 12325) and the first HPRT gene mutation assay (report no. ML-83-155) are classified as reliable with restrictions as some minor deviations were noted from OECD GD 476, however, these are not considered to affect the validity or integrity of the data. A second HPRT gene mutation assay (report no 31405 was reported as confidential information in Volume 4) and a third new HPRT study in Chinese hamster V79 cells was submitted (report no. 8441968). Both studies were considered to be acceptable and negative without and with metabolic activation. All available studies were run with and without metabolic activation to mimic *in vivo* liver metabolism. The available studies all reported no mutagenic properties of glyphosate in either the mouse lymphoma assay or in Chinese hamster ovary cells. Therefore, based on the currently available OECD-compliant studies, there is no evidence that glyphosate causes gene mutations in mammalian cells.

***In vitro* clastogenicity / aneugenicity / DNA damage and repair**

Five chromosome aberration studies were performed (report nos. CTL/P/6050, 434/015, IET 94-0143, 141918 and the study submitted as CA 5.4.1/029). The first four studies are classified as acceptable but with restrictions as some minor deviations were noted from OECD GD 473, however, these are not considered to affect the validity or integrity of the data. The fifth study (no report number, study submitted as CA 5.4.1/029) was not considered acceptable due to several deviations (incomplete historical control data, only 100-200 cells in metaphase were evaluated, no cytotoxicity at maximum concentration and inconsistencies regarding exposure duration and reporting deficiencies). This study is not further considered for classification purposes. The available studies were all run with and without metabolic activation to mimic *in vivo* liver metabolism. No evidence of clastogenic properties for glyphosate were found in either of the two studies using human lymphocytes (report nos. CTL/P/6050 and 141918) or the two studies in Chinese hamster lung cells (report nos. CTL/P/6050 and IET 94-0143).

A new *in vitro* micronucleus assay was submitted (report no. 8441969). The study was considered to be acceptable and was negative for induction of micronuclei in human peripheral lymphocytes in presence and absence of metabolic activation.

Seven studies investigating glyphosate in *in vitro* DNA damage assays are available. None of these studies were considered reliable (with or without restrictions). One of these studies (report no. IET 94-0141) is considered as supportive data only due to methodological shortcomings. This 'supportive' study, which was a DNA repair test (Rec assay), which also showed no DNA-damaging activity in the presences or absence of metabolic activation (report no. IET 94-0141). The remaining six studies are considered not acceptable due to several methodological deviations and were not further considered for classification purposes (report nos. 931564, M-645649-01-1, ET-78-241, 87BME014-E, 87BMS013-E, and 300/2)

In addition to those regulatory GLP-compliant studies, nine open-literature publications report *in vitro* findings on clastogenic and/or aneugenic properties of glyphosate (Santovito *et al*., 2018; Nagy *et al*., 2019; De Almeida *et al*., 2018; Kasuba *et al*., 2017; Kwiatkowska *et al.*, 2017; Townsend *et al.*, 2017; Roustan *et al,* 2014; Mañas *et al*., 2013 and Koller *et al.*, 2012).

The open-literature publication by Santovito *et al.* (2018, CA 5.4/006) describes an *in vitro* chromosome aberration (CA) assay and micronucleus (MN) assay in human lymphocytes. At physiological relevant concentrations of 0.025 to 0.5 µg glyphosate/mL the study showed a dose-dependent increased frequency of micronuclei in the human peripheral lymphocytes cultured in the presence of different glyphosate concentrations. Also, glyphosate induced a dose-dependent increase structural chromosome aberrations including chromatid and chromosome breaks, dicentric chromosomes and acentric fragments. This study was broadly compliant with OECD GD 473 and 487, however, several critical deficiencies were observed and therefore the study is classified as supportive information only. The following limitations were noted: 1) Treatment with glyphosate was initiated 24 hours after lymphocyte cultures were stimulated to divide, instead of the recommended 48 hours, consequently the cultures would not have been asynchronous. This could mean cells in some stages of the cell cycle may have been under-represented, whilst others over-represented. 2) Exposure to glyphosate was continuous for 28 hours in the chromosome aberration assay or 48 hours in the micronucleus assay. In contrast OECD test guidelines recommend maximum exposure of 1.5 cell cycles, equivalent to approximately 24 hours for lymphocyte cultures. 3) For both endpoints (chromosome aberration and micronucleus formation) the paper does not confirm if the slides were coded prior to analysis. 4) the glyphosate tested was not sufficiently characterized and thus, the influence of possible impurities cannot be assessed. 5) The stability of the test compound and the tested concentration were not analytically verified. And 6) no historical (positive and negative) control data is reported, therefore it is not possible to conclude whether proficiency of the lab is sufficiently proven. Overall, due to these methodological deficiencies the study is classified as supportive information and therefore the results of this publication should be treated with caution.

Kasuba *et al*. (2017, CA 5.4/007) studied effects of low doses of glyphosate on oxidative stress and DNA damage by a Comet and a micronucleus assay in the HepG2 cell line. The comet assay showed a statistically significant decrease in tail intensity after 4 hours treatment with no difference from control after 24 hours. In the cytokinesis block micronucleus (CBMN) assay, a non-statistically significant increase in micronuclei frequency was seen after 4 hours without a dose-effect relationship. After 24 hours, a decrease instead of an increase in MN frequency was reported. The nuclear bud frequency was statistically significantly elevated after 4 hours of exposure but was statistically significantly lower than control after 24 hours of exposure. The indicator tests for oxidative stress did not show a substance related effect. Overall, the results of the study do not indicate a genotoxic potential of glyphosate. The lack of statistical significance, reproducible effects as well as the fact that the control values in the comet assay and micronucleus assay seem to be highly variable limit the reliability of the study. Other deficiencies are that the stability of the test compound and the tested concentration were not analytically verified. Further, no

historical (positive and negative) control data is reported, therefore it is not possible to conclude whether proficiency of the lab is sufficiently proven. Based on these methodological deficiencies, the study is considered as supportive only.

A further six publications are available which mostly used an *in vitro* comet assay to study DNA damaging properties of glyphosate (Nagy *et al*., 2019, CA 5.4/003; De Almeida *et al*., 2018, CA 5.4/004; Kwiatkowska *et al*., 2017, CA 5.4/008; Suárez-Larios *et al.*, 2017, CA 5.4/009; Townsend *et al*., 2017, CA 5.4/010; and Koller *et al*., 2012, CA 5.4/013). All studies are classified as supportive information only, which will be explained later in the text. Five of these studies showed positive results in the comet assay, except for the study by Nagy *et al*. (2019, CA 5.4/003), which showed negative results. In three out of the five publications with positive results, DNA damage was reported concomitant with high, confounding cytotoxicity (De Almeida *et al.*, 2018, CA 5.4/004) or necrosis/apoptosis (Koller *et al.*, 2012, CA 5.4/013) and/or positive results were noted at rather high test concentrations as compared to physiologically relevant concentrations (De Almeida *et al*., 2018, CA 5.4/004 and Townsend *et al*., 2017, CA 5.4/010). In the study by Kwiatkowska *et al*. (2017, CA 5.4/008), the lowest *in vitro* dose (0.5 mM) which showed positive results was higher than the *in vivo* plasma concentration (0.3 mM) measured in guideline-compliant studies in animals dosed with 2000 mg/kg bw at which no evidence for DNA damage was identified. The study by Suárez-Larios *et al.* (2017, CA 5.4/009) showed the induction of DNA double strand breaks, however, without a clear dose-response relationship. All six studies are considered as supportive information only due to methodological shortcomings. In none of the studies reported on the stability of the test substance and the tested concentrations were not analytically verified. For several studies details on the active substance are missing (e.g. the purity) (Nagy *et al*., 2019, CA 5.4/003; Suárez-Larios *et al.*, 2017, CA 5.4/009), positive control data is missing (Nagy *et al*., 2019, CA 5.4/003) and/or no information is provided on historical control data in order to prove the proficiency of the lab (Nagy *et al*., 2019, CA 5.4/003; De Almeida *et al*., 2018, CA 5.4/004; Kwiatkowska *et al*., 2017, CA 5.4/008; Suárez-Larios *et al.*, 2017, CA 5.4/009 and Townsend *et al*., 2017, CA 5.4/010).

The open-literature study by Koller *et al.* (2012, KCA 5.4/013) described an *in vitro* comet assay (for which the limitations are discussed above) and an *in vitro* micronucleus assay in human buccal epithelial cells. The micronucleus assay was considered positive as different nuclear anomalies were measured. This study further demonstrated that there is a big difference in cytotoxicity between glyphosate and a Roundup formulation with the latter containing surfactant being more cytotoxic than glyphosate itself. Glyphosate was found to significantly increase tail intensity in the comet assay but without any further increase with increasing doses thereby indicating that the outcome was equivocal. In contrast, Roundup increased tail intensity in a dose dependent manner with increasing cytotoxicity and decreasing cell integrity. This indicates that there is a relationship between the cytotoxicity of Roundup and DNA instability. As the results may be confounded by high cytotoxicity and as the study is considered as supportive information only due to methodological shortcomings, the results of this publication should be treated with caution.

Roustan *et al.* (2014, CA 5.4/011) reported on the cytogenetic effect of two herbicides (glyphosate and atrazine), their metabolites (AMPA and DEA), and mixtures thereof studied in an *in vitro* micronucleus test in CHO-K1 cells. Only the results of glyphosate and AMPA tested alone are further considered here. Glyphosate and AMPA were tested with and without metabolic activation and under light irradiation. Also, the potency of glyphosate and AMPA to produce ROS was investigated. No statistically significant increase in the incidence of bi-micronucleated cells (BMC) was observed with glyphosate at concentrations up to 100 µg/mL in the dark and without metabolic activation. However, a statistically significant and dose-related increase in BMC was noted from 10 µg/mL in the presence of metabolic activation. With light irradiation, a statistically significant increase in BMC was noted for glyphosate at a concentration of 100 µg/mL. The results that glyphosate scored positive only with the presence of metabolic activation is somewhat surprising since glyphosate is essentially unmetabolized *in vitro* in the presence of a rat liver S9 homogenate. Moreover, these results are not corroborated by regulatory *in vivo* micronucleus tests in the mouse dosed up to more than 2000 mg/kg bw. Due to several methodological shortcomings (no positive control included, no historical control data were reported, purities not reported, test substance stability and test concentration not analytically verified) the study is classified as supportive information only and therefore interpretation of the results should be handled with caution.

Overall, the studies published in the literature may indicate positive *in vitro* comet assay and micronucleus assays to some extent. However, due to inconsistencies in methodology (e.g. cell lines used, exposure conditions, toxicity measurements, concentrations investigated (cytotoxic and/or physiological irrelevant concentrations), reproducibility and missing controls, no analytic verification of test substance stability and tested concentration, no HCD reported in order to prove lab proficiency for performing this specific assay) the toxicological relevance of the reported findings is unclear. When considered alongside the consistently negative findings in the regulatory mutagenicity/genotoxicity studies, it is concluded that DNA damage occurs rather secondary to other toxic cellular events than being the consequence of genotoxic potential of glyphosate. Furthermore, as demonstrated by the

consistent negative findings in *in vivo* models, provided that normal homeostasis mechanisms are not overwhelmed, glyphosate-induced secondary DNA damage does not occur *in vivo*.

Remark RMS: it is noted that several other *in vitro* public literature studies were discussed in the previous CLH report (BAuA, May 2016). Most studies were not submitted by the applicant as the publication date is >10 years before submission of the current dossier. For these studies, the RMS has included the information from the previous RAR (see below).

For the *in vitro* studies included in the previous CLH report but not submitted by the applicant as they were published over 10 ago, RMS has included the information from the previous RAR. See more details in Vol. 3 section B.6.4.4.16. RMS has not re-evaluated these studies:

In two studies by Lioi et al. (1998, ASB2013-9836 and ASB2013-9837) an increase in chromosome aberration (CA) and sister chromatid exchange (SCE) frequency was reported in human lymphocytes from 3 donors at concentrations between 5 and 51 µM and in bovine lymphocytes at concentrations between 17 and 170 µM. In the previous evaluation it was noted that the result of these studies are questionable as a number of well performed and validated studies *in vitro* mammalian cell and *in vivo* in mammals did not register comparable effects even in dose levels more than 10 times higher than the doses used in the studies described by Lioi et al. Three publications reported testing of technical glyphosate for micronucleus or chromosome aberration endpoints in cultured human lymphocytes (Manas et al., 2009, ASB2012-11892; Mladinic et al., 2009, ASB2012-11906; Mladinic et al., 2009, ASB2012-11907). Negative results for the micronucleus or chromosome aberration end points were observed in the absence of exogenous metabolic activation (S9) in all three publications. The maximum exposure concentration in the absence of S9 was in the range of 3-6 mM in these studies. It is noted that in the previous evaluation, the studies by Mladinic et al. (ASB2012-11907) and Mañas et al. (ASB2012-11892) were considered to be not reliable (Klimisch 3) and not relevant; the other study by Mladinic et al. (ASB2012-11906) was considered to be reliable with restrictions (Klimisch 2) and to be relevant with restrictions.

Bolognesi et al. (1997, Z59299) reported positive results from a micronucleus test in mouse bone marrow erythrocytes. Either glyphosate a.i. (declared as 99.9% pure) or a Roundup formulation were administered to Swiss mice once daily by the i.p. route on two consecutive days. Cell samples were harvested at 6 and 24 hours following the final dose. A weak positive effect was observed at total dose levels of 300 mg/kg bw (2 x 150 mg/kg bw/day) after 24 hours for glyphosate and of 450 mg/kg bw (2 x 225 mg/kg bw/day) at both sampling times for Roundup.
Further data in this publication indicated for high purity glyphosate a significant and dose-dependent increase in SCE frequency in human lymphocyte cultures obtained from two female donors from a concentration of 1000 µg/mL onwards. For Roundup, this effect became apparent even at lower concentrations of 100 and 330 µg/mL. However, this study was considered to not be reliable in the previous RAR: "*The test was not performed according to the current OECD guideline. In particular, the number of animals used (three male mice per dose group) was too low since a group size of at least five is recommended. A dose response cannot be assessed since only one dose level was included. The basis for statistical comparison is questionable since it is not clear when the six control animals were sacrificed because only one group mean value was indicated. Due to these deficiencies, this isolated positive finding is not considered to provide sufficient evidence to contravene the previously obtained negative results regarding the active substance."*

Monroy et al. (2005, ASB2012-11910) found positive SCGE result for two mammalian cell lines exposed to glyphosate for 4 hours at concentrations of 4.5-6.5 mM (GM39 cells) and 4.75-6.5 mM (HT1080 cells). It was noted in the previous RAR that "*results were found with exposures to mM concentrations of glyphosate. Although this dose level range is lower than the limit dose of 10 mM recommended for several in vitro mammalian cell culture assays, an even lower limit dos of 1 mM was recommended for human pharmaceuticals, particularly because of concerns about relevance of positive in vitro findings observed at higher dose levels. Concerns over the possibility of effects induced by toxicity have led to several suggestions for experimental and interpretive criteria to distinguish between genotoxic DNA-reactive mechanisms for induction of alkaline SCGE effects and cytotoxic or apoptotic mechanisms. One recommendation for the in vitro alkaline SCGE assay is to limit toxicity to no more than a 30 % reduction in viability compared to controls. Importantly, dye exclusion measurements of cell membrane integrity, such as those reported in some of the above publications may significantly underestimate cytotoxicity that could lead to alkaline SCGE effects. Other recommendations include conducting experiments to measure DNA double strand breaks to determine if apoptotic process might be responsible for alkaline SCGE effects. Measurement of apoptotic and necrotic incidence were only performed in one publication (Mladinic et al., 2009, ASB2012-11906) and these measurements indicated both apoptotic and necrotic processes occurring in parallel with observations of alkaline SCGE effects. These direct observations as well as the reported dose responses, consistently suggest that biological effects and cytotoxicity accompany the observations of DNA damage in vitro in mammalian cells and therefore confirm the likelihood that the observed effects are secondary to cytotoxicity and are thresholded."*

Overall conclusion on *in vitro* genotoxicity tests

In line with the conclusions of the previous CLH report (BAuA, May 2016) and the RAC opinion (March 2017), the standard regulatory GLP-compliant genotoxicity assays on glyphosate including bacterial Ames assays and mammalian cell gene mutation tests gave consistently negative results. Further, the majority of *in vitro* chromosomal aberration tests and micronucleus tests were negative. All studies performed according to GLP resulted in negative findings. Several *in vitro* indicator tests gave positive results for induction of SCE and DNA strand breaks (comet assay) mainly at cytotoxic concentrations but a negative result for induction of DNA repair (UDS). However, for all these studies several methodological shortcomings were identified. Thus, it is concluded that no obvious mutagenicity/genotoxicity could be evidenced for glyphosate based on acceptable *in vitro* data.

Remark RMS:
The applicant provided a justification for the 1 mM concentration threshold as a criterium for relevance of public literature publication. The following text is copied from RAR Vol 3 section B6.10 literature search.
"Some specific criteria were applied for articles on human health. In case of *in vitro* toxicity tests studies that tested beyond 1 mM were not considered to be relevant. The reason for this is because it is physiologically not possible to attain such concentrations in regulatory *in vivo* testing due to the limited oral bioavailability (appr. 20%), low dermal absorption and rapid excretion. Further justification on the selection of the 1 mM limit can be found in doc K-CA section 9.

The applicant provided the following justification in KCA-9 (report no 108689-CA9-1, 2020):
" *The limit of 1 mM has been based on the single dose oral pharmacokinetic data of a formulation containing 71.7% w/w glyphosate where an oral dose of 1,430 mg/kg bw in the rat gives plasma levels of 38.1 µg/mL or 0.225 mM after 2 hours. When extrapolated linearly (which is possible for glyphosate because it is not subject to hepatic metabolism) this gives plasma levels of 53.3 µg/mL or 0.315 mM at 2 hours after oral intake of 2,000 mg/kg bw and 107 µg/mL or 0.630 mM at 2 hours after oral intake of 4,000 mg/kg bw. A systemic concentration of glyphosate of 1 mM would then represent an oral dose of more than 6,000 mg/kg bw which is completely unreasonable for repeat dose experimental in vivo testing under today's OECD test guidelines. The ADI for glyphosate of 0.5 mg/kg bw/day corresponds with a daily systemic concentration of 0.17 µg/mL or 1 µM when a 60 kg person with 36 L extracellular fluid is considered with a glyphosate oral bioavailability of 20%."*

The RMS largely agrees with the above justification, however, a reference should be provided for the study in which an oral dose of 1,430 mg/kg bw (given as a formulation of 71.7% w/w glyphosate) resulted in plasma levels of 38.1 µg/mL in the rat. If the study is not already included in the dossier, the study should be submitted and evaluated. In addition, a further justification should be given on whether locally higher levels of glyphosate at cellular level could be reached (e.g. in intestinal epithelial cells and/or in the local lymphatic vessels of the intestinals).

In vivo genotoxicity/mutagenicity studies

***In vivo* studies in somatic cells**

An extensive database is available for glyphosate regarding *in vivo* genotoxicity in somatic cells, comprising micronucleus assays and chromosome aberration studies in mice or rats after oral or intraperitoneal application.

Fourteen *in vivo* micronucleus studies have been conducted with glyphosate, one in the rat (Report no. 23917 CA 5.4.2/014) and the remaining in the mouse. One micronucleus study in the mouse has been submitted as confidential information in Volume 4 where glyphosate with an impurity was administered (report no. 14613.402.078.14). In total, nine studies were GLP-compliant and performed according to OECD TG 474 and were therefore considered as either acceptable (report no. 14613.402.078.14 (Vol 4); report no. 485-1-06-4696 (CA 5.4.2/001); report no. 1479200 (CA 5.4.2/002); report no. 1158500 (CA 5.4.2/005)) or as acceptable but with restrictions (report no. 2060/014 (CA 5.4.2/007); report no. [redacted]-G12.79/99 (CA 5.4.2/008); report no. [redacted]/P/4954 (CA 5.4.2/009); report no. 889-MUT.MN (CA 5.4.2/010); report no. 12324 (CA 5.4.2/012); report no. 23917 (CA 5.4.2/014)). The remaining four studies were considered not acceptable due to the mentioned deviations from OECD TG 474; the studies were considered invalid for the evaluation (report no. [redacted]-3996.402.395.07 (CA 5.4.2/003 and CA 5.4.2/004); report no. RL33393/2007-3.0MN-B (CA 5.4.2/006); report no. 300/3 (CA 5.4.2/011); report no. not reported (CA 5.4.2/013)).

All *in vivo* micronucleus studies that were considered valid (either as acceptable or as acceptable but with restrictions) were negative and did not induce a statistically significant increase of micronuclei in the bone marrow,

except in two studies which will be discussed below. In the first study (report no. 889-MUT.MN (CA 5.4.2/010)) an equivocal result was obtained in high dose females. In this study, female mice were dosed at 5000 mg/kg bw/day administered on two consecutive days, which is above the current guideline dose of max 2000 mg/kg bw/day. Due to missing historical control data and a high variation in the % of polychromatic erythrocytes with micronuclei, the biological significance of the weak positive result observed in the females dosed at 5000 mg/kg bw/day is unclear. Considering this study, the following was stated in the previous CLH report (BAuA, May 2016): "*In contrast, a cytogenetic study conducted in the same laboratory and the same mouse strain under nearly identical conditions did not provide any evidence of chromosome aberrations even though test material of the same purity was applied at the same dose levels (890-MUT-CH.AB (CA 5.4.2/015)). In this second study of the same group, a certain degree of cytotoxicity to bone marrow cells at the highest dose level became apparent since the mitotic index was reduced. Although not measured in the preceding micronucleus test, such an effect could be expected to have occurred in the previous experiment, too, and cytotoxicity might have contributed to micronucleus formation. Last but not least, the study author also concluded that, under the conditions of the experiment, glyphosate was not mutagenic in the micronucleus test in mice."* The current RMS agrees with the conclusion of the previous CLH-report. Another MN study in mice using IP administration (report no. 2060/014 (CA 5.4.2/007)) reported a small but statistically significant increase in micronuclei observed at the highest dose but only at the 24 hour sample (but 48 hour data were clearly negative). The increase in micronuclei was within the range of the laboratory's historical control data and was accompanied by a reduction in the target cells (polychromatic erythrocytes). Based on this observation, the study authors postulated that this indicates that the increase in micronuclei was the consequence of a haematopoietic response to bone marrow toxicity rather than to a specific genotoxic effect. Together with the other clearly negative studies, these studies do not give rise to a concern for clastogenicity and/or aneugenicity of glyphosate.

For the *in vivo* micronucleus studies, target organ exposure to glyphosate should be proven. However, only toxicokinetic studies showing bone marrow exposure are available for the rat (e.g. report no. 6365-676/1 (CA 5.1.1/011), report no. 7006-676/2 (CA 5.1.1/012), report no. [redacted]-7215 (CA 5.1.1/014) and report no. [redacted] 332/951256 (CA 5.1.1/010), but not for the mice. One recent guideline-compliant *in vivo* micronucleus study was submitted as confidential information in Volume 4 (report no. 14613.402.078.14 (J-CA 5/028)). In this study bone marrow exposure of glyphosate proven by clinical signs (bristling, tachypnoea and motor incoordination) and by measurement of plasma levels of glyphosate at 24 h after application. In addition, several MN studies were conducted using intraperitoneal (IP) injection. While ensuring high systemic exposure, these caused clinical signs of toxicity consistent with systemic exposure. In one of these studies by IP administration, effects were noted which were considered to be due to bone marrow toxicity (report no. 2060/014 (CA 5.4.2/007)). Moreover, as also stated in the previous CLH-report, in a long-term study in rats (report No. 2060-0012, CA 5.5/001) the occurrence of hypoplasia in bone marrow was reported although this latter finding was confined to a very high dose. Overall, there is sufficient evidence to conclude that the target tissue in these studies, namely the bone marrow, was actually exposed to glyphosate. The same conclusion was drawn in the previous CLH report (BAuA, May 2016).

Two *in vivo* chromosome aberration studies have been conducted with glyphosate, one in mice by oral gavage (report no. 890-MUT-CH.AB (CA 5.4.2/015)) and one in rats by intraperitoneal (IP) injection (CA 5.4.2/016; report no. 830083). Although both studies are considered as supportive only, due to several deficiencies when compared with OECD 475 (2016), they provide some further evidence that glyphosate is not clastogenic *in vivo*.

Next to the above discussed regulatory guideline studies, four open-literature publications were submitted which were two *in vivo* micronucleus assays and two *in vivo* comet assays. The two *in vivo* micronucleus assays were performed with a similar protocol as OECD TG 474, however, the studies were classified as supportive information, due to several limitations. The first study by Ilyushina et al. (2018a, CA 5.4/002) negative test results were reported for a micronucleus test testing four batches of glyphosate (purity of respectively 95.7, 98.3, 95.1, and 95.8%) in mice at an oral dose level of 2000 mg/kg bw/day administered by gavage on 2 consecutive days. The same authors published a second paper in which three different technical batches of glyphosate were tested (purity of respectively 96.6 %, 95.8 % and 95.7 %) at concentrations ranging from 500 up to 2000 mg/kg bw/day similar to OECD 474 (*Ilyushina* et al., 2018b, CA 5.4/005). In the latter study, the authors reported that the tested samples of technical products showed different cytogenetic activities, with only one out of the three tested batches causing a statistically significant, dose-dependent increase in the frequency of micronuclei compared to the negative control. The authors postulated the presence of 0.13% formaldehyde in the respective batch as cause for the positive result although they did not provide any data to support their hypothesis. The RMS notes that this may be questionable whether 0.13% formaldehyde present in the test substance may cause micronuclei, also considering that for formaldehyde no evidence is known that indicates systemic mutagenicity of formaldehyde.

Remark RMS: in the previous CLH report four additional *in vivo* micronucleus studies were discussed (Mañas et al. (2009, ASB2012-11892), Bolognesi et al. (1997, Z59299), Rank et al. (1992, Z82234) and Chruscielska et al. (2000, ASB2013-9830)). These studies were not submitted by the applicant. As these studies were published over 10 ago,

RMS has included the information from the previous RAR. See more details in Vol. 3 section B.6.4.4.16. RMS has not re-evaluated these studies.

In the study by Mañas et al. (2009, ASB2012-11892) also an *in vivo* micronucleus assay was performed in mice at three dose levels via i.p. injection rendering statistical significance at 400 mg/kg bw (13.0±3.08 micronucleated erythrocytes/1000 cells, $p < 0.01$). In the previous evaluation (RAR 2015) this study was considered not reliable (Klimisch 3) as there were several guideline and reporting deficiencies.
Bolognesi et al. (1997, Z59299) reported positive results from a micronucleus test in mouse bone marrow erythrocytes. Either glyphosate a.i. (declared as 99.9 % pure) or a Roundup formulation were administered to Swiss mice once daily by the i.p. route on two consecutive days. Cell samples were harvested at 6 and 24 hours following the final dose. A weak positive effect was observed at total dose levels of 300 mg/kg bw (2 x 150 mg/kg bw/day) after 24 hours for glyphosate and of 450 mg/kg bw (2 x 225 mg/kg bw/day) at both sampling times for Roundup. In the previous RAR (2015), this study was considered to be not reliable (Klimisch 3) as there were several guideline and reporting deficiencies.
In the RAC opinion (RAC 40 opinion, 2017), the following was stated regarding the studies by Mañas et al. (2009) and Bolognesi et al. (1997): "*Two micronucleus tests showed positive results. In the first positive study (Mañas et al., 2009) Balb-C mice (5 per dose, sex unclear) were used. A statistically significant increase in micronucleated erythrocytes (% MN cells in controls 0.38 and at high dose 1.3) was reported at 24 hours after the animals had received two i.p. doses of 200 mg/kg bw glyphosate, administered 24 h apart. The two lower doses (2x50 or 2x100 mg/kg bw) were negative in this study. The study was reported by the DS to have some deviations from the OECD TG 474, the most problematic being that 1000 (instead of 2000) erythrocytes per animal were scored, and "erythrocytes" instead of immature or "polychromatic erythrocytes" (PCE) were scored for micronuclei. RAC notes that it is unclear whether the authors have counted mature or immature erythrocytes as they did not specify this in the article. RAC also notes that counting as few as 1000 PCE (assuming PCE were counted) would give results which are less reliable. For these reasons, the result from this study should be interpreted with care. In the second positive study (Bolognesi et al., 1997) an increase (0.075% in control; 0.14% at 6h and 0.24% at 24h) in micronuclei in mouse bone marrow cells following two i.p. doses of 150 mg/kg bw on two consecutive days was reported. The study is limited in its methodological description. However, it reports 4 animals (instead of five) in each of the glyphosate exposure groups, but counting of more cells (3000 vs 2000 NPCs per animal). The publication gives no reference to historical control data."*

In the study by Rank et al. (1993, Z82234) a micronucleus test in mouse bone marrow erythrocytes following single i.p. administration, Roundup as well as the IPA salt (i.e., a 1:1 mixture of glyphosate technical and isoproplyamine) proved negative up to the highest dose of 200 mg/kg bw. However, with Roundup but not with the glyphosate IPA salt alone, there was evidence of bone marrow cytotoxicity at this top dose level as indicated by a significantly lower percentage of polychromatic erythrocytes. During the previous evaluation (RAR 2015) the following was noted: "*According to the publication and to further information submitted by Monsanto, it is assumed that the Roundup formulation used was made of 48 % IPA salt, tallowamine surfactant, and water. The design of the micronucleus test was not in compliance with guideline requirements. A direct comparison between results obtained with the IPA salt and Roundup is not feasible since not exactly the same dose levels were used and since there was a difference in sampling time (24 and 48 h post dosing for the IPA experiment versus only at 24 h after administration of Roundup). The reported weak bone marrow cytotoxicity occurring already after single i.p. administration of 200 mg Roundup/kg bw (amount calculated as the IPA salt to facilitate comparison) may be considered a possible formulation-related effect when the observations in other micronucleus studies (see section I) are taken into consideration."*

The study by Chruscielska et al. (2000, ASB2013-9830) showed negative results in an *in vivo* micronucleus assay performed in mice at 300 mg/kg bw via i.p. injection with both the active as a formulation. The study did have several methodological deficiencies. It is noted that in the previous RAR (2015) the reliability of this study was not specifically indicated.

RMS comment regarding these four additional literature studies (which were copied from the previous RAR, 2015) and are summarized above: In the studies by Mañas et al. (2009) and Bolognesi et al. (1997) positive results were found, however both studies were considered not to be reliable due to guideline deviations and reporting deficiencies. The other two studies were negative for the active ingredient glyphosate. Overall, it is considered that the results from these four additional *in vivo* micronucleus assays do not change the overall conclusion. Based on the consistently negative findings in the regulatory guideline compliant *in vivo* micronucleus and chromosome aberration studies, it is concluded that glyphosate does not cause clastogenicity or aneugenicity in these studies.

In the first *in vivo* public literature study using the comet assay (Mañas *et al.*, 2013 (KCA 5.4/012)) a positive result was reported in liver and blood cells of *Balb C* mice after glyphosate (96% analytical grade) treatment at dose levels of 40 and 400 mg/kg bw/day for 14 days via drinking water. No statistically significant differences have been found in liver, kidney, lung and heart for all oxidative stress parameters measured with the exception of a decrease in SOD activity in the heart and an increase in CAT activity in the kidney at a daily glyphosate dose of 400 mg/kg bw. There was an increase in CAT activity in the lung but this was not statistically significant and did not show a dose-effect relationship. A statistically significant increase in severity of the elevated DNA damage parameters (tail intensity, tail length and tail moment) was reported for glyphosate with the exception of tail intensity in the liver at 40 mg/kg bw/day. No clear dose-effect relationship was evident for DNA damage parameters in blood after treatment with glyphosate. A dose-effect relationship was present for tail length and tail moment in the liver. This publication is considered as supportive information only as there were several limitations such as description of the method is very limited, sex of animals unknown, number of doses and scored nucleoids not in line with OECD TG 489, no positive controls or HCD provided to prove lab proficiency. Therefore, the results of the study showing an increase in DNA damage in liver and blood, with only two dose levels tested for glyphosate with few animals and without a dose-effect relationship in blood should be interpreted with caution. No comparable guideline-compliant studies are available on repeated oral dose administration of glyphosate during 14-days. During the previous assessment in the CLH report (BAuA, May 2016) the following was concluded for this study: "*More recently, Mañas et al. (2013, ASB2014-6909) reported a positive Comet assay in liver and blood cells of Balb C mice after glyphosate (96% analytical grade) administration at dose levels of 40 and 400 mg/kg bw/day for 14 days in drinking water. A clear dose response was seen only in the liver. The authors also reported evidence of oxidative stress. Taking into account that glyphosate proved negative in the UDS assay ([redacted], 1994, TOX9400697 (reported in the current assessment as report no. 931564 (CA 5.4.1/033)), the published findings in this indicator test are not considered to provide convincing evidence of an interaction with the DNA. Positive results in the alkaline elution assay may also occur as a result of toxic but non-mutagenic effects. In general, DNA damage end points such as SCE or alkaline SCGE are generally regarded as supplementary to the gene mutation and chromosome effects end point categories. DNA damage endpoints do not directly measure effects on heritable mutations or events closely associated with chromosome mutations. Stimulation of oxidative metabolism is not a sign of mutagenicity but may elucidate a possible mechanism behind toxic effects*." The RMS agrees with the conclusion of the previous CLH-report.

A second public literature study using the alkaline comet assay was reported by Milic *et al*. (2018, CA 5.3.1/010). In this study, glyphosate was orally administered to groups of 6 male rats at 0.1, 0.5, 1.75 and 10 mg/kg bw for 28 days. A significant increase in tail length and tail intensity in leucocytes and small (non-parenchymal cells, <30 µm of head length) and medium (parenchymal cells or hepatocytes, between 30 and 40 µm of head length) sized liver nuclei was observed. However, with the exception of tail length of small sized liver nuclei, no dose-effect relationship was evident. Moreover, tail intensity of the leucocytes could not be assessed because of the very high variability of the results. Also, oxidative stress markers in plasma and liver and cholinesterase activity in plasma revealed no dose-response relationship. Based on the information provided it is not possible to determine whether the acceptance criteria of the assay are met as no information is provided on the compatibility of the positive and negative controls with the laboratory's historical control database. Therefore it is not possible to determine whether the outcome of the Comet assay should be considered positive, negative or equivocal. The only conclusion that can be drawn is that with the exception of tail length of small sized liver nuclei, no dose-effect relationship was evident. Overall, based on overall quality of the reported data, especially missing dose-responses in several parameters, no conclusive decision on DNA damaging properties of glyphosate is possible.

Overall, the two public literature studies do not provide a clear and conclusive evidence of DNA damaging properties based on *in vivo* comet assays. In contrast, based on the extensive database of guideline-compliant *in vivo* somatic cell mutagenicity studies it is concluded that glyphosate is not genotoxic to rodents.

Remark RMS: In the previous CLH report some additional *in vivo* DNA damage studies were discussed (Bolognesi et al. (1997, Z59299) and Peluso et al., 1998, TOX1999-318). These studies were not submitted by the applicant. As these studies were published over 10 ago, RMS has included the information from the previous RAR. See more details in Vol. 3 section B.6.4.4.16. RMS has not re-evaluated these studies.

Bolognesi et al. (1997, Z59299) found a transient but significant effect towards DNA damage in liver and kidney in the alkaline elution assay after glyphosate (300 mg/kg bw) or Roundup (900 mg/kg bw) had been administered once by the i.p. route to mice. This assay may indicate the induction of DNA single-strand breaks and alkali labile sites. A test for DNA oxidative damage suggested glyphosate and the formulation Roundup to stimulate oxidative metabolism in the liver (glyphosate) or in the kidney (Roundup) at 24 hours after application. During the previous evaluation (RAR 2015) the following was noted: "*The data from the tests for DNA damage and stimulation of oxidative metabolism (Bolognesi et al., 1997, Z59299) are hardly to interpret since the results are given in summary figures only which are based on pooled individual data. There are reporting inconsistencies, e.g. it is not clear how*

many animals were actually used for testing. A positive control substance was not included. Taking into account that glyphosate proved negative in the UDS assay which is generally accepted to indicate a more frequent occurrence of DNA damage and repair (see section B.5.4.1.3 in the monograph), the published findings are not considered to provide convincing evidence of an interaction with the DNA. Positive results in the alkaline elution assay may also occur as a result of toxic but not-mutagenic effects. Stimulation of oxidative metabolism is not a sign of mutagenicity but may elucidate a possible mechanism behind toxic effects." It is noted that this study was considered to be not reliable during the previous evaluation (RAR 2015).

In a subsequent study from the same institute (Peluso et al., 1998, TOX1999-318), a low incidence of DNA adducts was found by means of the very sensitive ^{32}P-postlabeling technique in the liver and kidney of mice following single intraperitoneal administration of Roundup. All tested concentrations (400, 500 and 600 mg Roundup/kg bw, corresponding to 122, 152, and 182 mg glyphosate salt/kg bw) caused DNA adducts in both organs. A dose response was to be seen. In contrast, treatment with the vehicle (i.e., a DMSO/olive oil mixture) and with doses of 130 and 270 mg glyphosate IPA salt/kg bw did not result in DNA adduct formation. During the previous evaluation the following was noted: "*The results of Peluso (1998, TOX1999-318) and his group suggest a direct effect on the DNA. It has been shown that the observed effects were related to administration of the formulation only but not to glyphosate IPA salt. Biological significance of the results is equivocal. Generally, it is questionable whether findings after i.p. administration can be applied to more realistic exposure conditions. Of course, the occurrence of such effects also after oral intake would be much more relevant for human health evaluation. Furthermore, some deficiencies of this study make a definitive assessment difficult. It is rather equivocal what a low incidence of DNA adducts per animal as compared to no adducts in the control group actually means since a positive control substance was not included. The degree of variation between the animals is not known because only mean values for the groups comprizing of 3 to 6 mice were reported and individual values are not given but would be helpful for interpretation of the results. Another point of concern is the lacking information on toxicity. At least with Roundup, one could expect marked general toxicity when the observations reported from the micronucleus tests (see section I of this addendum) and from the acute intraperitonal toxicity studies (see section B.5.2.4 in the monograph) were taken into account. It is known that DNA adducts may be formed not only as a result of direct interaction of cellular DNA with chemicals but also occur naturally or can be even related to a treatment-dependent increase in endogenous metabolites. Thus, further characterisation of these adducts and clarification of their nature would be desirable."* It is noted that this study was considered to be not relible during the previous evaluation (RAR 2015).

Both studies (Bolognesi 1997 and Peluso 1998) seem to give an indication of possible DNA damage. However, due to methodological and reporting deficiencies both studies were considered not reliable in the previous RAR (2015). RMS is of the opinion that these studies do not alter the conclusion that based on the extensive database of guideline-compliant *in vivo* somatic cell mutagenicity studies it is concluded that glyphosate is not genotoxic to rodents.

***In vivo* studies in germ cells**

Genotoxic effects on germ cells were examined *in vivo* in three dominant lethal assays with rats and mice. These studies were considered to provide supportive evidence on mutagenic properties of glyphosate. Based on the available data, no genotoxic effect of glyphosate on germinal tissues could be evidenced in either rats or mice (report no. TOXI-888-DLT (CA 5.4.3/001-003); report no. not reported (CA 5.4.3/004); and report no. 401-064 (CA 5.4.3/005)).

In the first study, glyphosate did not induce dominant lethal effects in Wistar rats up to a dose level of 5000 mg/kg bw (report no. TOXI-888-DLT, CA 5.4.3/001-003). Briefly, investigation of the female uteri contents revealed an acute toxic effect of glyphosate at 5000 mg/kg bw after the first mating with treated males, but not after a single dose of 200 and 1000 mg/kg bw by oral gavage. The number and percentage of pregnant females and the number of implantations per dam were significantly lower than for control animals. In line with these findings, there was an increased incidence of early resorptions and of pre- and post-implantation losses in the animals of this group. In the first mating group, there were no adverse effects on the number of corpora lutea, the number of foetal and embryonic resorptions and the number of live implants per dam. Fertility indices during the remaining 9 study weeks were not considered to be affected by treatment. Some statistically significant effects were observed (e.g. number of implantations, number of early, foetal and embryonic resorptions, number of live implants and number of pre- and post-implantation losses), but those were observed among all dose groups and without any relation to dose or duration of treatment. Due to the identified methodological deficiencies (e.g. spacing factor between dose levels too high (5 instead of 2 – 4), too low number of implantations per group (< 400 implants), foetal body weights not recorded, no information on historical control data provided and the mean pre- and post-implantation losses per dam not calculated, but percentages of pre-implantation losses and percentages of post-implantation losses (corresponding to the dominant lethal factor) reported), the study was regarded as supportive information only.

In the second study, no mutagenic potential in germ cells was observed in CFY rats in a dominant lethal study after a 8-week repeated dietary administration in male rats (report no. not reported (CA 5.4.3/004)). Dose levels of 10, 30 and 100 mg glyphosate/kg food were applied, corresponding to mean test substance intakes of 6.8, 20.5 and 70.4 mg/kg bw/day. Investigations of the female uterine contents revealed no treatment-related findings and the percentage of post-implantation loss, corresponding to the dominant lethal factor, was comparable for all glyphosate and control groups. The study was considered supplementary due to serious reporting deficiencies (e.g. unknown purity of the test compound, equivocal number of males and females allocated to the individual test groups, number of implants < 400/group, dose levels appear to be too low for definitive assessment, HCD not reported).

In the last study, the mutagenic properties of glyphosate were investigated in CD-1 mice (report no. 401-064 (CA 5.4.3/005)). Ten male mice were treated with 200, 800 and 2000 mg glyphosate/kg bw (single dose) via gavage. Immediately after dosing, each male was paired with untreated virgin females (mating ratio 1:2) for 7 days followed by pairing with two new females for a second week. Investigations of the female uterine contents revealed no treatment-related findings. The number of pregnant females, the number of *corpora lutea* and implantation sites and the number of early and late resorptions were not affected for any mating interval upon treatment with glyphosate. There was a slight but statistically significant decrease in the number of viable foetuses in females of the 800 mg/kg bw group mated during study week 1 and in females of the 2000 mg/kg bw group mated during study week 3. As no increase in early foetal deaths were observed in these groups, the findings were not attributed to glyphosate treatment and considered to be incidental. Pre- and post-implantation losses and calculated dominant lethal factors were comparable for treated and control groups. No mutagenic potential was identified for glyphosate in the conducted dominant lethal assay. Due to several deviations to the current guideline (e.g. number of implantations per group < 400 implants per group, number of males and females used for mating too low, foetal body weights not recorded and no historical control data provided, dominant lethal frequency calculated based on raw data provided in the study report), the study was considered to provide supporting information.

Human data

As mentioned by RAC (RAC 40 opinion, 2017), ″some genotoxicity studies in human populations after occupational exposure to glyphosate-based herbicides (GHB) or exposure of bystanders/area residents exist, but their interpretation in regard to genotoxicity/germ cell mutagenicity of glyphosate is challenging”. RAC mentioned that, however, some evidence was suggested in two published studies (described below) which investigated populations believed to be exposed to glyphosate based formulations. Remark RMS: the applicant has not submitted these publications as the publication date is >10 years before submission of the current dossier. RMS has included the information from the previous RAR (2015) for these studies into Volume 3, section B.6.4.4.15, but has not re-evaluated these two studies.

Copied from the RAC-opinion / RAR 2015:
“Paz-y-Miño and co-workers (2007, refer to Vol 3 CA B.6.4.4.15) examined the consequences of aerial spraying with a glyphosate based herbicide added to a surfactant solution in the northern part of Ecuador. A total of 24 exposed (livingin with 3 km of areas sprayed) and 21 unexposed control individuals were investigated using the Comet assay 2 weeks to 3 months following intensive aerial spraying. The sprayed material was reported to be Roundup Ultra, a GBF containing 43.9% glyphosate, polyethoxylated tallowamine surfactant and a proprietary component, Cosmoflux 411F. Specific methods for collection, storage, and transport of blood samples are not described for either the exposed population or control group. The publication also does not indicate that slides were coded for scoring which consisted of visual classification into damage categories and measurement of DNA migration (tail length). Therefore documentation of the Comet assay was insufficient for assessment. There were fairly large differences in ages and sex distribution of the exposed and control populations but these did not appear to be statistically significant. The study reported increases in damaged cell categories and statistically significant increases in DNA migration (tail length) in the presumably exposed population. Interpretation of the results of this study should consider numerous reported signs of toxicity in the exposed population and the reported application rate of 24.3 litres/ha which was stated to be 20 times the maximum recommended application rate. Some of the reported human health effects described by Paz-y-Mino appear to be consistent with severe exposures noted in clinical reports of acute poisoning incidents with glyphosate formulations and other pesticide formulations (often self-administered) rather than typical bystander exposures. The factors related to either high surfactant exposure, unusual components in this formulation or other undocumented variables are likely to be confounding factors in this study. Further, individuals among the exposed group manifested clinical symptoms of toxicity after several exposures to aerial spraying which may by itself have an effect on generation of DNA single strand breaks. During the previous evaluation the study was classified as Klimisch code 3 (not reliable).

Bolognesi and co-workers (2009, refer to Vol 3 CA B.6.4.4.15) reported on results of a blood lymphocyte cytokinesis-block micronucleus study of individuals in areas treated with glyphosate formulations by aerial spraying

or manual application. Although the title of the publication contains the term “agricultural workers”, most of the populations studied do not appear to be agricultural workers who are involved in application of glyphosate-based formulations. The human lymphocyte culture and scoring methodology employed in the study appear to be generally consistent with commonly used and recommended practices for this assay. However, there is a significant question as to how long the blood samples used in the study were stored prior to initiating cultures and this may have affected the micronucleus numbers observed in the different sets of samples and populations. Also, the populations in the aerially sprayed regions had a second sampling a few days after the first sampling and this second sampling was not performed in the control populations. The publication reported a small increase in the frequency of binucleated cells with micronuclei and micronuclei per cell in samples collected from people living in three regions after spraying of glyphosate formulations compared with control values of samples collected just before spraying. However, the pattern of the increases did not correlate either with the application rate or with self-reported exposure. The largest post-spraying increase in binuclated cell micronucleus frequency was reported for a population with a much lower glyphosate active ingredient application rate and only 1 of 25 people in this region reported contact with sprayed glyphosate formulation. Increases in binucleated cell micronucleus frequency did not have a statistically significant relationship with self-reported exposure for two other populations. Some interpretative statements in Bolognesi et al. (2009) suggest a small transient genotoxic effect of glyphosate formulation spraying on frequencies of binucleated cells with micronuclei, but other statements indicate that causality of the observed effects could not be determined using reasonable criteria and that lack of exposure data precluded conclusions. This study has a combination of uncontrolled or inadequately characterized variables, such as uncharacterised exposure to ”genotoxic pesticides”, that would appear to preclude using the data to support any conclusion that exposure to glyphosate formulations affects binucleated micronucleus frequencies. Actually, the available data, while certainly limited in nature, support a conclusion that the observed effects do not appear to be attributable to glyphosate formulation exposure.

Other studies relevant for genotoxicity

In the previous RAC opinion (March 2017) several studies on oxidative stress were discussed. The following assessment was done by RAC (text copied from RAC opinion):
" *Measurements of DNA adduct levels and markers of oxidative stress may provide information on the potential genotoxic mode of action.*

Bolognesi et al. (1997) measured formation of the oxidative DNA lesion 8-hydroxy-2' –deoxyguanosine (8-OHdG) in liver and kidney from mice 8 h and 24 h following a single i.p. exposure to glyphosate (300 mg/kg bw). A statistically significant increase in 8-OHdG was reported in liver at 24 h, but not after 8 h and not in the kidney.

No increase in DNA adduct formation was detected by the 32P-postlabelling method following i.p. exposure to glyphosate isopropyl ammonium salt to mice at a single dose of 130 or 270 mg/kg bw (Peluso et al., 1998).

Oxidative stress is characterized by an imbalance between generation of reactive oxygen species and anti-oxidant defense mechanisms, and can be measured as an increase in markers of oxidative stress such as malondialdehyde (MDA) e.g. by the thiobarbituric acid reactive substances (TBARS) assay.

In a study by Mladinic et al. (2009) exposing isolated human whole blood samples to glyphosate in vitro, several markers of oxidative stress were examined. In this study an increase in plasma TBARS levels was demonstrated at the highest concentration of 580 μg/mL glyphosate. A modified version of the comet assay was used with addition of the human 8-oxoguanine DNA glycosylase (hOgg1) that recognises the oxidised DNA lesion 8-OHdG. No consistent increases in Ogg1-sensitive DNA lesions was revealed over the concentration range tested.

A few studies (Mañas et al., 2009 and 2013; Dai et al., 2016) have measured levels of lipid peroxidation byproducts (MDA/TBARS) as putative makers of oxidative stress following in vivo exposures of mice or rats to glyphosate. Significant changes in MDA or TBARS were not reported in mouse tissues to single or repeated administrations of glyphosate, although some differences in activities of antioxidant enzymes were reported (Mañas et al., 2009 and 2013). In a rat study (Dai et al., 2016) with doses up to 500 mg/kg bw/day for five weeks, no significant increases in testicular MDA levels or changes in anti-oxidant enzyme levels were reported. In addition, the IARC report and the RAR both refer to a study in rats by Astiz et al. (2009). This study measured effects on oxidative stress markers and oxidative defense systems in several tissues following repeated i.p. (10 mg/kg bw) glyphosate exposures three times a week for five weeks. TBARS concentrations in several tissues were increased (~doubled) in glyphosate exposed animals compared to the control animals, whereas plasma protein carbonyl levels were unaffected. In the RAR, this study is given Klimisch code 3 due to deficiencies in reporting, low number of animals per group (4 rats/group), and i.p. route of administration. RAC notes that only the unexposed control data and not the vehicle control data are presented and that the statistical evaluation seems to compare responses with the unexposed control data. The authors stated that they did not find any differences between data from the unexposed control group and the vehicle control group, but this is not shown.

In conclusion, the in vitro and in vivo data suggest that glyphosate may induce oxidative stress. However, increased levels of oxidative stress were not reliably demonstrated in the repeated dose studies where this was examined."

Remark RMS: For the studies mentioned on this subject in the RAC opinion that were published over 10 years ago, RMS has included copies from the evaluation in the previous RAR (2015). These can be found in Volume 3, section B.6.4.4.17. RMS has not re-evaluated these studies. In addition it is noted that in the previous RAR, the studies by Bolognesi *et al*. (1997), Peluso *et al*. (1998), Manas *et al*. (2009) and Astiz *et al*. (2009) were considered to be not reliable (Klimisch 3); the study by Mladinic et al. (2009) was considered to be reliable with restrictions (Klimisch 2). The study by Dai *et al*. (2016) is evaluated in Vol 3 section B.6.6.3.1 (KCA 5.6.1/023).

More recently, several non-standard studies investigated the effects of glyphosate on oxidative stress and DNA damage or methylation in diverse cell systems (HepG2 cells (Kasuba et al., 2017, B.6.4.4.7, CA 5.4/007), human peripheral blood cells (Kwiatkowska et al., 2017, B.6.4.4.8, CA 5.4/008) and CHO-K1 cells (Roustan et al., 2014, B.6.4.4.11, CA 5.4/011) under *in vitro* conditions. Further, the effect on oxidative homeostasis in mice after glyphosate administration was investigated in one study in several tissues including liver, kidney, lung and heart (Mañas et al., 2013, CA 5.4/012). All four studies were considered as supportive information due to methodological shortcomings. In general, the investigated endpoints like oxidative stress and/or oxidative DNA damage and induction of proteins involved in DNA recombination do not directly measure effects on heritable mutations or events closely associated with chromosome mutations. Especially stimulation of oxidative stress is not conclusively indicative for mutagenicity but may indicate a possible mechanism of toxicity and induced cellular biological effects. Alterations in DNA methylation may not necessarily be indicative of genotoxicity, in addition to the mostly reversible nature of the epigenetic modifications. The toxicological relevance of the results reported by Kwiatkowska et al. (2017, B.6.4.4.8, CA 5.4/008) in regard to classification purposes for germ cell mutagenicity remains unclear, especially as global methylation was reported to be decreased whereas methylation of specific DNA regions was increased.
Overall, the studies reporting rather biochemical and/or molecular events on DNA and protein level after glyphosate exposure are not considered to provide sufficiently conclusive evidences on genotoxicity. Therefore, they are not taken into account for classification of glyphosate for genotoxicity/mutagenicity. On the other hand, some of the *in vitro* and *in vivo* studies suggest that glyphosate may induce oxidative stress. However, increased levels of oxidative stress were not reliably demonstrated in the available studies.

2.6.4.2 Comparison with the CLP criteria regarding genotoxicity / germ cell mutagenicity

The criteria for classification for germ cell mutagenicity under Regulation 1272/2008 is as followed:

Category 1: Substances known to induce heritable mutations or to be regarded as if they induce heritable mutations in the germ cells of humans. The classification in Category 1A is based on positive evidence from human epidemiological studies. The classification in Category 1B is based on positive results from *in vivo* heritable germ cell mutagenicity tests in mammals or positive results from *in vivo* somatic cell mutagenicity tests in mammals, in combination with some evidence has potential to cause mutations to germ cells or positive results from tests showing mutagenic effect in the germ cells of humans, without demonstration of transmission to progeny.

Category 2: Substances which cause concern for humans owing to the possibility that they may induce heritable mutations in the germ cells of humans. The classification in Category 2 is based on: positive evidence obtained from experiments in mammals and/or in some cases from *in vitro* experiments, obtained from somatic cell mutagenicity tests *in vivo* in mammals or other *in vivo* somatic cell tests which are supported by positive results from *in vitro* assays.

Two studies from public literature with data from humans are available (see Table 51). These studies were already evaluated in the previous RAC opinion and RMS is of the opinion that this conclusion does not change:
"A limited number of biomonitoring studies have examined markers of possible genotoxicity in blood cells from humans exposed occupationally or from the general population in regions with high use of glyphosate. Some of these studies showed an apparently positive relationship between exposure to glyphosate and the levels of the markers being studied. However, all these studies were compromised by the lack of clear information about exposure to glyphosate itself and glyphosate-based formulations, and the extent to which other substances or lifestyle factors could have contributed to the findings. In some cases, the low numbers of subjects involved was also a factor. Although not completely negative, these studies do not provide sufficiently robust evidence of glyphosate genotoxicity to justify classification for this endpoint. The classification of glyphosate as Muta. 1A is not justified."

In line with the conclusions of the previous CLH report (BAuA, May 2016) and the RAC opinion (RAC 40, March 2017), the standard regulatory GLP-compliant genotoxicity assays on glyphosate including bacterial Ames assays and mammalian cell gene mutation tests gave consistently negative results. Further, the majority of *in vitro* chromosomal aberration tests and micronucleus tests were negative. All studies performed according to GLP resulted in negative findings. Several *in vitro* indicator tests gave positive results for induction of SCE and DNA strand breaks (comet assay) mainly at cytotoxic concentrations but a negative result for induction of DNA repair (UDS). However, for all these studies several methodological shortcomings were identified. Thus, it is concluded that no obvious mutagenicity/genotoxicity could be evidenced for glyphosate based on reliable *in vitro* data.

Regarding *in vivo* data in somatic cells:
Fourteen *in vivo* micronucleus studies have been conducted with glyphosate and RMS considers that overall, glyphosate does not induce micronuclei *in vivo*. Additional studies from public literature do not change this conclusion. Two *in vivo* chromosome aberrations assay which were negative provide further evidence that

glyphosate is not clastogenic *in vivo*.
Two public literature studies discussing Comet assays do not provide clear and conclusive evidence of DNA damaging properties. Based on the extensive database of guideline-compliant in vivo somatic cell mutagenicity studies it is concluded that glyphosate is not genotoxic to rodents.

Regarding *in vivo* data in germ cells:
These effects were examined in three dominant lethal assays with rats and mice. Based on these studies no genotoxic effect of glyphosate on germinal tissues could be evidenced in either rats or mice.

In line with the previous RAC opinion (RAC 40, March 2017) it is still considered that the mammalian *in vivo* database is sufficient and overall indicates that glyphosate does not warrant classification as Muta 1B.

Regarding Cat. 2:
As already indicated in the previous RAC opinion (RAC 40, March 2017):
"Classification in Category 2 is largely based on positive evidence obtained from somatic cell mutagenicity tests in mammals or other *in vivo* somatic cell genotoxicity tests which are supported by positive results from *in vitro* mutagenicity assays. Glyphosate is only metabolised to a very limited degree and is not a DNA reactive substance. Bacterial and mammalian gene mutation assays were all negative. Thus, the genotoxicity observed for glyphosate in some studies is likely to be caused by indirect mechanisms. Glyphosate appears to induce transient DNA strand breaks as observed in the *in vitro* and *in vivo* Comet assays. However, as glyphosate does not induce gene mutations and bone marrow mutagenicity is considered negative, their biological importance in relation to mutagenicity is equivocal. Further, it is unclear whether oxidative stress is of biological importance as a MoA for glyphosate as the data are equivocal. Taking all data into account, and based on the overall negative responses in the existing gene mutation and oral mutagenicity tests, RAC concludes that no classification of glyphosate for germ cell mutagenicity is warranted."

In line with the previous RAC opinion (RAC 40, March 2017) the RMS agrees with the previous conclusion that no Cat. 2 classification of glyphosate for germ cell mutagenicity is warranted.

2.6.4.3 Conclusion on classification and labelling for genotoxicity / germ cell mutagenicity

No classification for germ cell mutagenicity is warranted.

2.6.5 Summary of long-term toxicity and carcinogenicity [equivalent to section 10.9 of the CLH report template]

Table 53: Summary table of animal studies on long-term toxicity and carcinogenicity

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results** **- NOAEL/LOAEL** **- target tissue/organ** **- critical effects at the LOAEL**	**Reference**
OECD453, GLP Deviations[1] Rat, Wistar, male and female, 51/sex/dose (terminal), 15/sex/dose (interim) Study acceptable	Glyphosate, batch H05H016A, purity 95.7% 24-month dietary exposure at 0, 1500, 5000 and 15000 ppm (equal to 0, 85.5, 285.2, 1077.4 mg/kg bw/day in males and 0, 104.5, 348.6	<u>15000 ppm</u> - Increased alkaline phosphatase (up to +59% in males and +98% in females) - Changes in plasma electrolytes - Increased severity of adipose infiltration of the bone marrow in males - Change in mineral deposition within the kidney (lower incidence of pelvic/papillary deposition in males and females; and an increase in the corticomedullary deposition in females) - Skin effects including areas of necrosis/giant cell reaction to keratin and keratoacanthoma in males. Incidence of skin keratoacanthomas in males: 2/51, 3/51, 0/51, 6/51	Report No. 2060-0012 CA 5.5/001 [redacted] 2009

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL	Reference
	and 1381.9 mg/kg bw/day in females)	Salivary glands: Weight: not determined Histopathology: submaxillary gland only; no effect reported	
OECD 453, GLP Deviations[2] Rat, Wistar, male and female, 52/sex/dose (terminal), 12/sex/dose (interim) Study acceptable	Glyphosate, batch P30, purity 97.6% 24-month dietary exposure at 0, 2000, 6000 and 20000 ppm (equal to dose levels of 0, 121, 361 and 1214 mg/kg bw/day for males and 0, 145, 437 and 1498 mg/kg bw/day for females)	20000 ppm: - Minor changes in body weight (<10%) - Increase alkaline phosphatase (up to +70% in males; up to +87% in females), alanine aminotransferase (up to +59% in males), total bilirubin (up to +50% in males). Decreased plasma triglycerides (up to -24% in males) and cholesterol (up to -24% in males). - Hepatitis in males (8/64, 6/64, 9/64, 13/64). - Hepatocellular adenoma in males (0/64, 2/64, 0/64, 5/64 in males at 0, 2000, 6000, 20000 ppm). - Kidney papillary necrosis (0/64, 1/64, 0/64, 14/64 in males at 0, 2000, 6000, 20000 ppm; 0/64, 1/64, 2/64, 5/64 in females at 0, 2000, 6000, 20000 ppm), transitional cell hyperplasia (2/64, 3/64, 0/64, 5/64 in males at 0, 2000, 6000, 20000 ppm; 3/64, 1/64, 0/64, 1/64 in females at 0, 2000, 6000, 20000 ppm) and papillary mineralisation (1/64, 2/64, 0/64, 5/64 in males at 0, 2000, 6000, 20000 ppm; 1/64, 1/64, 0/64, 3/64 in females at 0, 2000, 6000, 20000 ppm). - Prostate prostatitis (13/64, 22/64, 23/64, 37/64 in males at 0, 2000, 6000, 20000 ppm) Salivary glands: Weight: not determined Histopathology: submandibular and parotid; no effect reported	Report No. [redacted]/PR1111 CA 5.5/002 [redacted] 2001
OECD 453, GLP Deviations[3] **Study unacceptable** Rat, Sprague-Dawley, male and female, 50/sex/dose (terminal), 20/sex/dose (interim)	Glyphosate, batch and purity not reported 24-months, dietary exposure at 0, 3000, 15000 and 25000 ppm (equivalent to 0, 150, 780, 1290 mg/kg bw/day in	25000/30000 ppm: - Decreased body weight gain in males (-12%) - Increased ALP (+83% in males, +117% in females) - Increased liver weight in females (+10%) - Increased kidney weight in females (+16%) Salivary glands: Weight: not determined Histopathology: not specified; no effect reported	Report No. 1231 CA 5.5/003 [redacted] 1997

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL	Reference
	males and 0, 210, 1060 and 1740 mg/kg bw/day in females) Interim group high dose at 30000 ppm (1920 mg/kg bw/day in males and 2540 mg/kg bw/day in females)		
OECD 453, GLP Deviations[4] Rat, Sprague-Dawley, male and female, 50/sex/dose (terminal), 30/sex/dose (interim) Study acceptable	Glyphosate, batch T-941209; T-950308, purity 97.56%; 94.61% 24-month dietary exposure at 0, 3000, 10000 and 30000 ppm (equal to 0, 104, 354, and 1127 mg/kg bw/day in males and 0, 115, 393, 1247 mg/kg bw/day in females)	<u>30000 ppm:</u> - Clinical signs including loose stool (incidences could not be determined due to grouped housing of animals), bradypnea (11/50 males compared to 3/50 males in control), mass (37/50 males compared to 22/50 males in control), soiled fur in perianal region (21/50 males compared to 10/50 males in control), decreases in incidence of tactile hair loss (0/50 males compared to 5/50 males in control), wound (0/50 males compared to 7/50 males in control) and hair loss (3/50 males compared to 12/50 males in control) - Decreased body weight (up to -10% in males and up to -6% in females) and decreased body weight gain. - Decreased urinary pH and protein - Increased caecum weight (+113% in males at week 104 and +127% in females at week 52 (+84% at week 104)) - Distention of caecum (0/18, 1/20, 1/18, 16/29 in males at 0, 3000, 10000, 30000 ppm), follicular hyperkeratosis of tail skin in males (7/76, 5/75, 2/80, 23/78 at 0, 3000, 10000, 30000 ppm), skin keratoacanthomas in males (4/76, 3/75, 0/80, 7/78 at 0, 3000, 10000, 30000 ppm), skin basal cell tumours (0/76, 0/75, 0/80, 4/78 in males) <u>10000 ppm:</u> - Increased caecum weight (+36% in males at week 26 (+32% at week 104); +42% in females at week 52 (+40% at week 104)) <u>Salivary glands:</u> Weight: not determined Histopathology: submandibular and parotid; no effect reported	Report No. [redacted] 94-0150 CA 5.5/004 [redacted] 1997

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL	Reference
OECD 453, GLP Deviations[5] Rat, Wistar, male and female, 50/sex/dose (terminal), 10-20/sex/dose (interim) Acceptable but with restrictions	Glyphosate, batch 60; 046, purity 96.8%; 96% 24-month dietary exposure at 0, 100, 1000 and 10000 ppm (equal to 0, 6.3, 59.4 and 595.2 mg/kg bw/day in males and 0, 8.6, 88.5 and 886.0 mg/kg bw/day in females)	<u>10000 ppm:</u> - Increased ALP in females (up to +108%) - Increased incidence of cataract (3/50, 4/50, 2/50, 7/50 males at 0, 100, 1000, 10000 ppm; 1/50, 4/50, 5/50, 4/50 females at 0, 100, 1000, 10000 ppm) - Increase in mandibular lymph node lymphoma in high dose males at terminal sacrifice (not significant). Incidence of mandibular lymph node lymphoma (dead and moribund sacrificed animals plus terminally sacrificed animals): 0/48, 0/35, 0/37 and 2/50 <u>Salivary glands:</u> Weight: not determined Histopathology: glands not specified; no effect reported	Report No. 886.C.C-R CA 5.5/005 [redacted] 1996
OECD 452, GLP Deviations[6] Rat, Wistar, male and female, 24/sex/dose Study acceptable	Glyphosate, batch P24, purity 95.6% 12-month dietary exposure at 0, 2000, 8000 and 20000 ppm (equal to 0, 141, 560 and 1409 mg/kg bw/day for males and 0, 167, 671 and 1664 mg/kg bw/day for females)	<u>20000 ppm:</u> - Clinical signs (increased urinary staining) - Decreased body weight (up to -7% in males and up to -5% in females; both not adverse) - Decreased food consumption (up to -9% in males and females; both not adverse) - Reduced cholesterol and triglycerides in males (-13% and -36% at week 27, respectively), increased bilirubin in males (+21% at week 14) and increased ALP (+86% in males and +88% in females at week 27) - Increase in focal basophilia of the acinar cells of the parotid salivary gland (see below for incidence) - Proliferative cholangitis in the liver - Prostatitis of the prostate gland <u>8000 ppm:</u> - Increase in focal basophilia of the acinar cells of the parotid salivary gland in females (see below) <u>Salivary glands:</u> Weight: not determined Histopathology: increase in focal basophilia of the acinar cells of the parotid salivary gland at 20000 ppm (10 minimal, 3 slight in M and 8 minimal, 5 slight and 2 moderate in F vs 2 minimal in M and 2 minimal in F controls) and	Report No. [redacted]/P/5143 CA 5.5/006 [redacted] 1996

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL	Reference
		8000 ppm (females only; 6 minimal vs 2 minimal in controls).	
OECD 453, GLP Deviations[7] Rat, Sprague-Dawley, male and female, 50/sex/dose (terminal), 35/sex/dose (interim) Study acceptable	Glyphosate, batch 229-JaK-5-1; 229-Jak-142-6, purity 98.9%; 98.7% 24-month dietary dose at 0, 10, 100, 300 or 1000 mg/kg bw/day.	<u>1000 mg/kg bw/day:</u> - Decreased body weight gain (-14% in males and -11% in females in the oncogenicity group after 104 weeks) - Increased ALP (+72% in males and +51% in females at week 102) - Reduced urinary pH - Decreased absolute or relative liver weight (up to -20% in males and up to -17% in females) - Increased absolute or relative salivary gland weight (up to +129% in males and up to +17% in females) - Cellular alterations of salivary glands (refer to table below) - Increased incidence of skin keratoacanthomas in males (5/50 compared to 1/50 in controls) <u>300 mg/kg bw/day:</u> - Increased ALP (+36% in males and +67% in females at week 78) - Decreased absolute or relative liver weight (up to -16% in males and up to -12% in females) - Increased absolute or relative salivary gland weight (up to +94% in males and up to +20% in females) - Cellular alteration of salivary glands (refer to table below) <u>100 mg/kg bw/day:</u> Non adverse effects noted: - Minor increase in ALP in females (+36%) - Minor decrease in relative liver weight (-8%) Adverse effects noted: - Slight increase in salivary gland weight (+12%) - Cellular alteration of salivary gland (refer to table below) Incidence of skin keratoacanthomas in males: 1/50, 2/25, 0/19, 0/21, 5/50 <u>Salivary glands:</u> Weight: dose-related increase in abs/rel parotid weight starting at 100 mg/kg bw/day in M at wk 52, but not at wk 104; combined sublingual and submaxillary gland weight was increased in males and females at top dose only Histopathology: parotid and mandibular (submaxillary) glands; refer to table below for results (cellular alteration is recorded when cells were larger and stained deeply basophilic).	Report No. 7867 CA 5.5/007-009 [redacted] 1993

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL	Reference

	Dose group (mg/kg bw/day)									
	0		10		100		300		1000	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Parotid – cellular alteration, oncogenicity group (wk 104)										
Grade +/-	4	1	4	2	8	2	3	2	4	**5**
Grade +	3	0	5	5	9	**9****	**21*****	**9****	**14****	**13*****
Grade ++	0	1	0	1	4	1	**17*****	**9***	**18*****	**18*****
Grade +++	0	0	0	0	0	0	0	1	0	2
Parotid – cellular alteration, toxicity group (wk 52)										
Grade +	0	0	0	0	**9****	2	**8****	**5***	4	**8****
Grade ++	0	0	0	0	1	0	4	3	**7****	**5***
Grade +++	0	0	0	0	0	0	0	0	4	1
Mandibular (submaxillary) – cellular alterations, oncogenicity group (wk 104)										
Grade +/-	7	2	5	0	10	3	14	1	9	6
Grade +	0	9	0	8	**12*****	9	**28*****	15	**22*****	**19***
Grade ++	0	0	0	0	0	0	0	2	0	1
Mandibular (submaxillary) – cellular alterations, toxicity group (wk 52)										
Grade +	0	0	0	-	1	2	**5***	0	**12*****	0

OECD 453, GLP Deviations[8] Rat, Sprague-Dawley, 50/sex/dose (terminal), 10/sex/dose (interim) Study acceptable	Glyphosate, batch XLH-264, purity 96.5% 24-month dietary dose at 0, 2000, 8000 and 20000 ppm (equal to 0, 89, 362 and 940 mg/kg bw/day for males and 0, 113, 457 and 1183 mg/kg bw/day for females)	<u>20000 ppm:</u> - Decreased body weight gain in females (-15% at week 1-7) - Increased incidence of cataract (1/29, 3/44, 6/25, 13/39 males at 0, 2000, 8000, 20000 ppm) and lens fibre degeneration (4/60, 6/60, 5/60, 8/60 at 0, 2000, 8000, 20000 ppm) - Increased ALP in females (+87% at 24 months) - Reduced urinary pH - Slight increase in liver weight in males (+13%) - Increased incidence of inflammation (2/58, 3/58, 5/59, 7/59 in males at 0, 2000, 8000, 20000 ppm; 0/59, 3/60, 9/60, 6/59 in females at 0, 2000, 8000, 20000 ppm) and hyperplasia (3/58, 3/58, 4/59, 7/59 in males at 0, 2000, 8000, 20000 ppm; 2/59, 3/60, 7/60, 6/59 in females at 0, 2000, 8000, 20000 ppm) of stomach squamous mucosa - Increased incidence of hepatocellular adenoma in males only (see incidences below) - Increased incidence of thyroid C-cell adenoma (see incidences below). <u>8000 ppm:</u> - Increased incidence of inflammation and hyperplasia of stomach squamous mucosa (for incidences see above) - Increased incidence of thyroid C-cell adenoma (for incidences see below).	Report No. [redacted]-10495 CA 5.5/010 [redacted] [redacted] 1990

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL	Reference
		Incidence of hepatocellular adenoma in males: 3/60, 2/60, 3/60, 8/60 Incidence of thyroid C-cell adenoma: Males: 2/60, 4/58, 8/58, 7/60 Females: 2/60, 2/60, 6/60, 6/60 Incidence of pancreatic islet cell adenomas in males: 1/58, 8/57, 5/60, 7/59 Incidence of skin keratoacanthomas in males: 1/59, 3/60, 4/60, 5/59 <u>Salivary glands:</u> Weight: not determined Histopathology: No histopathological changes were noted in the submandibulary glands whereas the parotid gland was not examined microscopically.	
OECD 451, GLP **Study unacceptable**, dose levels too low; low quality of study report Rat, Sprague-Dawley, 50/sex/dose	Glyphosate, XHJ-64, purity 98.7% 24-month dietary dose at 0, 30, 100 and 300 ppm (0, 3.05, 10.3, 31.49 mg/kg bw/day in males and 0, 3.37, 11.22 and 34.02 mg/kg bw/day in females)	No effect on body weight, food consumption, haematology, clinical chemistry, organ weight, non-neoplastic findings. Interstitial cell tumour of the testes: 0/50, 3/50, 1/50, 6/50 Pancreatic islet cell adenomas in males: 0/50, 5/49, 2/50, 2/50 <u>Salivary glands:</u> Weight: not determined Histopathology: mandibular; no effect reported	Report No. 77-2062 CA 5.5/011 [redacted] 1981
OECD 451, GLP Deviations: no histopathology on cervix and coagulating gland Mouse, CD-1, 51/sex/dose Study acceptable	Glyphosate, batch H05H016A, purity 95.7% 18-month dietary dose at 0, 500, 1500 and 5000 ppm (equal to 0, 71.4, 234.2 and 810 mg/kg bw/day in males and 0, 97.9, 299.5 and 1081.2 mg/kg bw/day in females)	No effect on body weight, food consumption, white blood cell count and organ weights. In males increase in malignant lymphoma: 0/51, 1/51, 2/51, 5/51 <u>Salivary glands:</u> Weight: not determined Histopathology: glands not specified; no effect reported	Report No. 2060-0011 CA 5.5/012-015 [redacted] 2009

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL	Reference
OECD 451, GLP Deviations: mortality observed once daily, food consumption not measured after 13 weeks, histopathology did not include cervix, Harderian gland, lacrimal gland, male mammary glands and vagina. Mouse, Swiss albino, 50/sex/dose Study acceptable	Glyphosate, batch 01/06/97, purity >95% 18-month dietary dose at 0, 100, 1000 and 10000 ppm (equal to 0, 14.5, 149.7 and 1454 mg/kg bw/day for males, and 0, 15.0, 151.2 and 1466.8 mg/kg bw/day for females	<u>10000 ppm:</u> - No adverse systemic effects - Slight increase in malignant lymphomas (not significant): 10/50, 15/50, 16/50 and 19/50 for males and 18/50, 20/50, 19/50 and 25/50 in females - Statistically significant increased incidence (Peto test) in mesenteric lymph node haemangioma in females: 1/50, 0/48, 0/48, 4/50 <u>Salivary glands:</u> Weight: not determined Histopathology: glands not specified; no effect reported	Report No. Toxi: 1559.CARCI-M CA 5.5/016 [redacted] 2001 Statistical re-analysis: B.6.5.12.1 CA 5.5/017 [redacted] 2017 Unacceptable due to errors in tumour incidences B.6.5.12.2 Re-analysis by AGG
OECD 451, GLP Deviations: mortality observed once daily; histopathology did not include cervix, lacrimal glands and male mammary gland. Mouse, CD-1, 50/sex/dose Study acceptable	Glyphosate, batch T-941209 and T-950308, purity 97.56% and 94.61% 18-month dietary dose at 0, 1600, 8000 or 40000 ppm (equal to 0, 165.0, 838.1 and 4348 mg/kg bw/day for males and 0, 153.2, 786.8 and 4116 mg/kg bw/day for females)	<u>40000 ppm:</u> - Clinical signs: pale-coloured skin (2/50, 3/50, 6/50, 10/50 in males; 4/50, 2/50, 6/50, 6/50 in females)), loose faeces (incidences not determined due to group housing of animals), tactile hair loss in males (0/50, 3/50, 3/50, 6/50), mass(es) at anus in males (0/50, 0/50, 0/50, 8/50) and decreased incidence of wounds (22/50, 16/50, 20/50, 6/50) and wetted fur (11/50, 9/50, 7/50, 4/50) in males. - Reduced body weight (gain) (up to -7% in males and up to -18% in females) - Reduced food consumption (overall group mean of -6% in males and -7% in females) - Increased incidences of distention of caecum (0/50, 0/50, 0/50, 14/50 in males; 0/50, 0/50, 0/50, 18/50 in females) - Increased caecum weight (+173-212%) - Increased relative kidney weight (females, 111%) - Increased incidence of anal prolapse (0/50, 0/50, 0/50, 5/50 in males; none in females) - Erosion/ulcer of the anus (observed in a total of 8 males; not assessed by a statistical method) - Increase in malignant lymphoma in males (see below) <u>8000 ppm:</u> - Reduced body weight (gain) (up to -10% in females)	Report NO. [redacted] 94-0151 CA 5.5/018-019 [redacted] 1997

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL	Reference
		Incidence of malignant lymphoma: Males: 2/50, 2/50, 0/50, 6/50 Females: 6/50, 4/50, 8/50, 7/50 <u>Salivary glands:</u> Weight: not determined Histopathology: glands not specified; no effect reported	
OECD 451, GLP Deviations[9] Mouse, CD-1, 50/sex/dose Study acceptable	Glyphosate, batch 206-JaK-25-1, purity 98.6% 24-month dietary exposure at 0, 100, 300 and 1000 mg/kg bw/day	No adverse findings observed. <u>Salivary glands:</u> Weight: not determined Histopathology: parotid, submaxillary, sublingual; no effect reported	Report No. 7793 CA 5.5/020-021 [redacted] 1993
Non-guideline, non-GLP Severe deviations noted including low number of animals and low quality study report **Study unacceptable** Mouse, Balb/c, 25/sex/dose (terminal), 5/sex/dose (interim)	Glyphosate, batch and purity not reported. 80-weeks dietary exposure at 0, 75, 150, 300 ppm (equal to0, 1.63, 3.35 or 5.87 mg/kg bw/day (males) and 0, 1.65, 3.35 or 5.42 mg/kg bw/day (females))	No indication of carcinogenic potential. However number of animals was too low and the background tumour incidences in controls was unusually low. <u>Salivary glands:</u> Weight: not determined Histopathology: not specified; no effect reported	CA 5.5/022, no report number provided. [redacted] 1988
OECD 451, conducted prior to GLP Deviations : no histopathology on coagulating gland, lacrimal glands, seminal vesicles and vagina Mouse, CD-1, 50/sex/dose	Glyphosate, batch NB 1782608/3 and NB 1782610/7, purity 99.7% 2-year dietary exposure at 0, 1000, 5000 and 30000 ppm (equal to 157, 814 and 4841 mg/kg bw/day for males and 190, 955 and	<u>30000 ppm:</u> - Reduced body weight (>10%) - Increased absolute and relative testis weight (+7% and +18%, respectively) - Hepatocyte hypertrophy in males only (18%, 10%, 6%, 34%) and necrosis in males only (0%, 4%, 4%, 20%) - Chronic interstitial nephritis in kidney (10%, 4%, 14%, 24% in males) and proximal tubule epithelia basophilia and hypertrophy in kidney (0%, 4%, 8%, 18% in females) - Kidney renal tubule adenoma in males (for incidences see below)	Report No. 77-2061 CA 5.5/023 [redacted] 1983

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL	Reference
Study acceptable	5874 mg/kg bw/day for females)	- Urinary bladder epithelium hyperplasia (slight to mild; 6%, 7%, 21%, 17% in males; 0%, 0%, 4%, 0% in females) <u>5000 ppm:</u> - Urinary bladder epithelium hyperplasia (slight to mild) in males (for incidences see above) Incidence of renal tubule tumours in male (PWG re-evaluation): Adenomas: 1/49, 0/49, 0/50, 1/50 Carcinomas: 0/49, 0/49, 1/50, 2/50 Combined: 1/49, 0/49, 1/50, 3/50 <u>Salivary glands:</u> Weight: not determined Histopathology: mandibular; no effect reported	
OECD 451, prior to GLP Severe deviations including surviving animals too low, no histopathology of animals that died during the study **Study unacceptable** Mice, CFLP, 50/sex/dose	Glyphosate, batch 14/980-03090380, purity not reported 18-month dietary exposure at 0, 100 and 300 ppm (equal to 0, 12.6 and 37.7 mg/kg bw/day for males and 0, 16.3 and 44.5 mg/kg bw/day for females)	No adverse effects noted. However, study unacceptable due to a wide number of deviations from the OECD test guideline and dose levels being too low. <u>Salivary glands:</u> Weight: not determined Histopathology: not specified; no effect reported	Report No. 8010 CA 5.5/024 [redacted] 1982 (original report) 1992 (revised translated version)

[1] Thyroid/parathyroid weight not measured. Histopathology did not include the cervix, coagulating gland and the lacrimal gland.

[2] Thyroid/parathyroid weight not measured. Histopathology did not include the coagulating gland and the vagina.

[3] A large number of parameters were missing in the haematological investigation, clinical chemistry and urinalysis. Organ weights of epididymides, heart, spleen, thyroid and uterus were not determined. In addition only the weight of 10 animals in the terminal sacrifice group were measured. Histopathology did not include harderian gland, cervix, coagulating gland, lacrimal gland and vagina. Except for the liver, kidney, lungs, testes, adrenals and ovaries only a limited number of animals were examined histopathologically in the low and mid dose.

[4] Prothrombine time and activated partial thromboplastin time were not investigated, organ weight measurement did not include epididymides, heart, ovaries, spleen, uterus and (para)thyroid. Histopathology did not include lacrimal gland.

[5]Individual animal weight exceeded the 20% rans, mortality observed once daily instead of twice, haematology did not include prothrombin time, activated prothrombin time and reticulocyte count, clinical chemistry did not include P, Cl, Na, K, cholesterol, bilirubin, and creatinine, urinalysis did not include osmolality/specific gravity and occult blood, organ weights did not include epididymides, heart, spleen, thyroid/parathyroid and uterus. In addition only 10 animals were included in organ weight measurements. Histopathology did not include Harderian gland, cervix, coagulating gland, lacrimal gland, rectum and vagina.

[6] Mortality observed once daily, blood samples not taken at start of the study but at week 14, organ weights did not include heart, ovaries, spleen, thyroid/parathyroid and uterus. Histopathology did not include coagulating gland, lacrimal gland, mammary gland of males and vagina.
[7] Haematological evaluation did not include prothrombin time and activated partial thromboplastin time, urinalysis did not include glucose, organ weights did not include (para)thyroid, histopathology did not include Harderian gland, cervix, coagulating glands, lacrimal glands, seminal vesicles and vagina.
[8] Animals were approximately 8 weeks old at the start of the study, haematology did not include prothrombin time and activated prothrombin time, volume of urine was not determined, organ weight measurements did not include adrenals, heart, ovaries, spleen, thyroid/parathyroid and uterus, histopathology did not include coagulating gland, lacrimal glands and vagina.
[9] Histopathology did not include Harderian gland, cervix, eye, coagulation glands, submandibular lymph nodes, lacrimal glands, seminal vesicles and vagina.

Table 54: Summary table of human data on long-term toxicity and carcinogenicity

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
Epidemiological study, public literature Study reliable with restrictions.	-	Pooled analysis of the case-control studies McDuffie *et al.* 2001 and De Roos *et al.* 2003 <u>Outcome evaluated:</u> Non-Hodgkin lymphoma (NHL) and subtypes of NHL <u>Population:</u> Ever use: 1690 cases, 5131 controls Duration of use: 1520 cases, 4183 controls Frequency of use and lifetime days of use: 898 cases, 2938 controls <u>Exposure:</u> Questionnaire by participants or by proxies. <u>Data analysis:</u> Unconditional multiple logistic regression. Adjustments: age at diagnosis, age at interview or death, state/province, sex, lymphatic or hematopoietic cancer in a first-degree relative, response by a proxy, and use of any personal protective equipment (PPE), use of other pesticides that have been associated with NHL (2,4-dichlorophenoxyacetic acid, dicamba and malathion. <u>Limitations:</u> Common limitations of case-control study, e.g. recall bias.	<u>Adjusted ORs (95%CI)</u> **Non-Hodgkin lymphoma (NHL) overall:** Ever use: 1.1 (0.8-1.5) ≤3.5 years: 1.3 (0.9-1.8) >3.5 years: 0.9 (0.6-1.4) ≤ 2 days/year: 0.7 (0.5-1.2) > 2 days/year: **1.7 (1.0-2.9)** ≤ 7 lifetime days: 0.9 (0.5-1.5) > 7 lifetime days: 1.1 (0.7-1.8) **Follicular lymphoma (FL):** Ever use: 0.7 (0.4-1.2) ≤3.5 years: 0.7 (0.3-1.3) >3.5 years: 0.6 (0.3-1.3) ≤ 2 days/year: 0.4 (0.2-1.1) > 2 days/year: 1.3 (0.6-3.2) ≤ 7 lifetime days: 0.6 (0.3-1.6) > 7 lifetime days: 0.8 (0.3-1.8) **Diffuse large B-cell lymphoma (DLBCL):** Ever use: 1.2 (0.8-1.9) ≤3.5 years: 1.6 (0.97-2.7) >3.5 years: 0.9 (0.5-1.7) ≤ 2 days/year: 0.7 (0.4-1.4) > 2 days/year: **2.1 (1.1-4.3)** ≤ 7 lifetime days: 0.8 (0.4-1.7) > 7 lifetime days: 1.1 (0.6-2.2) **Small lymphocytic lymphoma (SLL):** Ever use: 1.8 (0.9-3.7) ≤3.5 years: 1.4 (0.6-3.7) >3.5 years: 1.9 (0.8-4.8) ≤ 2 days/year: 1.3 (0.4-4.3) > 2 days/year: 2.3 (0.6-8.8) ≤ 7 lifetime days: 1.0 (0.2-4.8) > 7 lifetime days: 2.2 (0.7-6.9) **Other NHL subtypes:** Ever use: 1.5 (0.9-2.6)	Pahwa *et al.*, 2019 CA 5.5/033 B.6.5.18.8

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
			≤3.5 years: 1.8 (0.95-3.5) >3.5 years: 1.1 (0.5-2.5) ≤ 2 days/year: 1.1 (0.5-2.7) > 2 days/year: 1.6 (0.6-4.5) ≤ 7 lifetime days: 1.4 (0.6-3.5) > 7 lifetime days: 1.3 (0.5-3.3) Authors concluded that the results provided some limited, but inconsistent evidence of an association between NHL and glyphosate use. It should be noted that in the analysis of the subtypes of NHL there were at times only a limited number of cases (e.g. for SLL).	
Epidemiological study, public literature Study reliable.	-	The Agricultural Health Study (AHS), large prospective cohort study <u>Outcome evaluated:</u> Incident cancer diagnosis via cancer registries (all cancers, oral cavity, colon, rectum, pancreas, lung, melanoma, prostate, testicular, bladder, kidney, lymphohematopoietic, Hodgkin lymphoma (HL), non-Hodgkin lymphoma (NHL), non-Hodgkin lymphoma B-cell, chronic lymphocyte lymphoma, diffuse large B cell lymphoma, marginal-zone lymphoma, follicular lymphoma, multiple myeloma, non-Hodgkin lymphoma T cell, acute myeloid leukaemia, chronic myeloid leukaemia) <u>Population:</u> 54251 pesticide applicators recruited between 1993 and 1997 in Iowa and North Caroline. 44932 (82.8%) reported ever using glyphosate <u>Exposure:</u> Questionnaire by participants at enrolment and in a follow-up questionnaire after five years. Number of year and days were reported for 50 pesticides including glyphosate. Three metrics of cumulative lifetime exposure were created: ever/never use, lifetime days of	No increased risk for all cancers, cancer in lung, oral cavity, colon, rectum, pancreas, kidney, bladder, prostate, melanoma, testicular, all lymphohematopoietic cancers, HL, NHL, chronic myeloid leukaemia and multiple myeloma. For acute myeloid leukaemia a non-statistically significant increase was noted in the highest quartile intensity-weighted lifetime days of glyphosate use (RR 2.44, 95%- CI 0.94-6.32). When the incidence of acute myeloid leukaemia was assessed in relation to lagged intensity the effect was significant for the highest tertile of exposure with a 20-year lag period (**RR 2.04, 95%-CI 1.05-3.97**). However, it should be noted that a low number of cases was included in this subgroup (n = 15). For non-Hodgkin lymphoma (NHL) of T-cell subtype an elevated risk ratio was found for the 20-year lagged exposure (**RR of 2.97, 95%-CI 1.2-7.31**). However, it should be noted that a low number of cases was included in this subgroup (n = 9).	Andreotti *et al.*, 2018 B.6.5.18.10 CA 5.5/035

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
		use and intensity-weighted lifetime days. Data analysis: Poisson regression Adjustments: BMI, attained age, cigarette smoking status, packs of cigarettes smoked per day, alcohol drinks per month, family history of any cancer, state of recruitment, and the five pesticides most highly correlated with glyphosate, occupational exposure to solvents, gasoline, X-ray radiation and engine exhaust and pesticides linked to lymphohematopoietic cancers in previous AHS analysis. Limitations: 37% of participants did not respond to the follow-up questionnaire after five years. Exposure assessment is based on self-report instead of actual dose, therefore dose-response relationships must be carefully interpreted. In general, cohort studies are not prone to recall bias, however, the questionnaire itself contains questions that could entail recall bias, especially those that were used for exposure measurement matrices (e.g. questions on the use of specific pesticides). This is acknowledged by the authors that nondifferential misclassification bias may occur.		
Epidemiological study, public literature Study supportive to the Andreotti 2018 study	-	Systematic review and meta-analysis of six epidemiological studies on the relationship between non-Hodgkin lymphoma (NHL) and occupational exposure to pesticides. Outcome: Non-Hodgkin lymphoma and subtypes of NHL Limitations: Data extraction errors by the study authors that were identified in a subsequent meta-analysis by IARC working groups and by Chang and Delzell	The authors reported a positive association between glyphosate and NHL overall based on 6 studies (mRR = 1.5, 95%-CI 1.1-2.0) and for glyphosate and B cell lymphoma based on 2 studies (mRR = 2.0, 95%-CI 1.1-3.6).	Schinasi, L. and Leon, M.E., 2014 B.6.5.19.28

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
		(2016); a possible causal relationship was not discussed by the study authors; There is a more recent meta-analysis available using AHS data with extending cancer incidence follow-up through 2012 in North Carolina and 2013 in Iowa and incorporating additional exposure information from a follow-up questionnaire (Andreotti et al. 2018 (refer to B.6.5.18.10)). Therefore, this study by Schinasi is considered to be only supportive to the Andreotti study and is not considered further in the overall risk assessment.		
Epidemiological study, public literature Study reliable with restrictions	-	Pooled analysis of four case control studies (3 US studies and one Canadian study) in the North American Pooled project (NAPP). <u>Outcome:</u> Multiple myeloma (MM) <u>Population:</u> 547 MM cases, 2700 controls <u>Exposure:</u> Self-reported information on pesticide use, farming activities and demographic characteristics through interviews with participants. More detailed follow-up questions on pesticide use for those participants who reported pesticide use. <u>Data analysis:</u> Unconditional logistic regression Adjustment for: age, province/state, use of proxy respondent, and ever being diagnosed with any allergy, hay fever, or rheumatoid arthritis. <u>Limitations:</u> Typical limitations for case control studies. In addition, for Canadian part of the study response rate was quite low (58% for cases, 48% for controls). No adjustment made for other pesticide/chemical use, exposure to radiation or familiar history of cancers. Small sample	No significant increased ORs for multiple myeloma observed (for ever/never use, for less or more than 3 years of exposure and for less or more than 6 lifetime days of exposure to glyphosate). ORs decreased when proxy responders were removed from the analysis.	Presutti *et al.,* 2016 B.6.5.18.11 CA 5.5/036

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
		size in exposure groups for both cases and controls.		
Epidemiological study, public literature Study reliable with restrictions	-	Pooled analysis of three case-control studies. Outcome: Non-Hodgkin lymphoma Population: 650 cases and 1933 controls Exposure: Self-reported information on pesticide use or via proxy respondent. Data analysis: Standard logistic regression and hierarchical regression. Adjusted for age, study site and for use of other pesticides. Limitations: Typical limitations for case control studies. Cases with data missing information for any of the 47 pesticides were excluded (in contrast to the re-analysis of the data set in Pahwa, 2019). There was a fairly high number of proxy respondents (40% of cases, 31% of controls).	First stage logistic regression OR: 2.1 (95% CI 1.1-4.0) Second stage hierarchical regression OR: 1.6 (95% CI 0.9-2.8)	DeRoos *et al.,* 2003
Epidemiological study, public literature Study reliable	-	The Agricultural Health Study (AHS), large prospective cohort study Outcome evaluated: Incident cancer diagnosis via cancer registries (all cancers, oral cavity, colon, rectum, pancreas, lung, melanoma, lung, oral cavity, colon, rectum, pancreas, kidney, bladder, prostate, melanoma, all lymphohematopoietic cancers, Non-Hodgkin Lymphoma (NHL), leukaemia, multiple myeloma) Population: Pesticide applicators recruited between 1993 and 1997 in Iowa and North Caroline. 2088 cases for total cancer incidence (73.6% of cases ever used glyphosate) Exposure:	No increased risk for all cancers, cancer in lung, oral cavity, colon, rectum, pancreas, kidney, bladder, prostate, melanoma, all lymphohematopoietic cancers, NHL and leukaemia. For multiple myeloma relative risk of 1.1 (0.5-2.4) when adjusted for age and 2.6 (0.7-9.4) when adjusted for multiple confounders. There were indications in the fully adjusted cumulative exposure analyses of elevated multiple myeloma RRs in the higher exposure categories, though trend statistics were not significant. Study had limited power for multiple myeloma due to low number of cases. Missing data (exclusion of cases due to missing information on confounders) also limit the interpretation of the findings.	DeRoos *et al.,* 2005

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
		Questionnaire by participants at enrolment and in a follow-up questionnaire after five years. Exposure was limited to ever use. Data analysis: Poisson regression Adjustments: demographic and lifestyle factors, including age, education, cigarette smoking, alcohol consumption, family history of cancer in a first-degree relative, and state of residence, five pesticides most highly associated with glyphosate exposure (2,4-D, alachlor, atrazine, metolachlor, trifluralin). Limitations: Fairly low number of cases for multiple myeloma (n=32 for analyses without exposure-day metrics and n=19 for adjusted analyses of exposure-day metrics).		
Epidemiological study, public literature Study reliable	-	Re-analysis of the AHS database conducted with the aim to understand the results from De Roos *et al.* 2015 on the risks of multiple myeloma. The main difference is that the study did not exclude subjects with missing data.	No risk for multiple myeloma was observed.	Sorahan *et al.*, 2015
Epidemiological study, public literature Study of low reliability	-	Case-control study Outcome evaluated: Non-Hodgkin lymphoma (NHL) Population: 910 cases, 1016 controls Exposure: Exposure assessed based on questionnaire focussing on total work history, exposure to pesticides, solvents and other chemicals. For all pesticides, the number of years, number of days per year and length of exposure per day were questioned. Data analysis: Unconditional logistic regression. Adjustment: for age, sex and year of diagnosis/enrollment.	Univariate OR for NHL: 2.02 (95%-CI 1.10-3.71) Multivariate OR for NHL: 1.51 (95%-CI 0.77-2.94) OR less than 10 day use: 1.69 (95%-CI 0.70-4.07) OR more than 10 day use: 2.36 (95%-CI 1.04-5.37) OR latency period <10 yrs: 1.11 (95%-CI 0.24-5.08) OR latency period >10 yrs: 2.26 (95%-CI 1.16-4.40)	Eriksson *et al.*, 2008

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
		The unexposed category consisted of subjects unexposed to all pesticides Limitations: Typical limitations of case-control studies, e.g. recall bias and referral bias. Confounders such as family history of cancers and exposure to other pesticides was not adjusted for. The exposed group was compared to unexposed subjects (to all pesticide) which likely results in groups that are not comparable on potential covariates.		
Epidemiological study, public literature Study of low reliability	-	Pooled analysis of two Swedish case-control study Outcome evaluated: Non-Hodgkin lymphoma Population: 515 cases, 1141 controls Exposure: Questionnaire of occupational history and exposure to different chemicals (years and total exposure days). Unclear questionnaires were followed-up by telephone interview (unclear how often this occurred). Subjects with no pesticide exposure were used as the unexposed category. Data analysis: Univariate and multivariate conditional logistic regressions. Adjustments: not clearly reported. Limitations: Potential for recall bias as indicated by high ORs for virtually all pesticide evaluated. No adjustment for confounders such as medical history and lifestyle factors or other pesticide exposure. Statistical analysis compared to unexposed group not exposed to any of the evaluated pesticides leading to high potential for confounding effects. Statistical analysis not described in detail. Limited	Univariate analysis OR: 3.04 (95% CI 1.08-8.52) Multivariate analysis OR: 1.85 (95% CI 0.55-6.20) (multivariate variables not listed by study authors).	Hardell *et al.*, 2002

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
		number of exposed cases (N=8) and exposed controls (N=8)		
Epidemiological study, public literature Study reliable	-	Case-control study as part of the Nebraska Health Study II. <u>Outcome evaluated:</u> Adult glioma <u>Population:</u> 251 cases, 498 controls <u>Exposure:</u> Telephone interview of subjects or proxies on demographics, smoking and alcohol consumption, diet, family history of cancer, residential and occupational history, medical history and history of pesticide use. <u>Data analysis:</u> Unconditional logistic regression Adjustments: age, sex and respondent type. Other potential confounders were found to not change the ORs by more than 10% and were therefore not included in the analysis. <u>Limitations:</u> Typical limitations of case-control studies, e.g. recall bias. There was a fairly high number of proxy response, but this was considered in the analysis by calculating separate ORs.	OR all cases: 1.5 (95%-CI 0.7-3.1) OR self-responders: 0.4 (95%-CI 0.1-16) OR proxy-responders: 3.1 (95%-CI 1.2-8.2) Authors highlighted the striking difference between self and proxy respondents and indicated that the positive association may be due to misclassification of the exposure by proxy respondents.	Lee *et al.*, 2005
Epidemiological study, public literature Reliable with restrictions		Case-control study <u>Outcome evaluated:</u> Non-Hodgkin lymphoma (NHL) <u>Population:</u> 517 NHL cases, 1506 controls <u>Exposure:</u> Telephone questionnaires modified from the National Cancer Institute (NCI) telephone questionnaire. Questionnaire was validated in a pilot study in volunteer farmers and checked with purchase records from local agrochemical supplier. <u>Data analysis:</u>	Adjusted OR for any glyphosate exposure: 1.20 (95%-CI 0.83-174) OR for glyphosate use 0-2 days a year: 1.00 (95%-CI 0.63-1.57) OR for glyphosate use >2 days a year: 2.12 (95%-CI 1.20-3.73)	McDuffie *et al.*, 2001

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
		Ever/no use analysis: Logistic regression adjusted for age, province and statistically significant medical variables. Days of use analysis: stratified analysis adjusted for age and province. Limitations: Typical limitations of case control study, e.g. recall bias. No adjustment for medical confounders made in the ORs that were stratified for exposure duration. Low response rate, particular in controls (48%), questionnaire was not validated for all (occupational) groups.		
Epidemiological study, public literature Study of low reliability	-	Case control study. Outcome evaluated: Childhood leukaemia Population: 334 cases, 579 control Exposure: Face-to-face interview with parents on demographic data and data on known risk factors. Parents active in agriculture or livestock production completed an additional interview. Exposures were expressed as qualitative (yes, no), semiquantitative (unexposed, low exposure, high exposure) and quantitative metrics for specific pesticides and groups of pesticides. Data analysis: Unconditional crude and adjusted logistic regression Adjustment : urban or rural residence, X-ray exposure during pregnancy. Other potential confounders such as maternal age at conception, infectious diseases child, mother's tobacco and alcohol consumption were found to have low correlations and were not included. Limitations: Potential for recall bias as indicated by high ORs for	Elevated OR for all time points evaluated (year before conception, 1st trimester, 2nd trimester, 3rd trimester, 1st year of life). Paraquat, chlorothalonil glyphosate and 'others' were grouped.	Monge *et al.*, 2007

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
		virtually all pesticide groups evaluated. No specific exposure estimate for glyphosate, only combined chemical exposure.		
Epidemiological study, public literature Study of low reliability	-	Case control study <u>Outcome evaluated:</u> Lymphoid neoplasms (LN) <u>Population:</u> 244 non-Hodgkin lymphoma (NHL), 87 Hodgkin lymphoma (HL), 104 lymphoproliferative syndromes (LPS), 56 multiple myeloma (MM) cases and 456 controls <u>Exposure:</u> Self-administered questionnaire for socioeconomic characteristics, familial medical history, and lifelong residential and occupational histories. Face-to-face interview for personal and familial medical history, lifestyle characteristics, outdoor leisure activities and agricultural use (farmers and gardeners only). <u>Data analysis:</u> Unconditional logistic regression. Adjustments: age, center and socioeconomic category (blue or white collar). <u>Limitations:</u> Typical limitations noted for case control studies , e.g. recall bias. Uncertainty in the exposure assessment as repeat interviews were required due to insufficient information, but only 56.8% participated in the repeat interviews. No adjustments seems to be made for other pesticide exposures.	OR NHL: 1.0 (95% CI 0.5-2.2) OR HL: 1.7 (95% CI 0.6-5.0) OR LPS: 0.6 (95% CI 0.2-2.1) OR multiple myeloma: 2.4 (95% CI 0.8-7.3) OR all LN: 1.2 (95% CI 0.6-2.1)	Orsi *et al.*, 2009

NHL = Non-Hodgkin lymphoma, DLBCL = diffuse large B-cell lymphoma, FL = follicular lymphoma, SLL = small lymphocytic lymphoma, HL = Hodgkin lymphoma, LPS = lymphoproliferative syndromes, MM = multiple myeloma.

Table 55: Summary table of other studies relevant for long-term toxicity and carcinogenicity

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
Public literature study *In vitro* study Reliable without restriction	Glyphosate, purity 95%	Study evaluated the effect of glyphosate on selected epigenetic parameters and major cell cycle drivers in human peripheral blood mononuclear cells (PBMCs) by Real-Time PCR.	Glyphosate changed global DNA methylation pattern of the P21 and TP53 suppressor gene promoters but no change on P16, BCL2 and CCND1. Gene expression was decreased for P16 and TP53 and increased for BC12, CCND1 and P21. IT was noted that in most cases no clear concentration-response was observed for these effects. Regulation of transcription process is performed at many different levels and further and more global analysis are necessary.	Wozniak *et al.*, 2020
Public literature study *In vitro* study Reliable without restriction	Glyphosate, purity ≥96%	Study evaluated the effect of pesticides, including glyphosate, on lipid accumulation in differentiated adipocytes.	Glyphosate did not affect lipid accumulation	Biserni *et al.*, 2019
Public literature study *In vitro* and *in vivo* study Reliable with restrictions (only purity not reported)	Glyphosate, purity not reported	Study evaluated the effect of glyphosate on DNA methylation and tumorigenesis in non-neoplastic MCF10A cells. MCF10A cells were exposed to glyphosate *in vitro* at 10^{-11} M every three to four days over 21 days. Treated cells were investigated for DNA methylation. Treated cells were also injected subcutaneously in 7- to 8-week old Swiss nude mice to investigate tumour development. In addition, MCF10A cells transfected with microRNAs associated with breast-cancer were exposed as described above and also injected subcutaneously Swiss nude mice.	The study showed that exposure of MCF10A cells to a low dose of glyphosate (10^{-11} M) resulted in DNA hypomethylation with TET3 overexpression. The study concluded that glyphosate is not oncogenic by itself, but it acts as an oncogenic hit factor that, combined with another oncogenic hit, promotes the development of mammary tumours.	Duforestel *et al.*, 2019

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
		Positive control: UP peptide		
Public literature study *In vitro* study Reliable with restrictions	Glyphosate isopropylamine salt, purity ≥95%	Study evaluated the *in vitro* autophagic effect of Roundup, glyphosate and polyethoxylated tallow amine (POEA) on human A549 cells.	Glyphosate did not induce autophagic effects while POEA and Roundup containing POEA did.	Hao *et al.*, 2019
Public literature study *In vivo* study Reliable with restrictions	Glyphosate	Study evaluated the effect of glyphosate on multiple myeloma in Vk*MYC and wildtype (WT) mice. Chronic study: groups of 10 Vk*MYC and WT mice (exact number of animals not clearly reported) received 1.0 g/L glyphosate via drinking water. Subacute study: groups of 5 Vk*MYC and WT mice received glyphosate at 1, 5, 10 and 30 g/L via drinking water <u>Limitations:</u> - Number of animals low and not clearly reported - Daily intake in chronic study appear low compared to the guideline toxicity studies (90 mg/kg bw/day based on extrapolation from concentration in drinking water using default values for water consumption).	<u>Vk*MYC mice:</u> Glyphosate exposure resulted in reduced survival, increased spleen weight, change in splenocyte number. Higher IgG levels and haematological changes. Ostolytic bone lesions in the femoral shaft, hepatic fibrosis and collagen deposition, lung damage, renal obstruction by large casts, upregulation of activation-induced cytidine (AID). <u>WT mice:</u> Increased splenocyte number. Slightly increased IgG levels, slight haematological changes, less severe histopathological changes in the liver and lungs, renal obstruction by large casts	Wang *et al.*, 2019 B.6.5.18.9 CA 5.5/034

An overview of publications related to carcinogenicity that are classified by the applicant as "relevant but supplementary after detailed assessment of full-text article" is provided in Volume 3 CA Table B.6.10-2. Upon review of the titles and abstracts of articles assigned to this category by the RMS, several study summaries were requested by AGG to further justify the categorization of the information. The study summaries and justification provided by the applicant were reviewed by the RMS and are presented in Volume 3 CA B.6.5.18 (for publications related to carcinogenicity). For the three studies - indicated below in Table 2.6.5 - the RMS has set data gaps for the applicant to provide a full assessment including a relevance and reliability assessment. In addition a data gap is set to discuss these studies in the overall weight of evidence approach for carcinogenicity.

Table 2.6.5: Summary table of other studies for which RMS has set a data gap

Data requirement	Author	Year	Title
CA 5.9	Chang E. Et al	2016	Systematic review and meta-analysis of glyphosate exposure and risk of lymphohematopoietic cancers.

			RMS: A **data gap** was identified for providing a full assessment of the study including a relevance and reliability assessment (refer to Volume 1, section 2.6.5.1.2 Epidemiological studies). In addition, this study should be discussed in the overall weight-of-evidence approach for carcinogenicity.
CA 5.9	Leon M. E. Et al	2019	Pesticide use and risk of non-Hodgkin lymphoid malignancies in agricultural cohorts from France, Norway and the USA: a pooled analysis from the AGRICOH consortium. **RMS:** A **data gap** was identified for providing a full assessment of the study including a relevance and reliability assessment (refer to Volume 1, section 2.6.5.1.2 Epidemiological studies). In addition, this study should be discussed in the overall weight-of-evidence approach for carcinogenicity.
CA 5.9	Zhang L. Et al.	2019	Exposure to glyphosate-based herbicides and risk for non-Hodgkin lymphoma: A meta-analysis and supporting evidence. **RMS:** A **data gap** was identified for providing a full assessment of the study including a relevance and reliability assessment (refer to Volume 1, section 2.6.5.1.2 Epidemiological studies). In addition, this study should be discussed in the overall weight-of-evidence approach for carcinogenicity.

2.6.5.1 Short summary and overall relevance of the provided information on long-term toxicity and carcinogenicity

2.6.5.1.1 Animal studies

2.6.5.1.1.1 Rat studies (short summary of the studies)

The chronic toxicity and carcinogenic potential of glyphosate technical (batch H05H016A, purity 95.7%) was assessed in a 24-month feeding study ([redacted]., 2009; Report No. 2060-0012) with 51 male and 51 female Wistar rats at dietary concentrations of 0, 1500, 5000 and 15000 ppm (equal to achieved dose levels of 0, 85.5, 285.2, 1077.4 mg/kg bw/day in males and 0, 104.5, 348.6 and 1381.9 mg/kg bw/day in females). In addition a satellite group of 15 rats/sex/dose were included for interim sacrifice. The study was concluded in accordance with OECD 453 with the minor deviations that the thyroid/parathyroid were not weighed and histopathology did not include the cervix, coagulating gland and the lacrimal gland. Overall, the study was concluded to be acceptable.

Treatment related effects included elevations in alkaline phosphatase observed in the satellite group at 6 and 12 months and changes in plasma electrolytes (at 18 months only). The latter is considered of questionable toxicological significance mainly because effects were not seen at the 24-month observation point. In addition, a difference in the site of mineral deposition within the kidneys was observed. There was a lower incidence of pelvic/papillary deposition in both sexes and an increase in the corticomedullary deposition in females. At the same time there was a reduction in the incidence of renal pelvic hyperplasia; which is considered a consequence of decreased mineral deposition. An increase in severity of adipose infiltration into the bone marrow was observed. In high dose males, skin effects including areas of necrosis/giant cell reaction to keratin and keratoacanthoma was observed. The keratoacanthomas are further discussed below at 'Overall consideration of the tumour incidences'.

The NOAEL for systemic toxicity was concluded to be 5000 ppm (equal to 285.2 mg/kg bw/day in males and 348.6 mg/kg bw/day in females) based on the observed increase in alkaline phosphatase, increased severity of adipose infiltration of the bone marrow, kidney findings (which were concluded to be of equivocal relevance) and the skin effects including areas of necrosis/giant cell reaction to keratin and keratoacanthoma observed in high dose males.

In the second chronic toxicity and carcinogenicity study ([redacted] 2001; Report No. [redacted]/PR1111) glyphosate (batch P30, purity, 97.6%) was administered to groups of 52 male and 52 female Wistar rats at dietary concentrations

of with 0, 2000, 6000 and 20000 ppm (equal to dose levels of 0, 121, 361 and 1214 mg/kg bw/day for males and 0, 145, 437 and 1498 mg/kg bw/day for females). In addition, three satellite groups with 12 rats per sex each were included for interim sacrifice at 12 months. The study was conducted in accordance with OECD 453 with the minor deviations that the thyroid/parathyroid were not weighed and histopathology did not include the coagulating gland, gall bladder and the vagina. Overall, the study was concluded to be acceptable.

Treatment related findings in this study were found in the liver and kidney and were confined to animals (predominantly males) fed 20000 ppm glyphosate acid. There were a number of changes in males and females fed 20000 ppm, notably renal papillary necrosis, prostatitis, periodontal inflammation, urinary acidosis and haematuria, which may be attributed to the acidity of the test substance.

Regarding neoplastic findings an increased incidence of hepatocellular adenoma was observed in males (0/64, 2/64, 0/64, 5/64 at 0, 2000, 6000, 20000 ppm). The relevance of this finding is further discussed below at 'Overall consideration of the tumour incidences'. An increased incidence in hepatitis was noted in top dose males. The reported incidences were 8/64, 6/64, 9/64, 13/64 for doses 0, 2000, 6000 and 20000 ppm (no p-values available). The incidence at the top dose was above HCD mean (11.8%) but within HCD range (0-30%; HCD based on 5 studies from the same lab and in the same strain performed between 1998-2003). As the background incidence of hepatitis is highly variable and as the incidence is within HCD range, the relationship to treatment is doubted.

The NOAEL for systemic toxicity was concluded to be 6000 ppm (equal to 361 mg/kg bw/day in males and 437 mg/kg bw/day in females) based on the observed clinical chemistry changes and histopathological findings observed in the kidney, liver and prostate.

In a third carcinogenicity/chronic toxicity study in rats (Report No. 1231) groups of 50 Sprague-Dawley rats were treated with glyphosate (batch and purity not reported) at dietary concentration of 0, 3000, 15000 and 25000 ppm (equivalent to mean achieved dose levels of 0, 150, 780 and 1290 mg/kg bw/day (males) and 0, 210, 1060 and 1740 mg/kg bw/day (females)). In addition, 20 rats/sex/group were included for the interim sacrifice at week 52. These animals were treated at the same dose levels except for the high dose animals who were treated at 30000 ppm. The study showed a large number of deviations from OECD 453 in terms of the parameters examined. In addition, organ weight were only measured in 10 animals of the terminal sacrifice group and in the mid and high dose group only a small number of animals were examined histopathologically except for liver, kidneys, lungs, testes, adrenals and ovaries for which all animals were examined. It was also noted that the background tumour incidence appeared to be very low. For example, in control males only two neoplastic findings were noted, namely one seminoma in the testes and one fibroadenoma in mammary gland tissue. This puts into question the sensitivity of the animals used. Overall, the study was concluded to be unacceptable. The NOAEL of the study was concluded to be to be 15000 ppm (corresponding to 780 mg/kg bw/day in males and 1060 mg/kg bw/day in females) based on an increase in ALP (+83% in males, +117% in females) and increased kidney and liver weight in females at the LOAEL of 25000 ppm. No effect on tumour incidences was observed.

In the fourth carcinogenicity/chronic toxicity study in rats ([redacted], 1997; Report No. [redacted] 94-0150) groups of 50/sex/dose Sprague-Dawley rats were treated with glyphosate (batch T-941209; T-950308, purity 97.56%; 94.61%) at dietary concentration of 0, 3000, 10000 and 30000 ppm (equal to 0, 104, 354 and 1127 mg/kg bw/day for males and 0, 115, 393 and 1247 mg/kg bw/day for females). In addition, 30 rats/sex/group were included for interim sacrifice at 26, 52 and 78 weeks to study non-neoplastic histopathological changes. The study was conducted in accordance with OECD 453 with the exception that prothrombin time and activated partial thromboplastin time were not investigated. In addition, epididymides, heart, ovaries, spleen, and uterus and (para)thyroid weight were not measured and the gall bladder, and lacrimal gland and upper respiratory tract were not investigated histopathologically. Overall, the study was concluded to be acceptable.

Clinical observations consisted of loose faeces together with soiled fur in the perianal region in the high dose group as well as increased incidences of tail mass in the mid and high dose group. Moreover, decreases in body weight were observed in both sexes in the mid and high dose group along with a lower food consumption although the effect in the mid dose were only slight and not considered to be adverse. Necropsy supported the clinical signs of loose stool by increased incidences of distension of the caecum in the high dose group together with increased absolute and relative caecum weights in the mid and high dose group. Moreover, the increased incidences of thickened areas in the skin of the tail, corresponding to the increased incidences of tail mass, were histopathologically diagnosed as follicular hyperkeratosis in the mid and high dose group (7/76, 5/75, 2/80, 23/78 at 0, 3000, 10000, 30000 ppm). Skin keratoacanthoma and skin basal cell tumours were observed in the high dose group males (incidence of skin keratoacanthomas: 4/76, 3/75, 0/80, 7/78 at 0, 3000, 10000, 30000 ppm; incidence of skin basal cell tumours: 0/76, 0/75, 0/80, 4/78 at 0, 3000, 10000, 30000 ppm). An increased incidence of these tumours was only observed in males. The relevance of these findings is further discussed below at 'Overall consideration of the tumour incidences'.

The NOAEL for systemic toxicity was concluded to be 3000 ppm (equivalent to 104 mg/kg bw/day for males and 115 mg/kg bw/day for females).

In the fifth carcinogenicity study in rats ([redacted] 1996; Report No. 886.C.C-R) groups of 50 male and female Wistar rats received dietary doses of glyphosate (batches 60 and 046, purity 96.8% and 96%) at 0, 100, 1000 and 10000 ppm (equal to 0, 6.3, 59.4 and 595.2 mg/kg bw/day in males and 0, 8.6, 88.5 and 886.0 mg/kg bw/day in females) for a period of 24 months. In addition, one vehicle control with ten rats per sex and one high dose group with 20 rats per sex were included for interim sacrifice at the 12th month. The study was mainly conducted in OECD 453 although a large number of haematological and clinical chemistry parameters were not included. Moreover, organ weight measurements were only conducted in 10 animals instead of all. Based on these limitations the study was concluded to be acceptable but with restrictions.

There were no treatment-related deaths or clinical signs in any of the dose-groups. Moreover, there were no treatment-related effects on body weight gain or food consumption. The only treatment-related significant changes observed in haematological, biochemical parameters was an increase in ALP in high dose females. Gross pathology, organ weight data and histopathological examination demonstrated no treatment-related and dose-dependent effects except for an increase in cataract in high dose males. An apparent non-significant increase in mandibular lymph node lymphoma was seen in high dose males at terminal sacrifice with an incidence of 2/50 at the top dose, whereas in the incidences in the control, low and mid dose groups were 0/48, 0/35 and 0/37, respectively. **The applicant is asked to provide historical control data for the effect on mandibular lymph node lymphoma, if available. For the process under the Regulation (EC) No 1272/2008, the applicant is asked to submit the missing information during the public consultation period.** As 1) the incidence at the top dose is only 2 cases versus 0 cases in the other dose groups and controls, which is not a statistically significant increase and as 2) no increased incidences of this tumour type are seen in any of the other studies, the finding is considered an incidental (chance) finding and the relevance is not further discussed below at 'Overall consideration of the tumour incidences'. The NOAEL was concluded to be 1000 ppm (equal to 59.4 mg/kg bw/day in males and 88.5 mg/kg bw/day in females).

In a 12-month chronic toxicity study [redacted], 1996; report No. [redacted]/P/5143) groups of 24 male and female Wistar rats received glyphosate at dietary concentrations of 0, 2000, 8000 and 20000 ppm (equal to mean achieved dose levels of 0, 141, 560 and 1409 mg/kg bw/day for males and 0, 167, 671 and 1664 mg/kg bw/day for females). The study was conducted in accordance with OECD 452 with some minor deviations. Overall, the study was concluded to be acceptable.

A reduction in bodyweight was evident in animals receiving 20000 ppm glyphosate acid, which was however not considered adverse as the decrease was less than 10% compared with controls. There were no toxicologically significant or treatment-related effects on haematology, urine clinical chemistry or organ weights. An increase in ALP was observed at all dose levels. The effects at 2000 and 8000 ppm were slight and without accompanying pathological and therefore these changes were not considered to be adverse. Prostatitis was observed in high dose males and proliferative cholangitis of the liver in high dose females. In addition, an increased incidence of mild focal basophilia of the acinar cells of the parotid salivary gland was observed in both sexes which had received 8000 and 20000 ppm glyphosate acid.

For the interpretation of effects on salivary glands several aspects are considered in order to decide if the effect is adverse or not. The RMS considers salivary gland weight changes and histopathology (severity grade and incidence) along with dose-response and statistical significance. A histopathological finding which is statistically significant is not considered adverse if the severity grade of the finding is minor and there are no salivary gland weight changes. In the case there are no data for salivary gland weights, the effect on histopathology might be considered as potentially adverse in the absence of such data as a precautionary approach. Histopathology revealed an increased incidence and severity of focal basophilia of the acinar cells of the parotid salivary gland in both sexes at 20000 ppm (1409 mg/kg bw/day) at an incidence of 57% (males) and 75% (females) with a severity grade of minimal to slight (males) and minimal to moderate (females). At 8000 ppm (560 mg/kg bw/day) focal basophilia of parotid acinar cells were all of minimal severity and the incidence for males (12.5%) was comparable to that in the control group (8.7%), while the incidence for females (25%) was above the control group (8.3%). No statistical analyses were conducted and no historical control data are available. Since the salivary gland weights were not investigated in the study it is proposed to set the LOAEL at 8000 ppm based on the effect in females as a precautionary approach although the severity grade of findings observed at this dose level was minimal. At 2000 ppm no effects on parotid acinar cells were observed. Thus, the **NOAEL is set at 2000 ppm** (equal to 167 mg/kg bw/day in females).

No effect on neoplastic findings were observed in this study. However, it is noted that OECD 452 is not suited to evaluate the carcinogenic properties of active substances.

In the seventh chronic/carcinogenicity study in rat ([redacted], 1993; Report No. 7867) groups of 50 male and female Sprague-Dawley rats received dietary doses of glyphosate (batches 60 and 046, purity 96.8% and 96%) at 0, 10, 100, 300 and 1000 mg/kg bw/day. In addition, five groups of 35 rats/sex, receiving daily dietary doses of, 0, 10, 100, 300 or 1000 mg/kg bw/day. The study was conducted in accordance with OECD 453 with some minor deviations. Overall the study was concluded to be acceptable.

At 1000 mg/kg bw/day males and females had statistically significant reductions in body weight throughout the study. Reductions started at week one of dosing and were still apparent at week 104. The high-dose group males displayed the greatest reduction in body weights. Clinical chemistry evaluation indicated a treatment-related increase of ALP in males of the 1000 mg/kg bw/day dose group and females of the 300 and 1000 mg/kg bw/day dose groups, as well as reduced urinary pH in males at 1000 mg/kg bw/day. Organ weight data showed reduced relative liver weights in females at 100, 300 and 1000 mg/kg bw/day at interim kill in week 52, but not after 104 weeks.

For the interpretation of effects on salivary glands several aspects are considered in order to decide if the effect is adverse or not. The RMS considers salivary gland weight changes and histopathology (severity grade and incidence) along with dose-response and statistical significance. A histopathological finding which is statistically significant is not considered adverse if the severity grade of the finding is minor and there are no salivary gland weight changes. In the case where there are no data for salivary gland weights, the effect on histopathology might be considered as potentially adverse in the absence of such data as a precautionary approach.

Histopathology revealed a statistically significant increased incidence of parotid cellular alteration in both sexes at ≥100 mg/kg bw/day. Furthermore, a statistically significant increased incidence of submaxillary cellular alteration was found at ≥100 mg/kg bw/day in males and at ≥300 mg/kg bw/day in females. At 100 mg/kg bw/day the increased incidence of parotid cellular alteration observed in males was 67% at week 52 (compared to 0% in control) and 43% at week 104 (compared to 14% in control). The severity grade of the finding was minimal to moderate. In females, the increased incidence of parotid cellular alteration at week 52 was 13% (compared to 0% in control) and 24% at week 104 (compared to 14% in control). In males, at week 104 the increased incidence of submaxillary cellular alteration was 45% (compared to 14% in control), while no effects on submaxillary gland was observed at week 52 at this dose level. The severity grade of this finding was mild. No historical control data are available. Statistically significant increased parotid gland weights were observed in males (absolute weight: 56%, relative weight: 65%) at the dose level of 100 mg/kg bw/day at week 52 but not at week 104. At 10 mg/kg bw/day no effects were observed. Thus, the **NOAEL for systemic toxicity is set at 10 mg/kg bw/day** based on adverse effects on salivary glands (histopathological changes and organ weight changes) observed at 100 mg/kg bw/day.

An apparent increase in incidence of skin keratoacanthomas in males was observed at the top dose (5/50 compared with 1/50 in controls). The relevance of this finding is further discussed below at 'Overall consideration of the tumour incidences'.

In the eighth chronic toxicity/carcinogenicity study in rat ([redacted], 1990; Report No. [redacted]-10495), groups of 50 male and female Sprague-Dawley rats received glyphosate at dietary concentrations of , 2000, 8000 and 20000 ppm (equal to mean achieved dose levels of 0, 89, 362 and 940 mg/kg bw/day for males and 0, 113, 457 and 1183 mg/kg bw/day for females). The study was conducted in accordance with OECD 453 with some minor deviations mainly consisting of missing parameters which are required in the most recent version of the Guideline. Overall, the study was concluded to be acceptable.

There were no treatment-related effects on survival, clinical signs, food consumption, and haematology and clinical chemistry parameters except for an increase in ALP in high dose females. Reduced body weight (gain) was observed in high dose animals as well as increased absolute and relevant liver weight. Increased incidences of inflammation of the stomach mucosa in mid and high dose animals was observed. Pancreatic islet cell adenomas in low-dose males were not dose-related and considered incidental findings due to the lack of a dose-response relationship (1/58, 8/57, 5/60, 7/50 at 0, 2000, 8000 and 20000 ppm) and the lack of concomitant non-neoplastic findings. Increased incidences of cataractous lens changes in high-dose males were observed. An apparent increase in liver cell adenomas was observed in high dose males (8 versus 3 in controls) although no effect on non-neoplastic changes in the liver nor a progression to carcinomas was observed. The relevance of these findings is discussed below. In addition, an apparent increase in thyroid C-cell tumours (both sexes), pancreatic islet cell adenomas (in males) and skin keratoacanthomas (in males) was noted (refer to Table 53 for incidences). The relevance of these findings is further discussed below at 'Overall consideration of the tumour incidences'.

The NOAEL was concluded to be 2000 ppm (equal to 89 mg/kg bw/day in males and 113 mg/kg bw/day in females).

In the ninth carcinogenicity study in rat ([redacted], 1981; Report No. 77-2062) groups of 50 male and female Sprague-Dawley rats were treated with glyphosate (batch XHJ-64, purity 98.7%) for a period of 24-months. During the first week of the study, the test substance was administered at dose levels of 30, 100 and 300 ppm. For the remainder of the study, dose levels of 3.05, 10.30 and 31.49 mg/kg bw/day for the males, and 3.37, 11.22 and 34.02 mg/kg bw/day for the females were maintained. The dose levels selected are considered to be too low when compared to the other chronic studies and there was a lack of general systemic toxicity in the study. In addition, the study report was of poor quality. Therefore, the study was concluded to be unacceptable.

At the top dose, an increased incidence of interstitial cell tumours of the testes was observed (0/50, 3/50, 1/50, 6/50 at 0, 30, 100 and 300 ppm). In addition, an increased incidence of pancreatic island cell adenomas is seen among males of all dose groups compared with controls 0/50, 5/49, 2/50, 2/50 at 0, 30, 100 and 300 ppm. The

relevance of these findings is further discussed below at 'Overall consideration of the tumour incidences'.

In an US EPA assessment[4], another 2-year carcinogenicity study in rats was identified ([redacted], 1987) in which glyphosate was administered as a trimesium salt at low dose levels of up to 50 mg/kg bw/d. This study was not mentioned in the EU review on glyphosate before. According to the summuary provided in the US EPA assessment, there were no treatment-related increases in tumor incidences in the study. **For the process under Regulation (EC) No 1107/2009, the applicant is requested to provide the study by [redacted] (1987), if possible, and an assessment. For the process under the Regulation (EC) No 1272/2008, the applicant is asked to submit the missing information during the public consultation period.**

2.6.5.1.1.2 Mouse studies (short summary of the studies)

The carcinogenic potential of glyphosate technical was assessed in an 18-month feeding study in male and female CD-1 mice ([redacted], 2009, study report no. 2060-0011). Groups of 51 mice per sex received daily dietary doses of 0, 500, 1500 and 5000 ppm glyphosate technical (equal to 0, 71.4, 234.2 and 810 mg/kg bw/day in males and 0, 97.9, 299.5 and 1081.2 mg/kg bw/day in females). The study was conducted in accordance with OECD451 with some minor deviations and was considered to be acceptable.

There were no treatment-related deaths or clinical signs in any of the dose-groups. There were no treatment-related effects on body weight gain or food and water consumption noted. No significant treatment-related effects were noted on differential white blood cell counts in both sexes. The study was performed as a carcinogenicity study and only a limited of other toxicity endpoints were included. There were no treatment-related trends in the proportion of masses observed, number of mice affected or time to appearance of palpable masses. Gross pathology, organ weight data revealed no treatment-related effects. Histopathological evaluation revealed an apparent increase in malignant lymphoma in males (0/51, 1/51, 2/51, 5/51 at 0, 500, 1500 and 5000 ppm). In females no effect was observed (11/51, 8/51, 10/51, 11/51 at 0, 500, 1500 and 5000 ppm). The relevance of these findings is further discussed below at 'Overall consideration of the tumour incidences'.

As no adverse findings were observed up to the highest dose tested, a NOAEL of 810 mg/kg bw/day in males and 1081 mg/kg bw/day in females is derived, however, it should be noted that the study was conducted as a carcinogenicity study and only a limited of other toxicity endpoints were included. Therefore, this systemic NOAEL is of limited value.

In the second carcinogenicity in mice ([redacted], 2001, study report no. Toxi: 1559.CARCI-M) groups of 50 male and female Swiss albino mice received daily dietary doses of 0, 100, 1000 and 10000 ppm glyphosate technical (equal to an intake of 0, 14.5, 149.7 and 1454 mg/kg bw/day for males, and 0, 15.0, 151.2 and 1466.8 mg/kg bw/day for females). The study was conducted in accordance with OECD 451 with some minor deviations and was considered acceptable.

The survival after 18-month of treatment was 56, 60, 56 and 46% in males and 68, 68, 60 and 60% in females in the control through high dosage groups, respectively. There were no treatment-related effects on clinical signs, behaviour, eyes, body weight, body weight gain, food consumption, differential white blood cell counts, gross pathology or organ weight data. Degenerative changes in the heart were noted in high dose males, however, as the increase was not statistically significant and within HCD range, this findings was considered incidental. In males, the number of malignant lymphomas was slightly elevated in the high dose group compared to control (38% in high dose males compared to 20% in controls, and 50% in high dose females compared to 36% in controls). Using the Peto method, a significant trend was seen for mesenteric lymph node haemangioma in females (one-sided p-value of 0.004). The relevance of the observed increased incidences for these two tumour types in the context of the classification and labelling of glyphosate is further discussed below at 'Overall consideration of the tumour incidences'.

The systemic NOAEL was concluded to be 10000 ppm (equal to 1454 and 1467 mg/kg bw/day in males and females, respectively), the highest dose tested.

In a third carcinogenicity study ([redacted], 1997, study report no. [redacted] 94-0151) in CD-1 mice glyphosate was administered at dietary concentrations of 0, 1600, 8000 or 40000 ppm (equal to 0, 165.0, 838.1 and 4348 mg/kg bw/day for males and 0, 153.2, 786.8 and 4116 mg/kg bw/day for females) for a period of 18 months. The study was conducted in accordance with OECD 451 with some minor deviations and was considered acceptable.

In the high dose groups effects noted were an increased incidence of pale-coloured skin, loose faeces, reduced body weight, reduced food consumption, increased incidences of distention of caecum, increase in absolute and relative weights of the caecum and an increase in the incidence of anal prolapsed which was correspondent to

[4] Revised Glyphosate Issue Paper: Evaluation of Carcinogenic Potential EPA's Office of Pesticide Programs December 12, 2017. (https://downloads.regulations.gov/EPA-HQ-OPP-2009-0361-0073/content.pdf)

erosion/ulcer of the anus in histopathology. In the mid dose group reduced body weight (gain) was noted in females. An increase in malignant lymphoma was noted in high dose males. The relevance of these findings is further discussed below at 'Overall consideration of the tumour incidences'. The systemic NOAEL was concluded to be 1600 ppm, equal to 165.0 mg/kg bw/day for males and 153.2 mg/kg bw/day for females based on the decreased body weight gain in females at 8000 ppm. It is noted that the study was conducted as a carcinogenicity study an only a limited number of parameters for systemic toxicity were evaluated.

In the fourth carcinogenicity study ([redacted]., 1993, study report no. 7793) in CD-1 mice groups 50 mice per sex received daily dietary doses of 0, 100, 300 or 1000 mg/kg bw/day glyphosate technical for 24 months. The study was conducted in accordance with OECD 451 with some minor deviations and was considered to be acceptable.

There were no treatment-related deaths or clinical signs in any of the dose-groups. Body weight, food and water consumption did not differ significantly from the controls. Moreover, there were no treatment-related changes in differential blood count. At necropsy the incidence of lung masses was slightly higher in the 1000 mg/kg bw/day group but no treatment related effect on histopathological findings were observed. Organ weight data showed marginal increased thymus weights in males at 300 and 1000 mg/kg bw/day after 104 weeks, but not in females and without corresponding histopathological changes. Histopathological examination noted increased mineral deposit in the brain of high dose males. These changes were not considered toxicologically relevant. No treatment-related neoplastic lesions were observed at termination.

The NOAEL was concluded to be 1000 mg/kg bw/day, based on the absence of any adverse findings.

A fifth carcinogenicity study in mice is available ([redacted], 1988, reported at CA 5.5/022, no study report number). The study did not follow a specific test guideline and was not conducted under GLP. Some major limitations were noted including low dose levels, a very limited number of parameters investigated, the number of animals being too low and the lacking quality of the study report (e.g. missing individual animal data).

Overall the study was concluded to be unacceptable and no reliable conclusions can be made on the basis of the study. No effect on tumour incidences were observed although it is noted that the spontaneous tumour incidences appeared to be quite low with only one hepatocellular adenoma and one lung alveolar adenoma in both male and female controls.

In the sixth carcinogenicity study in mice ([redacted], 1983, study report no. 77-2061) groups of 50 male and 50 female CD-1 mice received glyphosate (batch NB 1782608/3 and NB 1782610/7, purity 99.7%) at dietary concentrations of 0, 1000, 5000 and 30000 ppm (equal to 157, 814 and 4841 mg/kg bw/day for males and 190, 955 and 5874 mg/kg bw/day for females). The study was conducted in accordance with OECD 451 with some minor deviations and was considered to be acceptable.

Mean body weights for the high-dose males were generally lower than in controls; differences from control were as great as -11 % (at Week 102) and were, for the most part, statistically significant. At the terminal sacrifice, the mean absolute and relative (to body and brain weights) weight of the testes were elevated for the high-dose group. Of the non-neoplastic findings, hepatic central lobular hypertrophy and necrosis was noted with increased incidence in the high-dose males. In addition a significant increase in chronic interstitial nephritis was noted in high dose males. Also, an increased frequency of epithelial hyperplasia in the urinary bladder of males in mid- and high dose males. In females an increase of proximal tubule epithelial basophilia and hypertrophy were observed in high dose females. An increase in renal tubule adenoma was observed in males (0/50, 0/50, 1/50, 3/50). The relevance of these findings is further discussed below at 'Overall consideration of the tumour incidences'.

Based on non-neoplastic histological changes affecting urinary bladder epithelium in male mice at 5000 ppm glyphosate in diet (814 mg/kg bw/day) and higher, the systemic NOAEL is considered the low dose of 1000 ppm (157 mg/kg bw/day).

A seventh carcinogenicity study is available in mice ([redacted], 1982, study report no. 8010). However, the study showed a wide number of limitations including dose levels being too low (max 300 ppm), only two dose levels being tested, lack of detail on test material and animals, body weight measured only monthly, no pathological examination on animals that died or were sacrificed during the study and the number of animals at termination being too low (11 to 23). Based on these limitations the study was concluded to be unacceptable.

In an US EPA assessment[5], another 2-year carcinogenicity study in CD-1 mice was identified ([redacted], 1987) in which glyphosate was administered as a trimesium salt at low dose levels of up to 991/1341 mg/kg bw/d. This study was not mentioned in the EU review on glyphosate before. According to the summary provided in the US EPA assessment, there were no treatment-related increases in tumor incidences in the study. **For the process under Regulation (EC) No 1107/2009, the applicant is requested to provide the study by** [redacted]

[5] Revised Glyphosate Issue Paper: Evaluation of Carcinogenic Potential EPA's Office of Pesticide Programs December 12, 2017. (https://downloads.regulations.gov/EPA-HQ-OPP-2009-0361-0073/content.pdf)

(1987), if possible, and an assessment. For the process under the Regulation (EC) No 1272/2008, the applicant is asked to submit the missing information during the public consultation period.

2.6.5.1.1.3 Rat and mouse studies - <u>overall consideration of the tumour incidences:</u>

A total of eight unpublished long-term feeding studies with the technical active ingredient in rats (Table 53 above) were submitted for evaluation of carcinogenic effect of glyphosate of which six were performed in compliance with OECD TG 453 (either fully acceptable or acceptable with restrictions). The remaining two [redacted], 1997, study report no. 1231; and [redacted], 1981, study report no. 77-2062) were flawed by serious deficiencies. Due to the strong limitations, these two studies cannot be considered suitable for the evaluation of carcinogenic properties of glyphosate to rats. However, since the study by [redacted] (1981) was subject to debate with regard to certain tumour types, it is taken here into consideration, along with the six guideline-compliant studies.

In mice, five long-term studies are available that may be considered valid according to current standards and were performed in compliance with OECD TG 451 (Table 53 above). Two other studies were submitted but did not comply with current standards ([redacted], 1988, reported at CA 5.5/022; and [redacted], 1982, report no. 8010). In both of them, there were serious reporting deficiencies and the top dose level was 300 ppm and, thus, much too low for meaningful evaluation of carcinogenic effect. No increase in any tumour type had been reported, but again, these studies are not suitable for the purpose of classification and labelling. Besides the seven studies in mice submitted by the applicant, another study was performed in 1999 by [redacted] This study was mentioned in the JMPR evaluation on glyphosate and concerns a 18-month feeding study in male and female CD-1 mice. According to the JMPR analysis, an increased incidence in malignant lymphomas was reported in female mice. However, as the study report is not available to the RMS, it is not possible to assess the reliability of the study and to check the raw incidence data. The results as presented in the JMPR assessment are given below.

All tumour types highlighted in Table 53 and in the study summaries above are considered in greater detail below. This means that incidences of these tumour types observed in all studies are reported together with the statistical calculations (as reported in the study report, by AGG own analysis and/or by statistical recalculation of the previous assessment for some studies (taken from CLH report 2016)). Both a pairwise comparison and a trend test are considered. In addition, for one study in mice ([redacted], 2001, study report no. Toxi 1559.CARCI-M), a Peto-analysis has been performed (refer to malignant lymphomas below). Where available, historical control data are reported for the selected tumour types in order to make a comparison with the natural background level.

For overall assessment, however, it must be further acknowledged that glyphosate is different from most other active substances in plant protection products because a number of comprehensive and high quality studies are available for nearly all toxicological endpoints. If dose levels are comparable, it would be expected that adverse effects were, at least to a certain extent, reproducible in other studies. A "weight of evidence" approach should and may be applied, therefore, as a general principle. Findings (including neoplastic) will be considered to have occurred by chance if they are not dose-related or cannot be confirmed at similar or higher dose levels in other studies.

Notwithstanding the conclusion of the EU 2001 evaluation, the opinion of the glyphosate renewal task force and of several other recent reviews of glyphosate (EFSA 2015, ECHA 2016, JMPR/WHO 2016, FSC Japan 2016, PMRA Canada, 2017, U.S. EPA 2019) where it was concluded that glyphosate is not a carcinogen, in 2015, a review of the scientific evidence by the International Agency for Research on Cancer (IARC) concluded that the evidence for the carcinogenicity of glyphosate was limited in humans but sufficient in experimental animals (rats and mice). IARC further concluded that glyphosate was probably carcinogenic in humans. In response to this IARC conclusion, the previous RMS has extensively discussed the carcinogenicity findings from animal and epidemiological studies.

The IARC conclusion has triggered a number of experts to investigate why there should be different conclusions from different investigating bodies (Crump *et al.*, 2020 (B.6.5.18.1) and Portier *et al.*, 2020 (B.6.5.18.2)).

In his paper Crump *et al.* (2020) point out that the animal carcinogenicity data on glyphosate are unusually extensive (≥15 long term rodent oral bioassays of glyphosate identified by U.S. EPA (2016), EFSA (2016) and IARC (2015). Each bioassay was conducted in both sexes, with each sex potentially having 40-60 unique tumour types, resulting in over 1000 potential statistical tests, which could easily result in many significant ($p \leq 0.05$) tumour increases occurring by chance alone – roughly 5%. Crump *et al.* (2020) have assessed the probability of false positives using a modification of the permutation approach of Farrar and Crump (1988 and 1990). The statistical method requires access to individual animal data on histopathological information and tumours, the length of time each animal was on test, and their doses. These criteria were met in 10 bioassays (4 mouse and 6 rat), which included all the bioassays

cited by IARC as showing evidence of carcinogenicity. The analysis made by Crump *et al.* (2020) shows that statistically significant effects on tumour incidences should be carefully evaluated for biological relevance as chance findings may occur.

Portier (2020) also provided an additional revised statistical evaluation and trend test analyses. The author asserts that his updated analyses in the publication support the IARC's conclusion of evidence of cancer in experimental animals. The study by Portier (2020) does not take into account the chance effect due to multiple testing as pointed out by Crump *et al.* (2020). Moreover, as also indicated in the OECD Guidance document 116 statistical significance is only part of the interpretation of the biological importance of a particular finding. Nevertheless, the tumour types showing statistically significant trends in the analysis by Portier (2020) were further taken into consideration (see below). One of the differences between the study by Portier (2020) and the analysis by AGG is that Portier used one-sided testing with a significance level of 0.05, whereas in the original study reports and the AGG analysis two-sided testing is applied with a significance level of 0.05 (which is equivalent to one-sided testing using a significance level of 0.025).

The statistical analyses provided by AGG are based on values reported in the original study reports, the statistical re-assessment of the data given in the previous CLH report (2016) and/or by AGG own statistical analysis. However, both one- or two-sided significance can be calculated, depending on the hypothesis to test. OECD Guidance Document 116 stipulates "*The choice of whether to use a one- or two-sided test should be made at the design rather than the analysis stage. A two-sided statistical hypothesis test tests for a difference from the negative control (in a pairwise comparison) in either direction. A one-sided comparison tests for a difference in only one pre-specified direction, but as a consequence has more power. In a carcinogenicity study, the expectation is often that the change will be an increase in tumours in the treated group so a one-sided test may be considered more appropriate, although this can be controversial. If the treatment could also be protective (i.e., reduce tumour incidence or delay it) then a two-sided comparison may be more appropriate*". In the AGG overall analysis on the tumour relevance, two-sided testing was applied as this is in line with how the statistical analysis was established in the study protocols of the available carcinogenicity studies.

A full evaluation of the complete carcinogenicity data package in the context of classification and labelling is provided below taking into account the findings by IARC, the previous assessment and the public literature assessment by Portier (2020). In the next section, a discussion is provided for the following tumour types:

1) Testes interstitial cell tumours in rats. This type of tumours was already discussed during the previous evaluation (CLH 2016 and RAC 2017) and highlighted in the publication by Portier (2020). Although there are no new findings (except updated historical control data), an assessment of the relevance of this tumour type is presented here again in order to provide a complete picture.

2) Pancreatic islet cell tumours in rats. This type of tumours was already discussed during the previous evaluation (CLH 2016 and RAC 2017) and highlighted in the publication by Portier (2020). Although there are no new findings, an assessment of the relevance of this tumour type is presented here again in order to provide a complete picture.

3) Thyroid C-cell tumours in rats. This type of tumours was already discussed during the previous evaluation (CLH 2016 and RAC 2017) and highlighted in the publication by Portier (2020). Although there are no new findings (except updated historical control data), an assessment of the relevance of this tumour type is presented here again in order to provide a complete picture.

4) Hepatocellular adenoma in rats. This type of tumours was already discussed during the previous evaluation (CLH 2016 and RAC 2017), however, only was study was considered ([redacted], study report no. [redacted]-10495). In the current assessment also a second study is taken into account in which an apparent increase is seen ([redacted], 2001) as highlighted by Portier (2020).

5) Pituitary adenoma in rats. The publication by Portier (2020) highlighted a statistically significant trend in the incidence of pituitary adenomas in male and female rats in the study by [redacted], 2009 (study report no. 2060-0012). This finding has not been previously discussed at EU level.

6) Skin basal cell tumours and 7) skin keratoacanthomas in rats. The publication by Portier (2020) highlighted a statistically significant trend in these types of tumours in male rats. Previously these findings have not been extensively discussed at EU level.

8) Malignant lymphoma in mice. In the previous assessment, this tumour type observed in mice was extensively discussed (CLH 2016 and RAC 2017). These tumours were also highlighted in the publication by Portier (2020).

As a new statistical analysis is available and updated historical control data have been provided, an assessment of the relevance of this tumour type is presented below.

9) Renal tubule tumours in male mice. Also this type of tumour have been extensively discussed during the previous assessment (CLH 2016 and RAC 2017). These tumours in mice were also highlighted in the publication by Portier (2020). As a new statistical analysis is available and updated historical control data have been provided, an assessment of the relevance of this tumour type is presented below. Although there are no new findings (except updated historical control data), an assessment of the relevance of this tumour type is presented here again in order to provide a complete picture.

10) Haemangiosarcoma in male mice and haemangioma in female mice. The haemangiosarcomas in male mice have been extensively discussed during the previous assessment (CLH 2016 and RAC 2017). These tumours in mice were also highlighted in the publication by Portier (2020). Although there are no new findings, an assessment of the relevance of this tumour type is presented here again in order to provide a complete picture. The mesenteric lymph node haemangioma observed in female mice in one study, have not been discussed before, but were highlighted by the publication from Portier (2020). Therefore an assessment is provided below.

1) Interstitial cell tumour of the testes in rats

The study by [redacted] (Report No. 77-2062, 1981), which is considered not acceptable as the dose levels are too low and due to a poor quality of the study report, reported an increased incidence of interstitial testicular tumours in rats with 6 out of 50 animals in the high dose group compared to 0 out of 50 in the control group (Table 2.6.5.1-1a). The difference was statistically significant using Fisher's Exact test ($p<0.05$). However, a clear dose-response relationship is lacking. The study author argued that this is a common tumour in aging rats and stated that historical control data showed that the incidence in the high dose group was only slightly outside the historical control range. As there was a lack of details on the historical control data (HCD) provided in the study report, the applicant was requested to provide further details on the HCD. The applicant replied that only for one contemporary chronic/carcinogenicity rat study conducted with Sprague-Dawley rats appropriate historical control data could be retrieved as for the remaining studies the data has been discarded. In this concurrent study, which was performed between 1980 and 1982, the incidence of testes interstitial cell tumours was 4/80 (5%) among controls. However, as HCD of only one study is available, this is of very limited value. The only comparison that can be made based on this very limited HCD is that the incidence of this tumour in the control group males that is lower (0%) than observed in this concurrent historical control data set and that the incidence in top dose males that is higher (12%). In addition, no dose-response effect is observed when all dose levels are taken into account.
Moreover, it is emphasized that the study is not considered acceptable, mainly because of the poor quality of the study report. More importantly, when considering all acceptable and guideline-compliant studies in rats, it is noted that no effect on interstitial cell tumours of the testis were observed in any of the other six carcinogenicity studies in the rat even though they were dosed at much higher dose levels (see Table 2.6.5.1-1b). In addition, no similar effects were observed in mice.
Overall, it concluded that there is no evidence that glyphosate induces interstitial testicular tumours. This conclusion is in line with the previous EU evaluation.

Table 2.6.5.1-1a: Interstitial cell tumours of the testes in male rats – overview of results in the different rat studies

Report No.; Test species; Dose levels	**Control**	**Low**	**Mid**	**Second mid dose**	**High dose**	**Fisher's exact test (high dose vs control)**
2060-0012 ([redacted] 2009) Wistar rats, 0, 1500, 5000 and 15000 ppm	2/51	3/22	1/18		1/50 (1077 mg/kg bw/day)	No significant difference
[redacted]/PR1111 ([redacted] 2001) Wistar rats 0, 2000, 6000 and 20000 ppm	5/63	2/63	2/63		2/64 (1214 mg/kg bw/day)	No significant difference

[redacted] 94-0150 ([redacted], 1997) Sprague-Dawley rats 0, 3000, 10000, 30000 ppm	3/75	2/75	0/80		2/78 (1127 mg/kg bw/day)	No significant difference
886.C.C-R ([redacted] 1996) Wistar rats 0, 100, 1000 and 10000 ppm	2/50	0/37	2/32		3/50 (595 mg/kg bw/day)	No significant difference
7867 ([redacted] 1993) Sprague-Dawley rats, 0, 10, 100, 300 and 1000 mg/kg bw/day	3/50	1/25	0/19	0/21	1/50 (1000 mg/kg bw/day)	No significant difference
[redacted]-10495 ([redacted] [redacted] [redacted] 1990) Sprague-Dawley rats, 0, 2000, 8000 and 20000 ppm	2/60	0/60	3/60		2/60 (940 mg/kg bw/day)	No significant difference
77-2062 ([redacted], 1981) Sprague-Dawley rats, 0, 30, 100 and 300 ppm Study **not** acceptable	0/50	3/50	1/50		**6/50** **(34.02 mg/kg bw/day)**	**$p < 0.05$**

Table 2.6.5.1-1b: Interstitial cell tumours of the testes in male rats – dose and incidence

Study		[redacted] 1981	[redacted] 1990	[redacted] 1993	[redacted] 1997	[redacted] 1996	[redacted] 2001	[redacted] 2009
Duration		26m	**24 m**	**24m**	**24m**	**24m**	**24m**	**24m**
Sex Strain		Male SD[1]	**Male SD**	**Male SD**	**Male SD**	**Male W[2]**	**Male W**	**Male W**
Dose mg/kg bw/day	**NOAEL / LOAEL (systemic)**							
0 (control)		0/50	2/60	3/50	3/75	2/50	5/63	2/51
3.05		3/50						
6.3						0/37		
10.3		1/50						
11	NOAEL			1/25				
31.49		6/50*						
59.4	NOAEL					2/32		
85.5								3/22
89	NOAEL		0/60					
104	NOAEL				2/75			
112	LOAEL			0/19				
121							2/63	
285.2	NOAEL							1/18
320				0/21				
354	LOAEL				0/80			
361	NOAEL						2/63	
362	LOAEL		3/60					
595.2	LOAEL					3/50		
940			2/60					
1077.4	LOAEL							1/50
1127					2/78			
1147				1/50				
1214	LOAEL						2/64	
p-value Fisher's Exact test		$p < 0.05$ (high dose only)	not sign	not sign	not sign	not sign	not sign	not sign

[1] SD: Sprague-Dawley; [2] W: Wistar

* p-value below 0.05 (statistically significant by pairwise comparison with controls)

2) Pancreatic islet cell tumours in rats

In the IARC evaluation of glyphosate a statistically significant increase in the incidence of pancreatic islet cell adenomas was noted in the study by [redacted] (study report no. [redacted]-10495) at two dose levels but there was no statistically significant positive trend nor progression to carcinoma. During the previous EU evaluation of glyphosate the RMS re-evaluated the results using Cochran-Armitage trend testing which confirmed the absence of a positive trend (p=0.1687, source CLH report 2016). The pairwise comparison by Fisher's Exact test revealed a significant increase over the control incidence but only at the low dose (p=0.030). There was no progression toward malignancy since the only carcinoma was observed in a single control male.

In addition, IARC reported a significant increase in the incidence of pancreatic adenoma in a second study ([redacted] 1981; study report no. 77-2062) for the low dose males. It should be noted that this study was considered to be unacceptable due to the low dose levels compared to the other carcinogenicity studies and the low quality of the study report. No positive trend over all dose groups was observed for the pancreatic adenomas and there was no clear indication of progression to carcinoma. The statistical re-evaluation which was conducted during the previous EU evaluation of glyphosate (CLH report 2016) confirmed a significant increase in only low dose animals for the pancreatic islet cell adenoma, but also revealed a positive trend (p=0.0496) for carcinomas although it should be noted this was based on a single incidence in high dose males compared to 0 in the control, low and mid dose groups.

There was no dose-response relationship observed in the two studies as indicated by the lack of a statistically

significant trend. In addition, in the five remaining carcinogenicity studies in the rat with even higher dose levels clearly no effect of pancreatic islet cell adenomas was observed (see Table 2.6.5.1-2b). There was no increase in pancreatic tumours in the females. The observed findings are therefore concluded to be incidental and not treatment related. This conclusion is in line with the previous EU evaluation.

Table 2.6.5.1-2a: Pancreatic islet cell adenomas in rats – overview of results in the different rat studies

Report No.; Test species; Dose levels	Sex	Control	Low	Mid	Second mid dose	High dose	Response Fisher's exact test (source CLH 2016)
2060-0012 ([redacted], 2009) Wistar rats, 0, 1500, 5000 and 15000 ppm	M	4/49	1/14	2/15		1/50	No significant difference
[redacted]/PR1111 ([redacted] 2001) Wistar rats 0, 2000, 6000 and 20000 ppm	M[1]	1/64	1/64	0/64		1/64	No significant difference
[redacted] 94-0150 ([redacted] 1997) Sprague-Dawley rats 0, 3000, 10000, 30000 ppm	M[1]	4/76	1/75	1/80		1/78	No significant difference
886.C.C-R ([redacted] 1996) Wistar rats 0, 100, 1000 and 10000 ppm	M	3/48	0/30	0/32		1/49	No significant difference
7867 ([redacted]1993) Sprague-Dawley rats, 0, 10, 100, 300 and 1000 mg/kg bw/day	M	7/50	1/24	2/17	2/21	1/49	No significant difference
[redacted]-10495, ([redacted] 1990) Sprague-Dawley rats, 0, 2000, 8000 and 20000 ppm	M	1/58 adenoma 1/58 carcinoma	8/57[a] adenoma 0/57 carcinoma	5/60 adenoma 0/60 carcinoma		7/59 adenoma 0/59 carcinoma	p = 0.030 (low dose) p = 0.062 (high dose) p = 0.1687 for trend
77-2062 ([redacted] 1981) Sprague-Dawley rats, 0, 30, 100 and 300 ppm	M	0/50 adenoma 0/50 carcinoma	5/49 [b] adenoma 0/50 carcinoma	2/50 adenoma 0/50 carcinoma		2/50 adenoma 1/50 [c] carcinoma	p = 0.027 / Cochran-Armitage trend test; p=0.0496 for carcinomas

[1] Includes interim sacrifice group

[a] Statistically significant increase compared with controls (Fishers's exact test; p=0.030) (source CLH report 2016) and statistically significant at $p \leq 0.01$ (Fisher exact test with Bonferroni inequality (source original study report)).

[b] Statistically significant increase compared with controls (Fishers's exact test; p=0.027) (source CLH report 2016).

[c] Statistically significant positive trend from carcinomas (Cochran-Armitage trend test; p=0.0496) (source CLH report 2016).

Table 2.6.5.1-1b: Pancreatic islet cell adenomas (and carcinomas) in rats – dose and incidence

Study		[redacted] 1981	[redacted] [redacted] 1990	[redacted] 1993	[redacted] 1997	[redacted] 1996	[redacted] 2001	[redacted] 2009
Duration		26m	**24 m**	**24m**	**24m**	**24m**	**24m**	**24m**
Sex Strain		Male SD[1]	**Male SD**	**Male SD**	**Male SD**	**Male W[2]**	**Male W**	**Male W**
Dose mg/kg bw/day	**NOAEL / LOAEL (systemic)**							
0 (control)		0/50 (0/50)	1/58 (1/58)	7/50	4/76	3/48	1/64	4/49
3.05		5/49* (0/50)						
6.3						0/30		
10.3		2/50 (0/50)						
11	NOAEL			1/24				
31.49		2/50 (1/50)						
59.4	NOAEL					0/32		
85.5								1/14
89	NOAEL		8/57* (0/57)					
104	NOAEL				1/75			
112	LOAEL			2/17				
121							1/64	
285.2	NOAEL							2/15
320				2/21				
354	LOAEL				1/80			
361	NOAEL						0/64	
362	LOAEL		5/60 (0/60)					
595.2	LOAEL					1/49		
940			7/59 (0/59)					
1077.4	LOAEL							1/50
1127					1/78			
1147				1/49				
1214	LOAEL						1/64	
Trend test p-value / p-value Fisher's Exact test		Adenomas: trend not sign / p = 0.027 (low dose) Carcinomas: trend p = 0.0496	p = 0.1687 for trend / p = 0.030 (low dose) p = 0.062 (high dose)	not sign	not sign	not sign	not sign	not sign

[1] SD: Sprague-Dawley; [2] W: Wistar ; (incidence): carcinomas; * p-value below 0.05 (statistically significant by pairwise comparison with controls)

3) Thyroid C-cell tumours in rats

In one study, an increase was noted in thyroid C-cell adenomas at the mid and high dose level in both males and females ([redacted]; study report no. [redacted]-10495). According to the study report, the increase was not statistically significant (tested with Fisher exact test with Bonferroni inequality). During the previous EU evaluation

of glyphosate the RMS re-evaluated the results using Cochran-Armitage trend. In this assessment, it was concluded that the increase in thyroid C-cell adenomas in females was not statistically significant in a pair-wise comparison (Fisher's exact test) but was weakly positive in a Cochran-Armitage trend test. No statistical significance was found when using pairwise comparison (Fisher's exact test). For males, the increased incidences of adenomas or combined adenomas/carcinomas were not statistically significant. The applicant provided historical control data for this finding. Based on this HCD, it is noted that the incidence in mid and high dose males and females are outside historical control data (males range 5.0-8.6% and females range 3.3%-8.5%; HCD from 3 studies between 1986 to 1989; Table 2.6.5.1-3a). There was no progression to carcinomas (reported incidences of carcinomas were 0, 2, 0, 1 in males and 0, 0, 1, 0 in females for the control, low, mid and high doses, respectively). Further, no effect on non-neoplastic precursors was observed in the study. In fact, the thyroid does not appear to be a target organ for glyphosate in any of the repeated dose toxicity studies in rats. Only in dogs, in the 1-year study a higher thyroid weight accompanied by C-cell hyperplasia was noted in males. No effect on thyroid C-cell adenoma was observed in any of the other studies (see Table 2.6.5.1-3b and -3c). Although the reported incidence is above historical control range, still the conclusions of the previous evaluation remain that the increased incidences are considered incidental and not treatment-related as in the other studies no thyroid C-cell adenomas. This conclusion is in line with the previous EU evaluation.

Table 2.6.5.1-3a: Thyroid C-cell adenoma in rats – overview of results in the different studies

Report No.; Test species; Dose levels	**Sex**	**Control**	**Low**	**Mid**	**Second mid dose**	**High dose**	**Fisher's exact test (high dose vs control) / Cochran-Armitage trend test (Source: CLH 2016)**
2060-0012 ([redacted] 2009) Wistar rats, 0, 1500, 5000 and 15000 ppm	M	9/51	1/14	1/13		3/51	Not analysed.
	F	5/51	1/18	1/15		0/51	Not analysed.
[redacted]/PR1111 ([redacted] 2001) Wistar rats 0, 2000, 6000 and 20000 ppm	M	2/63	1/63	1/63		0/64	Not analysed.
	F	4/63	0/63	0/64		2/64	Not analysed.
[redacted] 94-0150 ([redacted] 1997) Sprague-Dawley rats 0, 3000, 10000, 30000 ppm	M	6/76	10/74	5/79		6/78	Not analysed.
	F	4/78	7/78	8/76		4/78	Not analysed.
886.C.C-R ([redacted] 1996) Wistar rats 0, 100, 1000 and 10000 ppm	M	2/45	0/26	1/29		1/50	Not analysed.
	F	2/50	0/24	1/17		1/47	Not analysed.
7867 ([redacted] 1993) Sprague-Dawley rats, 0, 10, 100, 300 and 1000 mg/kg bw/day	M	9/50	1/21	1/17	2/21	9/49	Not analysed.
	F	8/50	1/27	1/29	2/29	7/49	Not analysed.
[redacted]-10495, ([redacted] 1990) Sprague-Dawley rats, 0, 2000, 8000 and 20000 ppm	M	2/60 (3.3%)	4/58 (6.9%)	8/58 (13.8%)		7/60 (11.7%)	No significant difference / No significant trend
HCD (3 studies, years 1986-1989): Males: 5/58 (8.6%), 4/60 (6.7%) and 3/60 (5.0%) Females: 5/59 (8.5%), 5/60 (8.3%) and 2/60	F	2/60 (3.3%)	2/60 (3.3%)	6/60 (10.0%)		6/60 [a] (10.0%)	No significant difference / p = 0.0435

(3.3%)							

[a] Statistically significant trend (Cochran-Armitage trend test; p = 0.0435; source CLH report 2016)

Table 2.6.5.1-3b: Thyroid C-cell adenoma in rats – dose and incidence males

Study		[redacted] 1990	[redacted] 1993	[redacted] 1997	[redacted] 1996	[redacted] 2001	[redacted] 2009
Duration		**24 m**	**24m**	**24m**	**24m**	**24m**	**24m**
Sex Strain		**Male SD[1]**	**Male SD**	**Male SD**	**Male W[2]**	**Male W**	**Male W**
Dose mg/kg bw/day	**NOAEL / LOAEL (systemic)**						
0 (control)		2/60	9/50	6/76	2/45	2/63	9/51
3.05							
6.3					0/26		
10.3							
11	NOAEL		1/21				
31.49							
59.4	NOAEL				1/29		
85.5							1/14
89	NOAEL	4/58					
104	NOAEL			10/74			
112	LOAEL		1/17				
121						1/63	
285.2	NOAEL						1/13
320			2/21				
354	LOAEL			5/79			
361	NOAEL					1/63	
362	LOAEL	8/58					
595.2	LOAEL				1/50		
940		7/60					
1077.4	LOAEL						3/51
1127				6/78			
1147			9/49				
1214	LOAEL					0/64	
Trend test p-value / p-value Fisher's Exact test		Not sign / Not sign	Not analysed	Not analysed	Not analysed	Not analysed	Not analysed

[1] SD: Sprague-Dawley; [2] W: Wistar

Table 2.6.5.1-3c: Thyroid C-cell adenoma in rats – dose and incidence females

Study		[redacted] 1990	1993	1997	1996	2001	2009
Duration		**24 m**	**24m**	**24m**	**24m**	**24m**	**24m**
Sex Strain		**Female SD[1]**	**Female SD**	**Female SD**	**Female W[2]**	**Female W**	**Female W**
Dose mg/kg bw/day	**NOAEL / LOAEL (systemic)**						
0		2/60	8/50	4/78	2/50	4/63	5/51
3.37							
8.6					0/24		
11.22							
12	NOAEL		1/27				
34.02							
88.5	NOAEL				1/17		
104.5							1/18
109	LOAEL		1/29				
113	NOAEL	2/60					
115	NOAEL			7/78			
145						0/63	
347			2/29				
348.6	NOAEL						1/15
393	LOAEL			8/76			
437	NOAEL					0/64	
457	LOAEL	6/60					
886.0	LOAEL				1/47		
1134			7/49				
1183		6/60					
1247				4/78			
1381.9	LOAEL						0/51
1498	LOAEL					2/64	
Trend test p-value / p-value Fisher's Exact test		0.0435 / Not sign	Not analysed	Not analysed	Not analysed	Not analysed	Not analysed

[1] SD: Sprague-Dawley; [2] W: Wistar

4) Hepatocellular adenoma in rats

In the previous CLH report the observed hepatocellular adenomas in male rats were already discussed where it was concluded that the effect was not considered attributable to glyphosate. However, compared to the previous evaluation the RMS considers that besides the study by [redacted] ([redacted]-10495), a second study ([redacted] 2001; study report no. [redacted]/PR1111) should be discussed in more detail as a potential treatment-related increase in hepatocellular adenomas was observed.

In the study by [redacted] ([redacted]-10495), high dose males showed an increase in hepatocellular adenomas (8/60 versus 3/60 in controls). The study report did not consider the effect to be statistically significant. However, re-analysis of the data during the previous EU evaluation of glyphosate (CLH report 2016) showed that the reported incidence was statistically significant using a Cochran-Armitage trend test (p=0.0171). It is noted that the incidence in high dose males of 13.3% was slightly above HCD mean of 11.1% (based on 3 studies performed between 1986-1989; same strain, same lab), however, clearly within the HCD range of 6.7%-18.3% (Table 2.6.5.1-4a). No statistical trend was observed when the incidences of adenoma and carcinoma were combined (p=0.0752). The incidence of carcinomas was 3/60, 2/60, 1/60 and 2/60 for the control, low, mid and high dose, respectively, which

results in a combined incidence of 6/60, 4/60, 4/60 and 10/60. In addition, no non-neoplastic precursors were observed in the liver.

In the other study by [redacted] ([redacted]/PR1111), hepatocellular adenoma was observed in 5 out of 64 animals (7.8%) compared to zero incidences in controls. The study report reported that the incidence at the top dose was not statistically significant using the Fisher's Exact test, however, the difference was statistically significant using the Peto-test for trend. The incidence in high dose males of 7.8% was slightly outside historical control data (range 0-5.8%, mean 1.5%; HCD from 5 studies between 1998 to 2003). It is noted that, although a statistical trend is observed, no clear-dose response is seen when comparing the incidences per dose group with 2 incidences at the low dose, 0 incidences at the mid dose and 5 at the high dose. Overall, there was no progression to carcinomas. However, an increased incidence in hepatitis was noted in top dose males. The reported incidences were 8/64, 6/64, 9/64, 13/64 for doses 0, 2000, 6000 and 20000 ppm (p-value to be added). The incidence of hepatitis at the top dose was above HCD mean (11.8%) but within HCD range (0-30%; HCD based on 5 studies from the same lab and in the same strain performed between 1998-2003). As the background incidence of hepatitis is highly variable and as the incidence is within HCD range, the relation to treatment is doubted.

The other four carcinogenicity studies in rat (two studies with Wistar rats, two studies with Sprague-Dawley rats) did not show an effect on hepatocellular adenomas (see Table 2.6.5.1-4b). In addition, no effect in females was observed in any of the studies (see Table 2.6.5.1-4c). Therefore, the majority of the carcinogenicity studies in the rat did not show a treatment-related effect on hepatocellular adenoma. The two studies in which a potential increase was observed no clear effect on non-neoplastic precursors was observed. In general, glyphosate shows low hepatoxicity based on an extensive data-set. Although the study by [redacted] ([redacted]/PR1111) showed an increase in hepatitis at the top dose, the relation with treatment was doubted as the background incidence of hepatitis is highly variable and as the incidence is within HCD range.
Based on the explanation above, the observed increase in hepatocellular adenomas is considered incidental and not related to treatment. This conclusion is in line with the previous EU evaluation.

Table 2.6.5.1-4a: Hepatocellular adenomas in rats – overview of results in the different rat studies

Report No.; Test species; Dose levels	Sex	Control	Low	Mid	Second mid dose	High dose	Fisher's exact test (high dose vs control) / Cochran-Armitage trend test (Source: CLH 2016)
2060-0012 ([redacted], 2009) Wistar rats, 0, 1500, 5000 and 15000 ppm HCD not available anymore	M	0/51	2/51	1/51	-	1/51	Not analysed
	F	1/51	0/51	1/51	-	1/51	Not analysed
[redacted]/PR1111 ([redacted] 2001) Wistar rats 0, 2000, 6000 and 20000 ppm HCD (5 studies, years 1998-2003) Males: mean 1.5%; range 0-5.8%	M[1]	0/64 (0%)	2/64 (3.1%)	0/64 (0%)	-	5/64 (7.8%)	No significant difference for Fisher exact test, but significant for Peto-test for trend (based on study report)
	F[1]	0/64	0/64	1/64	-	0/64	Not analysed
[redacted] 94-0150 ([redacted], 1997) Sprague-Dawley rats 0, 3000, 10000, 30000 ppm HCD not requested	M[1]	1/76	0/75	2/80	-	1/78	Not analysed
	F[1]	1/78	1/79	0/78	-	0/78	Not analysed
886.C.C-R ([redacted], 1996) Wistar rats 0, 100, 1000 and 10000 ppm HCD not requested	M	24/50	22/50	10/48	-	21/50	Not analysed.
	F	18/50	18/48	19/49	-	13/50	Not analysed
7867 ([redacted] 1993) Sprague-Dawley rats, 0, 10, 100, 300 and 1000 mg/kg bw/day HCD not requested	M	2/50	1/50	1/50	2/50	2/50	Not analysed
	F	0/50	1/50	3/50	1/50	2/50	Not analysed.
[redacted]-10495, ([redacted], 1990) Sprague-Dawley rats, 0, 2000, 8000 and 20000 ppm HCD (3 studies, years 1986-1989): Males: 11/60 (18.3%), 5/60 (8.3%) and 4/60 (6.7%); mean 11.1%	M[1]	Adenomas: 3/60 (5.0%) Carcinomas: 3/60	Adenomas: 2/60 (3.3%) Carcinomas: 2/60	Adenomas: 3/60 (5.0%) Carcinomas: 1/60	-	Adenomas: 8/60[a] (13.3%) Carcinomas: 2/60	Adenomas: p = 0.162 / p = 0.0171 p-trend combined adenomas + carcinomas

							p=0.0752
	F[1]	6/60	2/60	6/60	-	1/60	Not analysed

[1] Includes interim sacrifice group

Table 2.6.5.1-4b: Hepatocellular adenomas (and carcinomas) in rats – dose and incidence males

Study		[redacted] 1990	[redacted] 1993	[redacted] 1997	[redacted] 1996	[redacted] 2001	[redacted] 2009
Duration		**24 m**	**24m**	**24m**	**24m**	**24m**	**24m**
Sex Strain		**Male SD[1]**	**Male SD**	**Male SD**	**Male W[2]**	**Male W**	**Male W**
Dose mg/kg bw/day	**NOAEL / LOAEL (systemic)**						
0		3/60 (3/60)	2/50	0/76	24/50	0/64	0/51
3.05							
6.3					22/50		
10.3							
11	NOAEL		1/50				
31.49							
59.4	NOAEL				10/48		
85.5							2/51
89	NOAEL	2/60 (2/60)					
104	NOAEL			0/75			
112	LOAEL		1/5				
121						2/64	
285.2	NOAEL						1/51
320			2/50				
354	LOAEL			2/80			
361	NOAEL					0/64	
362	LOAEL	3/60 (1/60)					
595.2	LOAEL				21/50		
940		8/60 (2/60)					
1077.4	LOAEL						1/51
1127				1/78			
1147			2/50				
1214	LOAEL					5/64	
Fisher's exact test (high dose vs control) / Cochran-Armitage trend test		p = 0.162 / p = 0.0171 p-trend combined adenomas+ carcinomas p = 0.0752	Not analysed	Not analysed	Not analysed	No significant difference for Fisher exact test, but significant for Peto-test for trend (based on study report)	Not analysed

[1] SD: Sprague-Dawley; [2] W: Wistar
(incidence): carcinomas

Table 2.6.5.1-4c: Hepatocellular adenomas in rats – dose and incidence females

Study		[redacted] 1990	1993	1997	1996	2001	2009
Duration		**24 m**	**24m**	**24m**	**24m**	**24m**	**24m**
Sex Strain		**Female SD[1]**	**Female SD**	**Female SD**	**Female W[2]**	**Female W**	**Female W**
Dose mg/kg bw/day	**NOAEL / LOAEL (systemic)**						
0		6/60	0/50	1/78	18/50	0/64	1/51
3.37							
8.6					18/48		
11.22							
12	NOAEL		1/50				
34.02							
88.5	NOAEL				19/49		
104.5							0/51
109	LOAEL		3/50				
113	NOAEL	2/60					
115	NOAEL			1/79			
145						0/64	
347			1/50				
348.6	NOAEL						1/51
393	LOAEL			0/78			
437	NOAEL					1/64	
457	LOAEL	6/60					
886.0	LOAEL				13/50		
1134			2/50				
1183		1/60					
1247				0/78			
1381.9	LOAEL						1/51
1498	LOAEL					0/64	
Trend test p-value		Not analysed	Not analysed	Not analysed	Not analysed	Not analysed	Not analysed

[1] SD: Sprague-Dawley; [2] W: Wistar

5) Pituitary adenoma in rats

The publication by Portier *et al.* 2020 (refer to Vol 3 CA B.6.5.18.2) highlighted a statistically significant trend in the incidence of pituitary adenomas in male and female rats (one-sided p=0.045 and p=0.014, respectively) observed in the study by [redacted] 2009 (study report no. 2060-0012) which they considered as 'some evidence' for carcinogenicity. This finding has not been previously discussed at EU level. However, it is doubted whether a trend test including the low and mid doses is appropriate as the histopathological examination in this study was only performed for animals that died pre-terminally and that were moribund sacrificed. In addition, any lesions and/or palpable masses of terminally sacrificed rats of the low and mid dose groups were investigated. As tumour incidence in this study is given as incidence/number of animals investigated and not incidence/per total number of animals per group, the observed trend might be distorted. Further, to allow for a complete evaluation of all the available information the RMS has included the observations on pituitary adenomas for all carcinogenicity studies in the tables below. When considering the results from the available carcinogenicity studies in the rat together it is clear that pituitary adenomas are very common in rats and that no increases in incidence were seen in any of the other studies. No progression to carcinomas was observed and no effect on concomitant non-neoplastic findings were observed. Therefore, it is concluded that glyphosate has no effect on pituitary adenomas.

Table 2.6.5.1-5a: Pituitary adenoma in rats – overview of results in the different rat studies

Report No.; Test species; Dose levels	Sex	Control	Low	Mid	Second mid dose	High dose
2060-0012 ([redacted] 2009) Wistar rats, 0, 1500, 5000 and 15000 ppm	M	16/51	11/18	10/18		20/51
	F	24/51	23/28	16/25		32/51
[redacted]/PR1111 ([redacted] 2001) Wistar rats 0, 2000, 6000 and 20000 ppm	M[1]	18/64	17/63	18/64		19/63
	F[1]	47/63	44/63	46/63		49/63
[redacted] 94-0150 ([redacted] 1997) Sprague-Dawley rats 0, 3000, 10000, 30000 ppm	M[1]	38/76	40/75	33/80		42/78
	F[1]	54/78	54/79	47/77		52/78
886.C.C-R ([redacted], 1996) Wistar rats 0, 100, 1000 and 10000 ppm	M	3/49	4/30	3/31		5/49
	F	7/49	13/33	7/23		6/50
7867([redacted], 1993) Sprague-Dawley rats, 0, 10, 100, 300 and 1000 mg/kg bw/day	M	28/50	12/24	8/19	7/21	17/50
	F	33/49	19/28	19/29	25/30	30/49
[redacted]-10495, ([redacted], 1990) Sprague-Dawley rats, 0, 2000, 8000 and 20000 ppm	M	34/60	32/58	34/58		31/59
	F	46/60	48/60	46/60		34/59
77-2062 ([redacted], 1981) Sprague-Dawley rats, 0, 30, 100 and 300 ppm	M	16/48	19/49	20/48		18/47
	F	34/48	29/48	31/50		26/49

[1] Includes interim sacrifice group

Table 2.6.5.1-5b: Pituitary adenoma in rats – dose and incidence males

Study		[redacted] 1981	[redacted], 1990	[redacted] 1993	[redacted] 1997	[redacted] 1996	[redacted] 2001	[redacted], 2009
Duration		26m	24 m	24m	24m	24m	24m	24m
Sex Strain		Male SD[1]	Male SD	Male SD	Male SD	Male W[2]	Male W	Male W
Dose mg/kg bw/day	NOAEL / LOAEL (systemic)							
0 (control)		16/48	34/60	28/50	38/76	3/49	18/64	16/51
3.05		19/49						
6.3						4/30		
10.3		20/48						
11	NOAEL			12/24				
31.49		18/47						
59.4	NOAEL					3/31		
85.5								11/18
89	NOAEL		32/58					
104	NOAEL				40/75			
112	LOAEL			8/19				
121							17/63	
285.2	NOAEL							10/18
320				7/21				
354	LOAEL				33/80			
361	NOAEL						18/64	
362	LOAEL		34/58					
595.2	LOAEL					5/49		

940			31/59					
1077.4	LOAEL							20/51
1127					42/78			
1147				17/50				
1214	LOAEL						19/63	

[1] SD: Sprague-Dawley; [2] W: Wistar

Table 2.6.5.1-5c: Pituitary adenoma in rats – dose and incidence females

Study		[redacted] 1981	[redacted], 1990	[redacted], 1993	[redacted], 1997	[redacted] 1996	[redacted], 2001	[redacted], 2009
Duration		26m	**24 m**	**24m**	**24m**	**24m**	**24m**	**24m**
Sex Strain		Female SD[1]	**Female SD[1]**	**Female SD**	**Female SD**	**Female W[2]**	**Female W**	**Female W**
Dose mg/kg bw/day	**NOAEL / LOAEL (systemic)**							
0		34/48	46/60	33/49	54/78	7/49	47/63	24/51
3.37		29/48						
8.6						13/33		
11.22		31/50						
12	NOAEL			19/28				
34.02		26/49						
88.5	NOAEL					7/23		
104.5								23/28
109	LOAEL			19/29				
113	NOAEL		48/60					
115	NOAEL				54/79			
145							44/63	
347				25/30				
348.6	NOAEL							16/25
393	LOAEL				47/77			
437	NOAEL						46/63	
457	LOAEL		46/60					
886.0	LOAEL					6/50		
1134				30/49				
1183			34/59					
1247					52/78			
1381.9	LOAEL							32/51
1498	LOAEL						49/63	

[1] SD: Sprague-Dawley; [2] W: Wistar

6&7) Skin tumours in rats

The publication by Portier, 2020 (refer to Vol 3 CA B.6.5.18.2) highlighted skin basal cell tumours and skin keratoacanthomas in male rats as evidence for carcinogenicity of glyphosate. Previously these findings have not been extensively discussed at EU level in the context of classification and labelling.

6) Skin basal cell tumours

In the aforementioned publication by Portier, a positive trend for skin basal cell tumours in male Sprague-Dawley rats was reported for the [redacted] study (study report no. [redacted] 94-0150). This trend was confirmed by an external statistician upon request by AGG (p (two-sided) = 0.001 for the extended Mantel-Haenszel test (stratified Cochran-Armitage trend)). The study by [redacted] (study report no. [redacted] 94-0150) reported an incidence of 3 benign adenomas and 1 malignant carcinoma. The benign basal cell tumour is an elevated skin nodule that is thought to arise from hair follicles.
For this tumour type, an overview of the reported incidences in all available rat studies is provided in the table below. When looking at all studies together, the apparent increase in basal cell adenomas was only observed in one study in males ([redacted], [redacted] 94-015, 1997) and not in the three other studies with Sprague-Dawley rats nor in the three studies with Wistar rats. The applicant submitted historical control data for this finding, which comprised only control data obtained from two studies (Table 2.6.5.1-6a). In both studies no skin basal cell adenomas or carcinomas were reported among controls. However, it should be noted that HCD from only two studies is very limited. In the [redacted] study (1997), a statistically increased incidence of follicular hyperkeratosis is reported with an incidence of 29.5% (23/78) in top dose males and 8% in females (6/78) compared to 9.2% and 0% in controls for males and females, respectively. This finding might indicate a precursor effect.
In the study by [redacted] (report no [redacted]/PR1111, 2001) one carcinoma in the mid dose groups was observed. The applicant submitted HCD for this finding, showing that no skin basal cell adenomas or carcinomas were reported among controls, however, the database included only five studies, which is rather limited considering that these tumours are rare. Considering that the carcinoma was observed in the mid-dose only, thus lacking dose-response and that no carcinomas were observed in any of the other five studies, the single carcinoma is considered a chance finding by the RMS.
For the skin basal cell adenomas reported in the study by [redacted], the effect was confined to one study at the top dose in males (accompanied by follicular hyperkeratosis), whereas no effect was observed in the other five studies for which a similar dosing regime was applied (Table 2.6.5.1-6b). As very limited historical control data is available for this type of tumours (only two studies) it is difficult to put this finding into perspective. Moreover, no effect was observed in females nor in other species. Further, there is no plausible mechanism as no clear effects on skin upon systemic exposure to glyphosate were reported in the whole database (except for the follicular hyperkeratosis). Therefore this finding is considered of equivocal relevance and not sufficient for classification.

Table 2.6.5.1-6a: Skin basal cell tumours in male rat – Overview of all rat studies

Report No.; Test species; Dose levels	Sex	Control	Low	Mid	Second mid dose	High dose	Stratified Cochran-Armitage trend test (Source: AGG)
2060-0012 ([redacted], 2009) Wistar rats, 0, 1500, 5000 and 15000 ppm	M	1/51	0/51 (86 mg/kg bw/day)	0/51 (285 mg/kg bw/day)		0/51 (1077 mg/kg bw/day)	Not analysed.
[redacted]/PR1111 ([redacted], 2001) Wistar rats 0, 2000, 6000 and 20000 ppm HCD (5 studies, years 1998-2003) Males: all studies 0/52 (0%) for both adenoma and carcinoma	M[1]	1/64	0/64 (121 mg/kg bw/day)	2/64# (361 mg/kg bw/day)		1/63 (1214 mg/kg bw/day)	Not analysed.
[redacted]94-0150 ([redacted] 1997) Sprague-Dawley rats 0, 3000, 10000, 30000 ppm HCD (2 studies, years 1995-2000) Males: 0/50 (0%) for both studies	M[1]	0/76	0/75 (104 mg/kg bw/day)	0/80 (354 mg/kg bw/day)		4/78#[a] (1127 mg/kg bw/day)	p = 0.001
886.C.C-R ([redacted] 1996) Wistar rats 0, 100, 1000 and 10000 ppm	M	0/50	0/30 (6 mg/kg bw/day)	0/32 (59 mg/kg bw/day)		0/50 (595 mg/kg bw/day)	Not analysed.
7867 ([redacted], 1993) Sprague-Dawley rats, 0, 10, 100, 300 and 1000 mg/kg bw/day	M	1/50	0/25 (10 mg/kg bw/day)	0/19 (100 mg/kg bw/day)	0/21 (300 mg/kg bw/day)	0/50 (1000 mg/kg bw/day)	Not analysed.
[redacted]-10495, ([redacted] [redacted] 1990) Sprague-Dawley rats, 0, 2000, 8000 and 20000 ppm	M	0/59	0/60 (89 mg/kg bw/day)	0/60 (362 mg/kg bw/day)		1/59 (940 mg/kg bw/day)	Not analysed.
77-2062 ([redacted], 1981) Sprague-Dawley rats, 0, 30, 100 and 300 ppm Study **not** acceptable	M	0/49	0/48	0/49		1/49	Not analysed.

#Includes one carcinoma;

[a] P (two-sided) for trend = 0.001 (for the extended Mantel-Haenszel test (stratified Cochran-Armitage trend), source: statistical re-analysis by external statistician upon AGG request).

Table 2.6.5.1-6b: Skin basal cell tumours in male rat – dose and incidence males

Study		[redacted] 1981	[redacted] 1990	[redacted] 1993	[redacted], 1997	[redacted] 1996	[redacted], 2001	[redacted] 2009
Duration		26m	**24 m**	**24m**	**24m**	**24m**	**24m**	**24m**
Sex Strain		Male SD[1]	**Male SD**	**Male SD**	**Male SD**	**Male W[2]**	**Male W**	**Male W**
Dose mg/kg bw/day	**NOAEL / LOAEL (systemic)**							
0		0/49	0/59	1/50	0/76	0/50	1/64	1/51
3.05		0/48						
6.3						0/30		
10.3		0/49						
11	NOAEL			0/25				
31.49		1/49						
59.4	NOAEL					0/32		
85.5								0/51
89	NOAEL		0/60					
104	NOAEL				0/75			
112	LOAEL			0/19				
121							0/64	
285.2	NOAEL							0/51
320				0/21				
354	LOAEL				0/80			
361	NOAEL						2/64[$]	
362	LOAEL		0/60					
595.2	LOAEL					0/50		
940			1/59					
1077.4	LOAEL							0/51
1127					4/78[$]			
1147				0/50				
1214	LOAEL						1/63	
Trend test p-value		Not analysed	Not analysed	Not analysed	0.001	Not analysed	Not analysed	Not analysed

[1] SD: Sprague-Dawley; [2] W: Wistar
$ Includes one carcinoma

7) Skin keratoacanthomas

This tumour type was not extensively discussed during the previous EU renewal of glyphosate and not all studies were further considered in an overall weight-of-evidence approach. The publication by Portier (2020), however, highlighted increased incidences of skin keratoacanthomas in male rats as evidence for carcinogenicity of glyphosate (refer to Vol 3 CA B.6.5.18.2). Therefore, this tumour type is further discussed below. The reported incidences of skin keratoacanthomas in male rats are provided by the RMS in table 2.6.5.1-7 and further discussed below.

Increased incidences of skin keratoacanthomas were observed in male rats in four studies ([redacted], 2009; [redacted], 1997; [redacted], 1993; [redacted], 1990) whereas no such finding was observed in two other studies ([redacted], 2001; [redacted], 1993).

7.1 Lines of evidence for skin keratoacanthomas – tumour incidences

- In the Sprague-Dawley rat study by [redacted] 1997 the following results were found in males:

- Comparison to control: at high dose of 1127 mg/kg bw/d, frequency of skin keratoacanthomas was increased versus control (9.0% versus 5.3%).
- Dose-response: the increased incidence of skin keratoacanthomas was only observed in the high dose group and the incidence at the mid dose was 0%.

- Comparison to historical control data (HCD): HCD (2 studies: 4% and 8%) were exceeded at the high dose.

- **In the Sprague-Dawley rat study by [redacted] 1993** the following results were found in males:
  - Comparison to control: at high dose of 1000 mg/kg bw/d, frequency of skin keratoacanthomas was increased versus control (10% versus 2%).
  - Dose-response: the increased incidence of skin keratoacanthomas was only observed in the high dose group; the incidence at the low dose was 8% and the incidences at the two mid dose groups were 0% (low mid and high mid dose). However, it should be noted that not all animals from low and mid and high mid dose levels were examined (25, 19 and 21 animals per group, respectively; only the animals that died during the study or that were killed in extremis were investigated in these groups). Therefore, a dose response is not interpretable.
  - Comparison to historical control data (HCD): HCD mean and range (13 studies: mean 0.7%, range 0-6.1%) were exceeded at the high dose.

- **In the Sprague-Dawley rat study by [redacted], 1990** the following results were found in males:
  - Comparison to control: at the low, mid and high doses of 89, 362 and 940 mg/kg bw/d, frequency of skin keratoacanthomas was increased versus control (5.0%, 6.7% and 8.5% respectively versus 1.7%).
  - Dose-response: increasing number of skin keratoacanthomas were found with increasing doses, following a monotonic, non-linear curve.
  - Comparison to historical control data (HCD): HCD (3 studies: 1/6, 1/5 and 0/2) were exceeded at all tested doses. These HCD incidences are reported as incidence of animals for which histopathological examination of skin lesions was performed. As it may be assumed that is it highly unlikely that any skin lesions (which might be skin keratoacanthomas) have been missed by the study pathologist, then an assumption of an overall historical control incidence of 1 case per study (50 animals generally) might be reasonable.

- **In Wistar rat study by [redacted], 2009** the following results were found in males:
  - Comparison to control: at high dose of 1077 mg/kg bw/d, frequency of skin keratoacanthomas was increased versus control (11.7% versus 3.9%).
  - Dose-response: the increased incidence of skin keratoacanthomas was only observed in the high dose group and the incidence at the mid dose was 0%.
  - Comparison to historical control data (HCD): no HCD are available. HCD were requested for the purpose of this renewal but applicant informed that the data have been discarded.

- **In Wistar rat study by [redacted], 2001** the following results were found in males:
  - Comparison to control: frequency of skin keratoacanthomas was not increased versus control up to the high dose of 1214 mg/kg bw/d (1.6%, 0%, 1.6% and 1.6% in the control, low, mid and high dose groups, respectively).
  - Dose-response: no dose response was observed.
  - Comparison to historical control data (HCD): no HCD were requested by AGG.

- **In Wistar rat study by [redacted], 1996** the following results were found in males:
  - Comparison to control: skin keratoacanthomas were not observed in any of the control and treated groups up to 595 mg/kg bw/d (0% in each group).
  - Dose-response: not applicable.
  - Comparison to historical control data (HCD): no HCD were requested by AGG.

Table 2.6.5.1-7: Skin keratoacanthomas in male rat – overview of tumour incidences per dose level (as number and (as %)) observed in the carcinogenicity studies

Study		[redacted], 1981; 77-2062	[redacted], 1990; [redacted]-10495	[redacted] 1993; 7867	[redacted] 1997; [redacted]94-0150	[redacted], 1996; 886.C.C-R	[redacted], 2001; [redacted]/PR1111	[redacted], 2009; 2060-0012
Duration		26 m	24 m	24m	24m	24m	24m	24m
Sex Strain		Male SD[1]	Male SD	Male SD	Male SD	Male W[2]	Male W	Male W
Dose mg/kg bw/day	**NOAEL / LOAEL (systemic)**							
0		0/49 (0%)	1/59 (1.7%)	1/50 (2.0%)	4/76 (5.3%)	0/50 (0%)	1/64 (1.6%)	2/51 (3.9%)
3.05		0/48 (0%)						
6.3						0/30 (0%)		
10.3		0/49 (0%)						
11	NOAEL			2/25 (8.0%) [6]				
31.49		0/49 (0%)						
59.4	NOAEL					0/32 (0%)		
85.5								3/51 (5.9%)
89	NOAEL		3/60 (5.0%)					
104	NOAEL				3/75 (4.0%)			
112	LOAEL			0/19 (0%) [6]				
121							0/64 (0%)	
285.2	NOAEL							0/51 (0%)
320				0/21 (0%) [6]				
354	LOAEL				0/80 (0%)			
361	NOAEL						1/64 (1.6%)	
362	LOAEL		4/60 (6.7%)					
595.2	LOAEL					0/50 (0%)		
940			5/59 (8.5%)					
1077.4	LOAEL							6/51 (11.8%)
1127					7/78 (9.0%)			
1147				5/50 (10%) [7]				
1214	LOAEL						1/63 (1.6%)	
Trend test p-value (two-sided; AGG analysis)		Not analysed, no trend.	p = 0.15	p = 0.07	p = 0.21	Not analysed, no trend.	Not available – 0.774 if	Not available –

						extrapolated from 1-sided test	p = 0.06 if extrapolated from 1-sided test
Trend test p-value (one-sided; Portier analysis)	Not analysed, no trend.	p = 0.042	p = 0.047	p = 0.029	Not analysed, no trend.	p = 0.387	p = 0.03
Historical control data	-[3]	3 studies, years 1986-1989: 1/6, 1/5 and 0/2 [4];	13 studies, years 1989-1995 overall mean 0.7%; range 0-6.1%	2 studies, years 1995-2000: 2/50 (4%) and 4/50 (8%)	-[3]	-[3]	-[5]

[1] SD: Sprague-Dawley; [2] W: Wistar; [3] HCD not requested; [4] Historical control data reported as incidence of animals for which histopathological examination of skin lesions was performed. As it may be assumed that is it highly unlikely that any skin lesion (which might be a skin keratoacanthoma) would have been missed by the study pathologist, then an assumption of an overall historical control incidence of 1 case per study (50 animals generally) seems reasonable.[5] HCD not available anymore; [6] Lower number of animals investigated. Only the animals that died during the study or that were killed in extremis were investigated according to the study authors.

7.2 Lines of evidence for skin keratoacanthomas – statistical analysis

Pairwise comparisons
In none of the studies the incidences were significantly increased in a pairwise comparison based on the statistical analysis available in the study reports (2-sided testing).

Trend analysis
The p-values for trend tests were performed one-sided by Portier, 2020 and AGG reports two-sided tests. For skin keratoacanthomas, the following p-values are reported:

- In Sprague-Dawley rat study by [redacted], 1997:

Statistical test for male rats	p-value (reference)	Comment
Trend test Cochran Armitage	1-sided: 0.029 (Portier, 2020) 2-sided: 0.21 (AGG analysis)[1]	1-sided: <0.05 statistically significant 2-sided: >0.05 not statistically significant

[1] Stratified Cochran-Armitage trend test performed by AGG

- In Sprague-Dawley rat study by [redacted], 1993

Statistical test for male rats	p-value (reference)	Comment
Trend test Cochran Armitage	1-sided: 0.047 (Portier, 2020) 2-sided: 0.07 (AGG analysis)[1]	1-sided: <0.05 statistically significant 2-sided: >0.05 not statistically significant but borderline

[1] Stratified Cochran-Armitage trend test performed by AGG
It should be noted that in the study by [redacted] (1993) not all animals from low and mid and high mid dose levels were examined (25, 19 and 21 animals per group, respectively; only the animals that died during the study or that were killed in extremis were investigated in these groups). Therefore, performing a trend test is questionable.

- In Sprague-Dawley rat study by [redacted], 1990

Statistical test for male rats	p-value (reference)	Comment
Trend test Cochran Armitage	1-sided: 0.042 (Portier, 2020) 2-sided: 0.15 (AGG analysis)[1]	1-sided: <0.05 statistically significant 2-sided: >0.05 not statistically significant

[1] Stratified Cochran-Armitage trend test performed by AGG

A statistical analysis was conducted considering the combined incidences of skin keratoacanthomas observed in the three studies using Sprague-Dawley rats, excluding the low/middle doses of the study by [redacted] (1993). Based on the combined data, a significant trend (p=0.014) was observed using the extended Mantel-Haenszel test (stratified Cochran-Armitage trend, two-sided). It is noteworthy that this analysis was performed without correcting or testing for differences in background incidences among studies and was only performed for Sprague-Dawley rats and not for Wistar rats.

- In Wistar rat study by [redacted], 2009

Statistical test for male rats	p-value (reference)	Comment
Trend test Cochran Armitage	1-sided: 0.03 (Portier, 2020) 2-sided: not available – 0.06 if extrapolated from 1-sided test	1-sided: <0.05 statistically significant 2-sided: >0.05 not statistically significant but borderline

- In Wistar rat study by [redacted], 2001

Statistical test for male rats	p-value (reference)	Comment
Trend test Cochran Armitage	1-sided: 0.387 (Portier, 2020) 2-sided: not available – 0.774 if extrapolated from 1-sided test	1-sided: >0.05 not statistically significant 2-sided: >0.05 not statistically significant

- In Wistar rat study by [redacted], 1996

Statistical test for male rats	p-value (reference)	Comment
Trend test Cochran Armitage	Statistical analysis not performed, no incidence of skin keratoacanthomas in any group.	-

7.3 Overall Weight of Evidence approach for relevance of skin keratoacanthomas

In four studies out of the six acceptable rat studies increased incidences were observed at the high dose (in all three studies in Sprague-Dawley rats and in one of the three studies in Wistar rats). Dose-response is shown in one of the these studies ([redacted], 1990), however, it should be noted that this was not linear with the three-fold stepwise increase in dose-levels. When available, HCD are exceeded. The incidences were not significantly increased in a pairwise comparison based on the statistical analysis available in the study reports. Regarding trend analysis, statistical significance was demonstrated using one-sided Cochran-Armitage tests (Portier, 2020), whereas two-sided Cochran-Armitage tests were either not statistically significant or borderline significant.

Study	Lines of evidence – tumour incidences	Lines of evidence – statistical analysis
[redacted] 1997 SD rats	Increased incidences versus control (at high dose by 1.7-fold); no dose-response; HCD exceeded.	Statistically significant 1-sided trend test; not statistically significant 2-sided trend test
[redacted] 1993 SD rats	Increased incidences versus control (at high dose by 5-fold); dose-response not interpretable (animals from low and mid dose levels not examined); HCD exceeded.	Statistically significant 1-sided trend test; 2-sided trend test not statistically significant, but borderline
[redacted] 1990 SD rats	Increased incidence versus control at all tested doses (by 3-fold, 4-fold and 5-fold at the low, mid and high doses respectively); dose-response, but non-linear; HCD exceeded at all doses.	Statistically significant 1-sided trend test; not statistically significant 2-sided trend test
[redacted] 2009 Wistar rats	Increased incidences versus control (at high dose by 3-fold); no dose-response; HCD unavailable.	Statistically significant 1-sided trend test; 2-sided trend test not statistically significant, but borderline
[redacted], 2001 Wistar rats	No increased incidence; no dose-response; HCD not requested.	Not statistically significant trend test
[redacted] 1996 Wistar rats	Not observed in any of the control and treated groups.	Not available

Depending on the statistical method applied, the increased frequencies were either non-significant, borderline or significant. However, it should be noted that when performing trend tests, in the case that effects only occur at the highest dose, it is in fact the high dose levels that trigger the statistical significance in a trend test. Further, in the AGG analysis on the relevance of skin keratoacanthomas, two-sided testing was applied as this is in line with how the statistical analysis was established in the study protocols of the available carcinogenicity studies (refer to the general comment on the statistical analysis above at section 2.6.5.1.1.3).

In addition, in an overall Weight of Evidence approach, not only statistical significance but also other factors should be considered. The skin keratoacanthomas were only observed in one species of one sex. In the carcinogenicity studies in mice, no skin keratoacanthomas were reported. In addition, none of the studies in rats reported increased incidences in females. In addition, the increased incidences in skin keratoacanthomas were only observed at very high dose rates, which slightly exceeded the maximum recommend dose rate according to the OECD GD. The only exception is the study by [redacted] (1990) in which a dose-response is seen which is, however, not linear with the three-fold increase in dose levels. The reported incidences of skin keratoacanthomas at the control, low, mid and high doses of 0, 89, 362 and 940 mg/kg bw/d were 1/59, 3/60, 4/60 and 5/59, respectively). There was no statistically significant trend by 2-sided testing, only by 1-sided testing. The pairwise comparison of the incidence at each dose level vs control by Fisher exact test did not result in statistically significant differences (2-sided testing).

Skin keratoacanthoma is a benign tumour which is rather common in aged male rats (Zwicker *et al.*, 1992)[6]. According to this publication, these tumours are in general first observed at an average age of 549 days (range of 303-702 days). In the rat studies with glyphosate, this tumour type was also reported after approximately 550 days (based on the available data for [redacted], 2009; [redacted] 1997 and [redacted], 1993), which is in agreement with the publication by Zwicker *et al*. (1992). The probable cell of origin is the squamous cell (Evans, 1997 and Mecklenburg,

[6] Zwicker, Eyster, Sells and Gass (1992); Spontaneous skin neoplasms in aged Sprague-Dawley ratsToxicol Pathol 1992;20(3 Pt 1):327-40. doi: 10.1177/019262339202000303.

2013[7]). On histologic section, the tumour appears as a crater- or flask-like invagination forming one or a few cystic spaces which are often filled with keratinaceous debris, which are connected to the exterior by a pore. The tumour involves both the dermis and epidermis. The keratoacanthomas are commonly seen accompanied by hyperkeratosis of the squamous epithelium. However, neither in the long-term studies nor in the other studies epithelial hyperkeratosis is reported (except for follicular hyperkeratosis as reported in the study by [redacted] as discussed in the previous section on skin basal cell tumours). Moreover, in the available studies no malignant squamous cell carcinomas were reported.

No plausible underlying mechanism is currently identified. In humans, this type of benign skin tumours is associated with multiple exposure to sunlight. Whereas in rats, which are most likely only exposed to artificial light, the cause of keratoacanthomas is unknown. However, traumas and genetic predisposition are factors that may contribute to the development of this type of skin tumour in rats. No explanation/rationale have been provided on the fact that increased incidences of keratoacanthomas were found following oral exposure. The relation with glyphosate exposure remains, therefore, unknown.

Overall, when considering that:
- The increased incidence in skin keratoacanthomas were observed at very high dose rates, which slightly exceeded the maximum recommend dose rate of 1000 mg/kg bw/day according to the OECD guideline. The only exception is the study by [redacted] (1990) in which an apparent dose-response is seen which, but not linear with the three-fold increase in dose levels.
- Even at this high dose rate (≥ 1000 mg/kg bw/day), it is still a relatively rare tumour with 6/51 (12%) as the highest incidence;
- In one study in Wistar rats ([redacted], 2001) at the same high lose level (1214 mg/kg bw/day) no increase in skin keratoacanthomas was seen;
- Although the incidences exceeded the background incidence (for which limited information is available for most of the studies), no statistically significant differences were observed (either by pairwise comparison or by trend analysis; 2-sided testing);

Together with the following factors:
- The skin keratoacanthomas were only observed in one species (rat) of one sex (males);
- The tumour is a benign tumour, which is rather common in aged male rats;
- No non-neoplastic precursor effects were observed; and
- No malignant squamous cell carcinomas were reported;

the RMS considers that the apparent increase in **skin keratoacanthomas is not of sufficient relevance for classification and labelling**.

[7] Evans MG, Cartwright ME, Sahota PS, Clifford CB.(1997). Proliferative lesions of the skin and adnexa of rats. ISI In: Guides for Toxicologic Pathology. STP/ARP/AFIP, Washington, CD
Mecklenburg (2013). Proliferative and Non-Proliferative Lesions of the Rat and Mouse Integument. J Toxicol Pathol. 2013; 26(3 Suppl): 27S–57S.

8) Malignant lymphoma in mice

Malignant lymphomas in mice were already extensively discussed during the previous renewal of glyphosate. In the current renewal the findings from the carcinogenicity studies were re-evaluated and an analysis of these findings in an overall weight of evidence approach is performed below.

8.1 Lines of Evidence for Malignant Lymphomas – tumour incidences

- In mouse study by [redacted], 2001, some additional elements in comparison to previous renewal allow further detailed evaluation of this study. For this study, an additional trend test (Peto analysis) was performed (by the applicant and by AGG) and updated historical control data (HCD) was provided request by AGG (HCD provided from 8 studies performed between 1996—2002). For this study, the following results were found:

For male mice:

- Comparison to control: at all dose levels the frequency of malignant lymphomas (ML) was increased versus control i.e., respectively 30%; 32%; and 38% for 15; 151; 1454 mg/kg/day versus 20% in the control group. It is noted that control presents a relatively high background level of ML, but increases at low, intermediate and high doses were 1.5-fold, 1.6-fold and 1.9-fold higher, respectively.
- Dose-response: increasing number of ML were found with increasing doses *i.e.*, following a monotonic, but non-linear curve, which might indicate a dose-response.
- Comparison to historical control data (HCD): HCD min—max range is 6—30% and the mean is 15.8%. In all groups, including the control group, the incidences are above mean ML HCD frequencies. The maximum HCD incidence is exceeded in the intermediate and high dose groups. It is noted that the updated HCD provided an identical range compared with the previous evaluation, however, the updated mean is slightly lower (new HCD mean 15.8%, previous mean HCD 18.4%).

For female mice:

- Comparison to control: there is slight increase of ML frequencies at 15 and 151 mg/kg/day versus control i.e., respectively 40% and 38% versus 36% in control. At high dose of 1467 mg/kg/day frequency of ML is 50% i.e., greater than 36% in control.
- Dose-response: there is no monotonic response with increasing doses for female rats.
- Comparison to historical control data (HCD): HCD min—max range is 14—58% and the mean is 33%. In contrast to male mice, for none of the tested doses in female mice the HCD is exceeded.

- In mouse study by [redacted] 2009, no HCD are available. HCD were requested for the purpose of this renewal but applicant informed that the data have been discarded. The following results were found:

For male mice:

- Comparison to control: at doses of 71; 234 and 810 mg/kg/day ML frequencies are respectively 2%; 4% and 10%, all exceeding control incidence (0%).
- Dose-response: there are increasing number of ML with increasing doses of glyphosate i.e., following a monotonic curve, hence dose-response is shown.
- Comparison to historical control data (HCD): no HCD available, however, considering the fact the ML are not rare tumours, the observed frequency of 0% in the control group is rather low.

For female mice:

There is neither exceedance of response from tested doses versus control nor clear dose-response (respectively 16%; 20% and 22% respectively for 98; 300 and 1081 mg/kg/day versus 22% for control).

- In mouse study by [redacted] 1997, details on historical control data have been provided for male mice upon request by AGG. The following results were found:

For male mice:

- Comparison to control: only the very high dose tested of 4348 mg/kg/day showed increased ML frequency versus control i.e., 12% versus 4%, which is 3-fold greater.

- Dose-response: no dose response is found with ML frequencies of 4%; 0% and 12% respectively for doses 165; 838 and 4348 mg/kg/day. It is noted that the gap between intermediate dose and high dose is very large.
- Comparison to historical data (HCD): HCD were provided for 7 studies. Over these 7 studies the min—max range is 3.8%—19.2% and the overall mean 7.0%. However, it is noted that 6 studies out of the 7 are all below 6% and only one has a much higher incidence, with a value of 19.2%. The latter study might be an outlier and if excluded the min—max range would be 3.8%—6% and the mean 4.92%. In this case, ML frequency of 12% from the high dose would exceed the maximum from HCD.

For female mice:

- Comparison to control: a slight increase is observed compared to control (12%) for intermediate (16%) and high (14%) doses, but not for low dose (8%).
- Dose-response: no dose-response is observed.
- Comparison to historical data (HCD): HCD were provided for 7 studies. For these 7 studies the min—max range is 7.8%—26.9% and the overall mean 15.7%.

- In mouse study by [redacted], 1989, the following results were found:

For male mice:

- Comparison to control: only comparison of the high dose versus control can be performed, with frequencies 6 out of 50 animals versus 4 out of 50 animals, respectively. It should be noted that not all animals from low and mid dose levels were examined (25 and 21 animals per group, respectively; only the animals that died during the study or that were killed in extremis were investigated in these groups), therefore no comparison can be made for these dose groups.
- Dose-response: there is no dose response.
- Comparison to historical data (HCD): no HCD requested by AGG.

For female mice:

- Comparison to control: only comparison of the high dose versus control can be performed, with frequencies 13 out of 50 animals versus 14 out of 50 animals, respectively. It should be noted that not all animals from low and mid dose levels were examined (34 and 24 animals per group, respectively; only the animals that died during the study or that were killed in extremis were investigated in these groups), therefore no comparison can be made for these dose groups.
- Dose-response: there is no dose response.
- Comparison to historical data (HCD): no HCD requested by AGG.

- In mice by [redacted], 1983, the following results were found:

The study by [redacted], 1983 (Report No. 77-2061) did not use the term malignant lymphoma in the description of the effects. However, malignant tumours in the lymphoreticular system were reported which do not show an effect up to a dose levels of 4841 mg/kg bw/day in males and females. If a more recent histopathological nomenclature would have been used, malignant lymphoma was covered by this data. There was no dose-response in either sex. No HCD was requested by AGG.

Table 2.6.5.1-8a: Malignant tumours in the lymphoreticular system ([redacted], 1983)

Type of tumour	Sex	Control	Low	Mid	High dose
Dose level		**0**	**1000 ppm**	**5000 ppm**	**30000 ppm**
Lymphoblastic lymphosarcoma with leukaemia	M	1	4	3	2
Lymphoblastic lymphosarcoma without leukaemia	M	0	1	0	0
Composite lymphosarcoma	M	1	0	1	0
Lymphoreticular neoplasms (total)	**M**	**2/48**	**5/49**	**4/50**	**2/49**

Lymphoblastic lymphosarcoma with leukaemia	F	1	4	5	1
Lymphoblastic lymphosarcoma without leukaemia	F	0	1	0	3
Composite lymphosarcoma	F	4	1	1	6
Granulocytic leukaemia [a]	F	0	3	0	0
Lymphoreticular neoplasms (total)	F	5/49	9/49	6/49	10/49

[a] it should be noted that granulocytic leukaemia are not lymphomas.

- In mouse study by [redacted], 1999, no historical control data are available and incidences are reported only for female mice. This study was reported in the 2016 evaluation by JMPR, however, as the study report is not available to the RMS, only very limited data is available for this finding. It should be noted that an extremely high top dose was applied in this study (8690 mg/kg bw/day).

For female mice:

- Comparison to control: the intermediate (8%) and high (12%) dose groups show greater incidences of ML versus control (6%).
- Dose-response: there is no dose response shown.
- Comparison to historical data (HCD): no HCD available.

Table 2.6.5.1-8b: Malignant lymphomas in male rat – overview of tumour incidences per dose level (as number and (as %)) observed in the carcinogenicity studies

Study		**[redacted], 1983** [a]	**[redacted], 1993**	**[redacted], 1997**	**[redacted], 2009**	**[redacted], 2001**
Duration		**24m**	**24m**	**18m**	**18m**	**18m**
Sex Strain		**Male CD-1**	**Male CD-1**	**Male CD-1**	**Male CD-1**	**Male Swiss**
Dose mg/kg bw/day	**NOAEL / LOAEL (systemic)**					
0		2/48 [a]	4/50	2/50	0/51	10/50
14.5						15/50
71.4					1/50	
98			2/25 [#]			
149.7						16/50
157	NOAEL	5/49 [a]				
165.0	NOAEL			2/50		
234.2					2/51	
297			1/21 [#]			
810	NOAEL				5/51	
814	LOAEL	4/50 [a]				
838.1	LOAEL			0/50		
988	NOAEL		6/50			
1454	NOAEL					19/50
4348				6/50		
4841		2/49 [a]				
Fisher's exact test (high dose vs control)	2-sided testing (CLH 2016)	Not available	p = 0.741[##]	p = 0.269	p = 0.056	p = 0.077
	1-sided testing (Portier 2020)	Not available	Not reported	Not reported	**0.01< p ≤0.05 (Portier, 2020)**	**0.01 < p ≤ 0.05 (Portier, 2020)**
Cochran-Armitage trend test	2-sided testing (CLH 2016)	Not available	p = 0.0760[##]	**p = 0.0085**	**p = 0.0037**	p = 0.0655; *p = 0.092 (Combined Peto test)*
	1-sided testing (Portier 2020)	p = 0.754	p = 0.087	**p = 0.016**	**p = 0.0007**	p = 0.064; ***p = 0.046 (Combined Peto test)***
Historical control data		- [1]	- [1]	7 studies, years 1993-1998) Males: mean 7.0%; range	- [2]	8 studies, years 1996-2002) Males: mean 15.8%;

				3.9-19.2%		range 6-30%

[a] Lymphoreticular neoplasms
[#] Limited number of animals investigated in the low and mid dose groups.
[##] Statistics in CLH report (2016) based on incidences / 50 animals per dose group, instead of the incidences/number of animals investigated.
[1] HCD not requested; [2] HCD not available anymore

Table 2.6.5.1-8c: Malignant lymphomas in female rat – overview of tumour incidences per dose level (as number and (as %)) observed in the carcinogenicity studies

Study		[redacted], 1983 [a]	[redacted], 1993	[redacted], 1997	[redacted], 2009	[redacted] 1999[1]	[redacted], 2001
Duration			**24m**	**18m**	**18m**		**18m**
Sex Strain			**Female CD-1**	**Female CD-1**	**Female CD-1**	**Female CD-1**	**Female Swiss**
Dose mg/kg bw/day	**NOAEL / LOAEL (systemic)**						
0		5/49	14/50	6/50	11/51	3/50	18/50
15.0							20/50
93.2						1/50	
97.9					8/51		
102			12/34 [#]				
151.2							19/50
153.2	NOAEL			4/50			
190	NOAEL	9/49 [b]					
298			9/24 [#]				
299.5					10/51		
786.8	LOAEL			8/50			
909						4/50	
955	LOAEL	6/49					
1000	NOAEL		13/50				
1081.2	NOAEL				11/51		
1466.8	NOAEL						25/50
4116				7/50			
5874		10/49					
8690						6/50	
Fisher's exact test (high dose vs control)	2-sided testing (CLH 2016)	Not available	p = 1.000	p = 1.000	p = 1.000	Not significant in a pairwise comparison (JMPR 2016)	p = 0.225

	1-sided testing (Portier 2020)	Not available	Not reported	Not reported	Not reported	Not significant in a pairwise comparison (JMPR 2016)	Not reported
Cochran-Armitage trend test	2-sided testing (CLH 2016)	Not available	p = 0.4831	p = 0.2971	p = 0.3590	Statistically significant in a trend test (JMPR 2016)	p = 0.068; *p = 0.174 (Combined Peto test)*
	1-sided testing (Portier 2020)	p = 0.070	p = 0.484	p = 0.294	p = 0.353	p = 0.050	p = 0.070 *p = 0.087 (Combined Peto test)*
Historical control data		- [2]	- [2]	- [2]	- [3]	- [3]	8 studies, years 1996-2002: Females: mean 33.0%; range 14-58%

[a] Lymphoreticular neoplasms; [b] including 3 cases of granulocytic leukaemia, which are not lymphomas.
[1] As reported by JMPR, study not available to RMS; [2] HCD not requested; [3] HCD not available anymore;
Limited number of animals investigated in the low and mid dose groups.
Statistics in CLH report (2016) based on incidences / 50 animals per dose group, instead of the incidences/number of animals investigated.

8.2 Lines of Evidence for Malignant Lymphomas – statistical analysis

For malignant lymphomas, the following p-values are reported:

- In mouse study [redacted], 2001:
For male mice, there are statistically significant results if one-sided testing is applied. For female mice, there are no statistically significant results.

Summarized statistics for [redacted] 2001

Statistical test for male mice	**p-value**	**Comment**
High dose versus control Fisher exact test	1-sided: 0.01<p≤ 0.05 (Portier, 2020) 2-sided: 0.077 (CLH, 2016)	1-sided: <0.05 statistically significant 2-sided: >0.05 not statistically significant but borderline
Trend test Cochran-Armitage	1-sided: 0.064 (Portier, 2020) 2-sided: 0.0655 (CLH, 2016)	1-sided: >0.05 not statistically significant but borderline 2-sided: >0.05 not statistically significant but borderline
Peto analysis	1-sided: 0.046 2-sided: 0.092 (AGG analysis, Vol 3 CA 6.5.12.2)	1-sided: < 0.05 statistically significant 2-sided: >0.05 not statistically significant
Statistical test for female mice	**p-value**	**Comment**
High dose versus control Fisher exact test	2-sided: 0.225 (CLH, 2016)	2-sided: > 0.05 not statistically significant
Trend test Cochran-Armitage	1-sided: 0.070 (Portier, 2020) 2-sided: 0.068 (CLH, 2016)	1-sided: >0.05 not statistically significant but borderline 2-sided: >0.05 not statistically significant but borderline
Peto analysis	1-sided: 0.087 2-sided: 0.174 (AGG analysis, Vol 3 CA 6.5.12.2)	1-sided: >0.05 not statistically significant 2-sided: >0.05 not statistically significant

A statistical re-assessment of the [redacted] study was performed by the applicant ([redacted], 2017; refer to Vol 3 CA B6.5.12.1) as some issues were identified in the statistical analysis performed in the original study report. [redacted] (2017) re-performed the Peto-analysis used in this study. A Peto-analysis is a sort of a trend analysis that takes into account differences in intercurrent mortality within dose groups. A more detailed explanation is given by AGG in Vol 3 CA B.6.5.12.2. Although the method applied by [redacted] (2017) is technically correct and could largely be reproduced by AGG, the re-analysis is not acceptable as some errors were noted in the tumours incidences used in the statistical calculations. In turn, AGG performed a new Peto-analysis based on corrected tumour incidences (refer to Vol 3 CA B.6.5.12.2 for details) and the resulting p-values from this analysis are given in the table above.

- In mouse study by [redacted], 2009:

For male mice, there are statistically significant results if one-sided testing is applied and on one occasion if two-sided testing is applied. For female mice, there are no statistically significant results.

Summarized statistics for [redacted], 2009

Statistical test for male mice	**p-value**	**Comment**
High dose versus control Fisher exact test	1-sided: 0.01<p≤0.05 (Portier, 2020) 2-sided: 0.056 (CLH, 2016)	1-sided: <0.05 statistically significant 2-sided: >0.05 not statistically significant but borderline
Trend test Cochran-Armitage	1-sided: 0.007 (Portier, 2020) 2-sided: 0.0037 (CLH, 2016)	1-sided: <0.05 statistically significant 2-sided: < 0.05 statistically significant
Statistical test for female mice	**p-value**	**Comment**
High dose versus control Fisher exact test	2-sided: 1.000 (CLH, 2016)	2-sided: >0.05 not statistically significant
Trend test Cochran-Armitage	1-sided: 0.353 (Portier, 2020) 2-sided: 0.3590 (CLH, 2016)	1-sided: >0.05 not statistically significant 2-sided: >0.05 not statistically significant

- In mouse study by [redacted], 1997:

For male mice, there is one statistically significant result. For female mice, there are no statistically significant results.

Summarized statistics for [redacted], 1997

Statistical test for male mice	**p-value**	**Comment**
High dose versus control Fisher exact test	2-sided: 0.269 (CLH, 2016)	2-sided >0.05 not statistically significant
Trend test Cochran-Armitage	1-sided: 0.016 (Portier, 2020) 2-sided: 0.0085 (CLH, 2016)	1-sided: <0.05 statistically significant 2-sided: <0.05 statistically significant
Statistical test for female mice	**p-value**	**Comment**
High dose versus control Fisher exact test	1.000 (CLH, 2016)	2-sided >0.05 not statistically significant
Trend test Cochran-Armitage	1-sided: 0.294 (Portier, 2020) 2-sided: 0.2971 (CLH, 2016)	1-sided: >0.05 not statistically significant 2-sided: >0.05 not statistically significant

- In mouse study by [redacted], 1993:

It should be noted that in the study by [redacted] (1993) not all animals from low and mid dose groups were examined only the animals that died during the study or that were killed in extremis were investigated in these groups).

Summarized statistics for [redacted], 1993

Statistical test for male mice	**p-value**	**Comment**
High dose versus control Fisher exact test	2-sided: 0.741 (CLH, 2016)	1-sided: >0.05 not statistically significant; compares 6/8 animals versus 4/4 animals
Trend test Cochran-Armitage	1-sided: 0.087 (Portier) 2-sided: 0.0760 (CLH, 2016)	1-sided: >0.05 not statistically significant 2-sided: >0.05 not statistically significant but borderline; limited number of animals low and mid groups
Statistical test for female mice	**p-value**	**Comment**
High dose versus control Fisher exact test	2-sided: 1.000 (CLH, 2016)	2-sided: >0.05 not statistically significant; compares 13/14 animals versus 14/14 animals
Trend test Cochran-Armitage	1-sided 0.484 (Portier, 2020) 2-sided 0.4831 (CLH, 2016)	1-sided: >0.05 not statistically significant 2-sided: >0.05 not statistically significant

- In mouse study by [redacted], 1983

Summarized statistics for [redacted], 1983

Statistical test for male mice	**p-value**	**Comment**
High dose versus control Fisher exact test	Not available	-
Trend test Cochran-Armitage	1-sided: 0.754 (Portier, 2020) 2-sided: not available – 1.000 if extrapolated from 1-sided test	1-sided: >0.05 not statistically significant 2-sided: >0.05 not statistically significant
Statistical test for female mice	**p-value**	**Comment**
High dose versus control Fisher exact test	Not available	-
Trend test Cochran-Armitage	1-sided: 0.070 (Portier, 2020) 2-sided: not available – 0.140 if extrapolated from 1-sided test	1-sided: >0.05 not statistically significant 2-sided: >0.05 not statistically significant

- In mouse study by [redacted], 1999:

Only data of females were reported, which provides limited or no evidence.

Statistical test for female mice	**p-value**	**Comment**
High dose versus control Fisher exact test	Not reported as a value (CLH, 2016)	Not statistically significant (JMPR 2016)
Trend test Cochran-Armitage	1-sided: 0.050 (Portier, 2020) 2-sided: Not reported as a value (CLH, 2016)	1-sided: ≤0.05 statistically significant 2-sided: <0.05 statistically significant (JMPR 2016)

8.3 Overall Weight of Evidence approach for relevance of malignant lymphomas

Malignant lymphomas are one of the most common neoplasms in mice, with generally higher incidences in females than in males. Four out of five studies were performed in CD-1 mice, whereas one study was performed in Swiss mice. In addition, a sixth study is available ([redacted], 1999), however, for this study in CD-1 mice only incidence data for females is available, but no study report. In Swiss mice, the background incidences of ML appear to be higher than in CD-1 mice.

In males, in three out of five studies, higher incidences are associated to glyphosate administration. Dose-response is shown in two out of the five studies ([redacted], 2001 and [redacted], 2009). When available HCD, are exceeded. A Peto analysis, which is a trend test that takes intercurrent mortality into account was performed for one study ([redacted], 2001), which did show a statistically significant increase, but only for 1-sided testing. A trend test gave significant results in 2 out of five studies, however, possibly driven by low control incidences and borderline significant results were obtained in two other studies.

For females, incidence data are available for five studies (and only limited information from a sixth study). There are no clear greater incidences and no dose-response shown. There are no statistically significant patterns.

Study	Tumor incidence	Statistical analysis
[redacted], 2001	For male: increased incidences versus control (all doses); dose-response; HCD exceeded For female: slight increase versus control; no dose-response; below HCD	For male: pairwise: 1-sided statistically significant, 2-sided not statistically significant, borderline. Trend test: not statistically significant, borderline (1- and 2-sided); statistically significant 1-sided in the newly Peto analysis For female: not statistically significant (1- and 2-sided)
[redacted], 2009	For male: increased incidences versus control (all doses); dose-response; HCD unavailable For female: no increase versus control; no dose-response; HCD unavailable	For male: statistically significant trend (1- and 2-sided), pairwise statistically significant (1-sided), 2-sided not statistically significant, borderline For female: not statistically significant (1- and 2-sided)
[redacted], 1997	For male: increased incidence versus control (at high dose by 3-fold); dose-response unclear; HCD exceeded if outlier study discarded For female: slight increase of high versus control; no dose-response	For male: statistically significant trend test (1-and 2-sided), For female: not statistically significant
[redacted], 1993	For male: only comparison high dose versus control neither increase nor dose-response For female: only comparison high dose versus control neither increase nor dose-response	For male: not statistically trend test 1-sided and 2-sided, but 2-sided borderline For female: not statistically significant
[redacted] 1983	For male and female: no increase versus control; no dose-response; HCD unavailable	For male and female: not statistically significant
[redacted], 1999	For female mice: increase at intermediate and high-doses; dose-response unclear; HCD unavailable	For female: statistically significant trend test (1-and 2-sided)

Depending on the statistical method applied, the increased frequencies were either non-significant, borderline or significant. In the AGG analysis on the relevance of malignant lymphomas in mice, two-sided testing is applied as this is in line with how the statistical analysis was established in the study protocols of the available carcinogenicity studies (refer to explanation above at section 2.6.5.1.1.3). Further, it should be noted that when performing trend tests, in the case that effects only occur at the highest dose, it is in fact the high dose levels that trigger the statistical significance in a trend test. This is the case for the studies by [redacted] (2009) and [redacted] (1997) as these studies showed statistically significant increases with dose for male CD-1 mice in the trend test but a rather low or even "zero" incidence in the control groups might be behind this finding. In addition, for the study in mice by [redacted] it should be noted that not all animals from low and mid dose groups were examined. In these dose groups, only the animals that died during the study or that were killed in extremis were investigated. Therefore, performing a trend test on this data is rather questionable.

In addition, as also indicated in OECD GD 116 and in the previous EU evaluation, statistical significance is not the only criteria to decide if an effect is treatment-related. For the assessment of the biological significance of these findings, it is important to consider that malignant lymphomas are among the most common spontaneously occurring neoplasms in the mouse. To allow for a final conclusion the biological significance of the observed tumour rate, the database as a whole in the species and the respective strains (i.e. historical control data on the background incidence of a given tumour type) and aspects such as dose selection and dose response should be considered. These points were already extensively discussed during the previous EU evaluation. In the current assessment no new findings were identified compared to the previous evaluation.

During the previous evaluation (CLH 2016), the background incidence in Swiss mice was extensively discussed ([redacted] study). The AGG has now received HCD of 8 studies (in total 400 untreated control animals per sex) instead of data from 5 studies during the previous evaluation. The RMS notes that the updated HCD has only slightly changed the mean value, but not the range.
During the previous evaluation (CLH 2016) the following was added considering the high background incidence of this tumour type in Swiss or Swiss-derived strains of mice and the possible role of oncogenic viruses: "*Nonetheless, it seems well in line with information that was found in the literature providing confirmation that Swiss mice are prone to developing lymphoreticular tumours. According to older articles, control incidences in male mice of Swiss or Swiss-derived strains may reach 18–27.5% and exceed 36% in females (Sher, 1974, Z22020; Roe and Tucker, 1974, ASB2015-2534; Tucker, 1979, Z83266). In a more recent publication, Tadesse-Heath et al. (2000, ASB2015-2535) even mentioned a nearly 50% lymphoma (mostly of B cell origin) incidence in a colony of CFW Swiss mice but also emphasised the contribution of widespread infections with murine oncogenic viruses to the high but remarkably variable incidence of tumours of the lymphoreticular system in this species. This problem is known for long and was often addressed in the past in textbooks of virology or mouse pathology. Already more than 30 years ago, Wogan and Pattengale (1984, ASB2016-889) described the contradictory situation as follows: "The role of oncogenic viruses in many hematopoietic tumours in mice is well established. Virtually all spontaneous or induced lymphomas which have been studied in mice contain oncogenic viruses. It is also recognized that oncogenic viruses and chemicals can act synergistically on cells in vitro and in vivo to cause tumour formation. This can be manifested by either increased incidence, decreased latency, or both. This raises the important issue as to whether a chemical which induces lymphoma in mice requires the presence of a murine oncogenic virus. If so, perhaps the induction of this tumour in mice would not be relevant to human carcinogenic risk. However, since it is possible that many other species, including man, carry undetected oncogenic virus which may act with chemicals to increase tumour burdens, considerations of viral carcinogenesis do not totally resolve the questions concerning the significance of mouse lymphoma in safety testing, except to point out that the prevalence of oncogenic viruses in mice may make them highly susceptible to the induction of lymphoma, leukaemia, and perhaps other neoplasms."*
No information is available on possible abundance of oncogenic viruses in the mouse colonies from which the animals used in the glyphosate studies were obtained. During a teleconference (TC 117) on carcinogenicity of glyphosate hold by EFSA (EFSA, 2015, ASB2015-12200), it was mentioned by an U.S. EPA observer that the [redacted] (2001, ASB2012-11491) study had been excluded from U.S. EPA evaluation due to the occurrence of viral infection that could influence survival as well as tumour incidences, especially those of lymphomas. However, in the study report itself, there was no evidence of health deterioration due to suspected viral infection and, thus, the actual basis of EPA's decision is not known." As no information is available on the possible abundance of oncogenic viruses in the mice colony that was used for carcinogenicity testing in the study by [redacted] and as there are no indications that the mice in this study had a suspected viral infection, it is not clear whether or not this could have had effect on the outcome of the study. In addition, it is noted that the survival among all dose groups was relatively low in this study (62% in the control group, 64% in the low dose, 58% in the mid dose and 53% in the high dose groups, both sexes combined). However, as the reason for this high mortality is not known and/or whether there is any relation with the suspected viral infection as discussed above, it is unclear whether or not this could have introduced any uncertainly in the findings related to malignant lymphomas.

As indicated above dose selection and dose response in the individual studies should be considered (refer to Table 2.6.5.1-8b/c). As already indicated in CLH report and adopted by RAC, the results between the studies are rather variable. In the studies by [redacted] (2009) and by [redacted] (1993) in CD-1 mice, comparable top doses of 810 or 1000 mg/kg bw/day were administered and a similar incidence of malignant lymphoma was noted in high dose males (5/51 or 6/50, respectively). However, the control group incidences were clearly different (0/51 vs. 4/50) resulting in a positive trend test in the study by [redacted] (2009) only. In the study by [redacted] (1997), which was also performed in CD-1 mice, a dose of 4348 mg/kg bw/day was applied as a maximum. The incidence in malignant lymphomas of 6/50 at the top dose level was similar to what was seen in the two studies mentioned before even though the applied dose was by four to five times higher. This is surprising since a more pronounced increase would be expected if it was a treatment-related effect. Whereas in another long-term study in CD-1 mice by [redacted] (1983) in which an even higher dose of 4841 mg/kg bw/day was fed without an increase in lymphoreticular tumours in general. It should be noted, however, that in this study malignant lymphoma was not mentioned as a

particular pathological entity but it can be reasonably assumed that such tumours have been reported as "lymphoreticular neoplasia" (refer to section above). Therefore, it was concluded in the previous CLH report (2016) that if all four studies in CD-1 mice are taken together, there is no consistent dose response. The current RMS agrees with this conclusion as no other new information that would change this conclusion was identified.

Considering the background incidence of malignant lymphoma in CD-1 mice, based on the concurrent control data and the historical control data it is noted that the incidence is higher in females than in males for both strains. In addition, the background incidence is lower in CD-1 mice than in Swiss mice (refer to the discussion above). For the studies in CD-1 mice reliable historical control data on malignant lymphoma incidence from the performing laboratories is available only for one of the three studies ([redacted], 1997). During the previous assessment (CLH 2016) a comparison with incidence data from the open literature or from industry databases has been made. However, this comparison should be made with caution.

In the previous CLH report (2016) is was overall concluded that "*On the balance, based on uncertainties with regard to partly contradictory study outcomes depending on the statistical method applied, inconsistent dose response in the individual studies, and a highly variable tumour incidence as suggested by historical control data, it is not likely that glyphosate has induced malignant lymphoma in mice. A possible role of oncogenic viruses should not be ignored. Moreover, human relevance of such an effect, if occurring only as a high-dose phenomenon as it was the case here, is considered equivocal."*
The current RMS agrees with the previous assessment and conclusions as outlined above. In the current assessment, the study by [redacted] (1999) has been added (study report not available to RMS). In this study, an apparent increase in malignant lymphoma was observed in female mice (6/50 versus 3/50) at a very high dose level of 50000 ppm (8690 mg/kg bw/day). However, as in the other studies in CD-1 females no increases in malignant lymphomas were observed, as the increase is only slight and as this finding occurred at a very high dose level, which is 8- to 9-fold higher than the maximum recommended dose level of 1000 mg/kg bw/day according to the OECD TG 453 (2009), this finding is considered of very limited relevance. The current assessment did not yield any other new findings that were not already taken into account during the previous assessment.

In addition, RAC (2017) concluded the following for the malignant lymphomas in CD-1 mice:

"No significant increases in malignant lymphomas were found in the mouse studies when assessed by the pairwise Fisher's exact test. However, in two of the five studies, a significant positive trend for malignant lymphoma incidences in males was reported. In two studies, increases were observed that were not statistically significant. In the fifth and oldest of the studies, the term malignant lymphoma was not used, but there was no statistically significant increase in lymphoreticular neoplasms reported in this study in response to glyphosate exposure. Thus, the lymphoma incidences in male mice show a slight, but clearly variable increase. Further, no increase in treatment related non-neoplastic lymph nodes were reported, thus supporting the conclusion that the tumours were of a spontaneous nature. The biological and human relevance of the findings is uncertain for the following reasons:

i) the maximum incidences were regarded to be within the historical control range for the CD-1 mice, although adequate historical control data were not available for all studies;

ii) the increases in malignant lymphoma incidences appeared to be confined to the high dose groups in the CD-1 mice;

iii) the incidence of malignant lymphomas is known to be related to the age of the animals. However, significant associations between exposure to glyphosate and induction of malignant lymphomas were not observed in the 24-month studies. Furthermore, there was no reduction in overall survival in the exposed groups;

iv) no parallel increases were observed in female CD-1 mice. It is known that female CD-1 mice are usually more prone to develop spontaneous malignant lymphoma than male mice (Son and Gopinath, 2004, ASB2015-2533). The lymphoma incidences were generally higher in females than in males, but no glyphosate related increases were seen in female CD-1 mice."

Based on the weight of evidence approach presented above, the RMS agrees that the biological and human relevance of the findings is uncertain. The RMS largely agrees with the above reasoning, however, it is added that only for one of the two studies showing a significant trend appropriate historical control data is available. Nevertheless, this is not considered to change the overall conclusion. More important is the fact that overall no dose-response concordance is seen between studies. The RMS further adds that, although an increase in malignant lymphoma incidence in females was seen in one 'new' study by [redacted] (1999; study report not available to RMS), this finding is not considered to change the conclusion. This is because the dose administered was extremely high (refer to discussion above) and it therefore not considered of relevance for the overall evaluation. No other new information was identified during the renewal evaluation which would change this conclusion.

To summarize:

- In three mouse studies, a slightly higher incidences in the rather common malignant lymphoma in males was seen [redacted], [redacted] and [redacted], 2001);
- [redacted] study: Swiss mice, overall no significant trend was observed and together with the high variability in the background incidence, the apparent increase in malignant lymphomas is not considered treatment-related;
- [redacted] and [redacted]: CD-1 mice an increase in malignant lymphomas. Only trend sign, but not the pairwise comparison;
- Only for one of the three studies in CD-1 mice HCD is available, showing that the incidence at the top dose level was within HCD range ([redacted], 1997);
- Two studies showed no increased incidences ([redacted] 1993 and [redacted], 1983);
- Variability in background incidence was shown based on the (limited) historical control data;
- The increases in malignant lymphoma incidences appeared to be confined to the high dose groups in the CD-1 mice which were around or above the OECD limit dose of 1000 mg/kg bw/day;
- No clear dose-effect concordance between studies was observed;
- The incidence of malignant lymphomas is known to be related to the age of the animals. However, significant associations between exposure to glyphosate and induction of malignant lymphomas were not observed in the 24-month studies. Furthermore, there was no reduction in overall survival in the exposed groups;
- No parallel increases were observed in female CD-1 mice. It is known that female CD-1 mice are usually more prone to develop spontaneous malignant lymphoma than male mice. The lymphoma incidences were generally higher in females than in males, but no glyphosate related increases were seen in female CD-1 mice;
- The study by [redacted] (1999; study report not available to RMS) in which an increased incidence in females was noted, is considered of limited relevance as the increase is only slight and as this finding occurred at a very high dose level, which is 8- to 9-fold higher than the maximum recommended dose level of 1000 mg/kg bw/day according to the OECD TG 453 (2009). Therefore, the previous conclusion that no parallel increases were reported in females remains;
- No increase in treatment-related non-neoplastic lymph nodes were reported, thus supporting the conclusion that the tumours were of a spontaneous nature;

Considering all the above arguments, the increased incidences malignant lymphomas in male CD-1 mice are not considered to be treatment-related when a weight of evidence approach was applied. The very different dose levels in all the studies and the dose-specific incidences were taken into account as well as the high variability in spontaneous occurrence of this tumour type together with the statistical uncertainties. In addition, the current assessment did not find any new information that would change the outcome of the previous evaluation.

9) Renal tubule tumours in male mice

In one of the mouse studies ([redacted], 1993; study report no. 77-2061) there was an increase in renal tubule adenoma and carcinomas in males (3/50 versus 1/49 in control). In the original study report all animals were reported to have renal tubule adenomas. However, according to the CLH report (2016) a re-evaluation of the histopathological slides by a Pathology Working Group (PWG) was conducted during the first evaluation of the study by EPA which concluded one adenoma and two carcinomas (PWG report not available to the RMS). An overview of the observed incidence is provided in the table below. Although the increase was not statistically significant by pairwise comparison, the effect was significant when a trend analysis was performed using Cochran-Armitage during the previous evaluation of glyphosate (refer to Table 2.6.5.1-9). The applicant provided a statement that historical control data are not available anymore.

Table 2.6.5.1-9a: Renal tumours in male CD-1 mice ([redacted], 1993), based on the original study report and the re-evaluation by PWG. Fisher's exact test was performed for the pairwise comparison (with p-value between brackets). A trend analysis was performed using Cochrane Armitage, with p-value in a separate row.

Dose (mg/kg bw/day)	N	Original report	Re-evaluation by PWG		
		Adenoma	Adenoma	Carcinoma	Combined
0	49	0	1	0	1
157	49	0 (1.000)	0 (1.000)	0 (1.000)	0 (1.000)
814	50	1 (1.000)	0 (0.495)	1 (1.000)	1 (1.000)
4841	50	3 (0.242)	1 (1.000)	2 (0.495)	3 (0.617)
Trend test (p-value		0.0080	0.2473	0.0370	0.0339

In the study by [redacted] (study report no. 94-0151; 1997) two renal tubule adenomas were observed at the high dose in males. Also for this study, the effect was significant when a trend analysis was performed using Cochran-Armitage, but not statistically significant by pairwise comparison (source CLH, 2016). The incidence at the top dose in this study is above the HCD mean and HCD range (mean 0.3%, range 0-2%; based on 7 studies performed between 1993-1998).

In the other two studies in CD-1 mice, no significant increase in renal tubule adenomas was observed in studies with top dose levels of roughly 800 and 1000 mg kg/bw day ([redacted], 2009, study report no. 2060-0011 and [redacted], 1993; study report no. 7793, respectively). The increase in renal tumour incidences in CD-1 mice was thus only observed in two studies with extremely high dose levels (4841 mg/kg bw/day and 4348 mg/kg bw/day), which is far above the recommended maximum dose of 1000 mg/kg bw/day according to OECD TG 453.

Regarding progression in malignancy, two carcinomas were observed in the study by [redacted], 1983; study report 77-2061) at the top dose and one in the mid dose; while the study by [redacted] (study report 7793, 1993) noted one carcinoma in both control and low dose. As also stated above it should be noted that it is difficult to distinguish between benign and malignant renal tumours and therefore the combined incidences are likely to represent the most accurate numbers.

No renal tubule tumours were observed in female CD-1 mice. No increase was reported in related preneoplastic lesions (renal tubular hyperplasia or necrosis) in male mice. In the study by [redacted] (1983), non-neoplastic kidney pathology in the form of chronic interstitial nephritis was reported to be increased, but is not considered to be a precursor for renal tubular cell adenoma.

In a study with another mice strain (Swiss mice, [redacted], 1997, report # Toxi 1559.CARCI-M), one renal tubule adenoma was observed in the mid dose and two adenomas in the high dose group males. According to the original study report the effect was not statistically significant by z-test. During the statistical evaluation conducted during the previous EU evaluation the effect was found to be statistically significant using a Cochran-Armitage trend test, but not with a pairwise comparison using the Fisher's Exact test (refer to Table 2.6.5.1-9b). The increase at the mid (3.8%; 1/26) and high dose (4.0%; 2/50) was above HCD mean, but within HCD range (mean 2.0%; range 0-6%, based on 8 studies using the same strain of mice, from the same lab, years 1996-2002). No concomitant non-neoplastic findings were observed in the kidney in males. No statistically significant increase in renal tumours nor non-neoplastic findings were seen in female Swiss albino mice.

Table 2.6.5.1-9b: Renal tumours in male mice – Overview of all mice studies

Report No.; Test species; Dose levels	Control	Low	Mid	High dose	Fisher's exact test (high dose vs control) / Cochran-Armitage trend test (Source: CLH 2016)
77-2061 ([redacted], 1983) CD-1 mice, 24-month study 0, 1000, 5000 and 30000 ppm HCD not available anymore	1/49	0/49 (157 mg/kg bw/d)	1/50[#] (814 mg/kg bw/d)	3/50[##] (4841 mg/kg bw/d)	For combined adenoma and carcinoma p = 0.617 / p = 0.0339
7793 [redacted]n, 1993) CD-1 mice, 24-month study 0, 100, 300 and 1000 mg/kg bw/day HCD not requested	2#/50	2#/50 (100 mg/kg bw/d)	0/50 (300 mg/kg bw/d)	0/50 (1000 mg/kg bw/d)	No significant increase
94-0151 ([redacted], 1997) CD-1 mice, 18-month study 0, 1600, 8000 and 40000 ppm HCD (7 studies, years 1993-1998) Males: 1/49 in one study, other six studies 0/50 or 0/52; mean 0.28%, range 0-2%	0/50 (0%)	0/50 (0%) (165 mg/kg bw/d)	0/50 (0%) (838 mg/kg bw/d)	2/50 (4%) (4348 mg/kg bw/d)	p = 0.495 / p = 0.0078
2060-0011 ([redacted], 2009) CD-1 mice 18-month study 0, 500, 1500 and 5000 ppm HCD not requested	0/51 (0%)	0/51 (0%) (71 mg/kg bw/d)	0/51 (0%) (234 mg/kg bw/d)	0/51 (0%) (810 mg/kg bw/d)	No significant increase
Toxi 1559.CARCI-M ([redacted], 2001) Swiss mice 18-month study 0, 100, 1000, 10000 ppm HCD (8 studies, years 1996-2002) Males: mean 2.0%; range 0-6%	0/50 (0%)	0/26 (0%) (15 mg/kg bw/d)	1/26 (3.8%) (151 mg/kg bw/d)	2/50 (4%) (1454 mg/kg bw/d)	p = 0.495 / p = 0.039

[#]Includes one carcinoma, [##]Includes two carcinomas

Table 2.6.5.1-9c: Renal tumours in male mice – dose and incidence

Study		[redacted], 1983	[redacted], 1993	[redacted], 1997	[redacted] 2009	[redacted], 2001
Duration		**24m**	**24m**	**18m**	**18m**	**18m**
Sex **Strain**		**Male** **CD-1**	**Male** **CD-1**	**Male** **CD-1**	**Male** **CD-1**	**Male** **Swiss**
Dose mg/kg bw/day	**NOAEL / LOAEL (systemic)**					
0		1/49	2/50[#]	0/50	0/51	0/50
14.5						0/26
71.4					0/51	
98			2/50[#]			
149.7						1/26

157	NOAEL	0/49				
165.0	NOAEL			0/50		
234.2					0/51	
297			0/50			
810	NOAEL				0/51	
814	LOAEL	1/50[#]				
838.1	LOAEL			0/50		
988	NOAEL		0/50			
1454	NOAEL					2/50
4348				2/50		
4841		3/50[##]				
Fisher's exact test (high dose vs control) / Cochran-Armitage trend test		For combined adenoma and carcinoma p = 0.617 / p = 0.0339	Not sign.	p = 0.495 / p = 0.0078	Not sign.	p = 0.495 / p = 0.039

#Includes one carcinoma, ##Includes two carcinomas

The two studies in CD-1 mice in which an increase in renal tubule tumours were seen ([redacted], 1983, study report no. 77-2061 and [redacted], 1997, study report no. 94-0151) used very high dose levels (>4000 mg/kg bw/day), which is well in excess of the limit dose for carcinogenicity testing as recommended by OECD guidance document 116. Since the other CD-1 mice studies were conducted at lower dose levels (up to 1000 mg/kg bw/day) the lack of an effect on renal tubules tumours in these studies cannot address the potential concern for a treatment-related effect (Table 2.6.5.1-9c). In the study in Swiss mice ([redacted], 1997, study report no. Toxi 1559.CARCI-M), a statistically significant trend was seen for renal tubular adenomas, however, as the incidence was within historical control range (0-6%) of this strain, this is therefore considered incidental and not treatment-related.

In the previous CLH report (2016) some argumentation was provided that the high dose levels (>4000 mg/kg bw/day) in the two studies in CD-1 mice may have exceeded the MTD:

'*Dose levels of >4000 mg/kg bw per day were well in excess of the limit dose for carcinogenicity testing (1000 mg/kg bw per day) as recommended by OECD guidance document 116. The OECD test guideline 451 for carcinogenicity studies does not give a precise recommendation but states that the highest dose level should elicit signs of minimal toxicity, with depression of body weight gain of less than 10%. However, in the studies by [redacted] et al. (1997, ASB2012-11493) and by [redacted] (1983, TOX9552381), however, the body weight gain in high dose males was decreased by more than 15% compared to controls. Mean terminal body weight of top dose males in the [redacted] (1983, TOX9552381) study was by 11% lower than in the controls. In addition, there were gastrointestinal signs and lesions in the first and a significant increase in central lobular hepatocyte hypertrophy and central lobular hepatocyte necrosis suggesting some liver toxicity in the second study (see Table 30). Of particular interest was the observation of some kidney pathology in the study by [redacted] (1983, TOX9552381). There was a positive trend for chronic interstitial necrosis in males with 12/50 affected in the high dose group versus 5/49 in the control. In females, there was a dose-related increase in proximal tubule epithelial basophilia and hypertrophy which were not seen among untreated control animals at all. Another finding in the urogenital tract in the same study was slight to mild urothelial hyperplasia in the bladder in mid and high dose males. The percentage of affected animals accounted for 6% in both the control and low dose groups but for 20% in the mid dose and for 16% in the high dose group. Even though there was no clear dose response, it may be assumed that glyphosate (acid) when administered at high doses might produce mucosal irritation. To conclude, there is some evidence that the MTD was exceeded in both studies at the highest dose level at which the number of tumour-bearing mice was slightly increased.'*

The current RMS agrees that the dose levels were quite high and seem to be near and possible beyond the maximum tolerable dose (MTD). Generally, a decrease in body weight gain of more than 15% and mean terminal body weight of 11% is not considered to be beyond the MTD by the RMS. However, in the study by [redacted] (1997, report no 94-0151) gastrointestinal effects occurred at the top dose consisting of cecum distention, anal prolapse, erosion/ulcer in the anus, regressive hyperplasia of mucous epithelium of the large intestine which may be due to the low pH of glyphosate as a decrease in urinary pH was also observed in the study. In the study by [redacted] (1983, report no 77-2061), liver central lobular hepatocyte hypertrophy as well as necrosis were observed. Moreover, the kidney showed chronic interstitial nephritis (in males) and proximal tubule epithelial basophilia and hypertrophy (in

females). These effect may also be due to the low pH of glyphosate although there is not sufficient evidence to support this.

In addition, in the previous CLH report (2016) is was stated that it cannot be clearly distinguished whether the small increase in a rare renal tumour in mice at exaggerated dose levels that have been applied for 18 or 24 months could be attributed to glyphosate itself and its toxicity, was due to long-lasting renal excretion of large amounts of an otherwise more or less inert substance or rather a chance event. The whole database, quantitative (dose) and mechanistic considerations as well as historical control data should be taken into account. During the previous assessment, a comparison has been made with historical control data from other studies, however, this is no longer considered appropriate.

As outlined above in the section on mutagenicity, a genotoxic mode of action of glyphosate is unlikely. Further, as already discussed previous CLH report (2016) the kidney is not a clear target organ and occurrence of non-neoplastic lesions in the kidney was confined to an exaggerated dose level in the study by [redacted] (1983) in mice (see paragraph above) and to papillary necrosis in a long-term study in male Wistar rats receiving 1200 mg/kg bw/day ([redacted], 2001). In other long-term study in rats ([redacted], 2009) a change in mineral deposition within the kidney was observed. This comprised a lower incidence of pelvic/papillary deposition in males and females; and an increased incidence in the corticomedullary deposition in females. In the long-term study by [redacted] (1997) an increased relative kidney weight was reported in females (11% increase compared with concurrent controls). The only kidney-related finding in the short-term studies was an increase in relative kidney weight in both sexes at dose levels far above the limit dose of 1000 mg/kg bw/day (3706 mg/kg bw/day in males and 4188 mg/kg bw/day in females; study report no. 434/016 (1996)). On the other hand, after oral absorption, glyphosate is chemically unchanged eliminated in the urine (see section on toxicokinetics and metabolism above) and glyphosate acid is a known irritant to the eyes (see section above). However, it is questionable if irritation would sufficiently explain tumour formation in the kidney.

The findings of renal tubule tumours were extensively discussed during the previous EU evaluation. The following arguments were made on why the effect should not be considered in the classification of glyphosate:

- The effects occurred at very high dose levels above the OECD-recommended limit of 1000 mg/kg bw/day and exceeding the MTD.
- If the whole database is taken into consideration it is clear that the top dose incidences are comparable to those observed in controls and low dose groups in the other studies or are only slightly higher (RMS: argument no longer considered appropriate as only HCD should be considered which is obtained from studies using the same strain of mice from the same test facility gathered from a period of 5 year as closely matching the period that the study was performed. Refer to RMS comment below)
- No pre-neoplastic kidney lesions were observed.
- There is no plausible mechanism.

In the RAC opinion (2017) the findings were summarized as follows:
" *Low, but elevated incidences of renal tumours were reported at the high doses exposures in three of the five mouse carcinogenicity studies (Table above). The increases in renal tumours were not statistically significant in pairwise comparisons (Fisher's exact test), but when the Cochran-Armitage trend-test was used, statistical significance was reported in these studies*."

" *All kidney tumours were observed at termination. No increase was reported in related preneoplastic lesions (renal tubular hyperplasia or necrosis) in male mice. In the study by [redacted] (1983), non-neoplastic kidney pathology in the form of chronic interstitial nephritis was reported to be increased, but is not considered to be a precursor for renal tubular cell adenoma*."

" *Renal adenomas and carcinomas are rare tumours in CD-1 mice. Spontaneous control incidences for CD-1 male mice obtained from [redacted] report a mean incidence of 0.24 and a range of 0-4% for adenoma and a mean incidence of 0.14 and a range of 0-2% for carcinoma from studies initiated between 1987 and 2000 ([redacted], 2005, ASB2007-5200). The incidences in the high dose CD-1 mice are at the upper end or slightly outside the control range for renal adenomas/carcinomas. Historical control data from the test facility (as cited in the EPA report, 2015) for the [redacted] (1983) study, had a range between 0 and 3.3%. No historical control data were available to RAC for renal tumours from the test facilities for the [redacted] (1997) or [redacted] (2001) studies."*
Note by RMS: this argument is no longer considered valid as only HCD should be considered which is obtained from studies using the same strain of mice from the same test facility gathered from a period of 5 year as closely matching the period that the study was performed". No appropriate HCD data is available for the [redacted] (1983) study, however, for [redacted] study (1997), valid HCD was provided. The incidence at the top dose

was outside HCD range in this study, however the relevance of this finding is limited as this dose was above the maximum recommended dose (according to OECD TG 453 (2009)). The increased incidence of renal adenomas in the study by [redacted] (2001) was within HCD range.

" *In two of the five studies, no renal tumours were reported at the two highest doses and in two studies, adenomas/carcinomas were reported in the control groups. Furthermore, no increase in renal tumours was reported in female mice. There was a positive trend in male mice, but the findings were not consistent across all studies. RAC notes that although the p-value determined in the trend test in the study by [redacted] (1997) indicated that the finding was statistically significant, there were only two adenomas among the 200 males examined in this study*."

" *In two of the three positive studies ([redacted]., 1997 and [redacted], 1983), increased tumour incidences were only observed at very high doses (>4000 mg/kg bw/d) at which the body weight gain in males were decreased compared to controls by up to 11% and 15% in the [redacted] (1983) and the [redacted] (1997) study, respectively. The OECD TG 451 for carcinogenicity studies does not give a precise top dose recommendation, but states that the highest dose level should elicit signs of minimal toxicity, with depression of body weight gain of less than 10%. RAC therefore gives less weight to the findings at these very high dose levels*".
In the RAC opinion (2017) the following was concluded:
'The human relevance of the renal tumours at very high doses is considered to be low and the overall evidence for the increase in renal tumours having been caused by glyphosate is considered insufficient for classification.'

The current RMS did not find any new information which would change the outcome of this conclusion.

10) Haemangiosarcoma in male mice / haemangioma in female mice

In two of the mice studies ([redacted], 1993; study report no 7793 and [redacted], 1997, study report no 94-0151) an increase in haemangiosarcoma was observed in high dose males. These effects were statistically significant using a trend test, but not with a pairwise comparison.

A full overview of the haemangiosarcomas observed in all CD-1 mice is provided in the tables below (Tables 2.6.5.1-10a and 10b). In the study with Swiss mice ([redacted], 2001; report number Toxi 1559.CARCI-M) no effect on haemangiosarcoma was observed in males with only two incidences in the mid dose group (for one animal in the liver and for another animal in the mesenteric lymph node).

Table 2.6.5.1-10a: haemangiosarcomas in male mice – Overview of all mice studies

Report No.; Test species; Dose levels	**Control**	**Low**	**Mid**	**High dose**	**Fisher's exact test (high dose vs control) / Cochran-Armitage trend test (Source: CLH 2016)**
77-2061 ([redacted], 1983) CD-1 mice 0, 1000, 5000 and 30000 ppm	0/48	0/49	1/50	0/49	Not analysed
7793 ([redacted], 1993) CD-1 mice, 0, 100, 300 and 1000 mg/kg bw/day	0/50	0/50	0/50	4/50 (8%)	p = 0.059 / p = 0.0004
94-0151 ([redacted], 1997) CD-1 mice, 0, 1600, 8000 and 40000 ppm	0/50	0/50	0/50	2/50 (4%)	p = 0.495 / p = 0.0078
2060-0011 ([redacted], 2009) CD-1 mice 0, 500, 1500 and 5000 ppm	2/51	1/51	2/51	1/51	Not analysed
Toxi 1559.CARCI-M ([redacted], 2001) Swiss mice 0, 100, 1000, 10000 ppm	0/50	0/20	2/28	0/50	Not analysed

Table 2.6.5.1-10b: haemangiosarcomas in male mice – dose and incidence

Study		[redacted], 1983	[redacted], 1993	[redacted], 1997	[redacted], 2009	[redacted], 2001
Duration		**24m**	**24m**	**18m**	**18m**	**18m**
Sex **Strain**		**Male** **CD-1**	**Male** **CD-1**	**Male** **CD-1**	**Male** **CD-1**	**Male** **Swiss**
Dose mg/kg bw/day	**NOAEL / LOAEL (systemic)**					
0		0/48	0/50	0/50	2/51	0/50
14.5						0/20
71.4					1/51	
98			0/50			
149.7						2/28
157	NOAEL	0/49				
165.0	NOAEL			0/50		
234.2					2/51	
297			0/50			
810	NOAEL				1/51	
814	LOAEL	1/50				
838.1	LOAEL			0/50		
988	NOAEL		4/50			
1454	NOAEL					0/50
4348				2/50		
4841		0/49				
Fisher's exact test (high dose vs control) / Cochran-Armitage trend test		Not analysed	p = 0.059 / p = 0.0004	p = 0.495 / p = 0.0078	Not analysed	Not analysed

Historical control data was provided for the study by [redacted], 1993 (6 studies terminated between September 1988 and September 1991) which showed a mean incidence of 3.3% with a range of 0-8%. Therefore, the reported incidence at the top dose was within the historical control range. No historical control data was available for the [redacted] study. Historical control data from [redacted] ([redacted], 2005 available online) showed haemangiosarcoma (whole body) in 8 out of 52 studies with a range of 1.67-12.00%. Moreover, the incidence reported in the [redacted] study is within the incidence reported in the control group of the study by [redacted] (study report number 2060-0011).

Overall, it is considered that the effect in males is unlikely to be treatment-related as the effect observed in the two studies were within HCD and no effect was observed in the other two studies with CD-1 mice nor in the study with Swiss mice. This conclusion is in line with the previous EU evaluation.

In females, a statistically significant trend for mesenteric lymph node haemangioma was seen in the study with Swiss mice ([redacted], 2001; report number Toxi 1559.CARCI-M) with 4/50 incidences at the top dose against 1/50 in the control group and none in the low and middle dose. This trend was also highlighted in the publication by Portier (2020). However, this apparent effect was not seen in the other carcinogenicity studies in female mice. As the increase in haemangioma at the top dose was only seen in females in one study, this finding is considered incidental and not treatment-related.

Overall conclusion tumour incidences rat and mouse studies

Refer to section 2.6.5.2 Comparison with the CLP criteria regarding carcinogenicity

2.6.5.1.2 Epidemiological studies

2.6.5.1.2.1 General introduction to the epidemiological studies

A number of epidemiology studies over the last decade have focused on pesticide exposure and associated health outcomes. Publications vary in the scope of their conclusions regarding either pesticides in general, certain classes of pesticides and in some cases individual insecticides, herbicides or fungicides. While some of these publications specifically mention glyphosate, others are focussed more on pesticide use in general. It is noted that also epidemiological studies beyond the scope of a 10 year literature search were included in the dossier in order to allow a comprehensive evaluation and to take all relevant data into account. Most studies were already discussed during the previous renewal of glyphosate and/or discussed in the CLH report (2016) or in the report by RAC (2017). It is further noted that the studies by Andreotti *et al.* 2018 (B.6.5.18.10) and Pahwa *et al*. (2019, B.6.5.18.8) are new public literature studies that have not been discussed before in the context of classification and labelling of glyphosate.

Epidemiological studies are the only source of information on carcinogenicity of glyphosate. However, one of the main difficulties is that it is not possible to distinguish between effects of the active substance glyphosate and its co-formulants since humans are always exposed to plant protection products and their residues, but hardly ever to the active substance alone. Furthermore, as humans are exposed to a great number of environmental chemicals, it is difficult to attribute health effects including cancer to directly to exposure to glyphosate.

When assessing and interpreting the relevance of the findings from epidemiological studies, an essential consideration is the exposure assessment. Any suggested association between health outcomes and possible exposure to an active substance may be speculative, if exposure cannot be confirmed and quantified.

The available epidemiological studies investigating the relation between exposure to glyphosate-based formulations and carcinogenicity outcomes can be divided in two categories: case-control studies and prospective cohort studies. In short, a case-control study is a study in which the investigators select persons with a certain type of cancer (‘cases’) and a control group of persons without this disease (‘controls’). Then the investigators look back in time to compare the exposure – in this case to glyphosate-based formulations – of the cases compared with controls. The outcome parameter is an odds ratio (OR). An OR represents the odds that an outcome will occur given a particular exposure, compared to the odds of the outcome occurring in the absence of that exposure. In this case, the OR can be used to determine whether a particular exposure to glyphosate is a risk factor for a certain cancer type. If the calculated OR = 1, then the exposure does not affect odds of outcome. If the OR < 1, the exposure is associated with a lower odds of outcome and if the OR > 1, the exposure is associated with higher odds of outcome. Together with the OR an 95% confidence interval (95%-CI) is provided. The 95%-CI is used to estimate the precision of the OR. A large 95%-CI indicates a low level of precision of the OR, whereas a small 95%-CI indicates a higher precision of the OR. It is important to note that an OR that is statistically significant does not include the value of ‘1’ in their confidence intervals. Conversely, OR confidence intervals (CIs) that include the value ‘1’ are not statistically significant.

Another type of epidemiological study used for investigating the possible relation between glyphosate exposure and certain types of cancer is a prospective cohort study. In this type of study, a large group of persons defined by different exposures to glyphosate is followed over time and the incidence of certain types of cancers is observed. By dividing the cumulative incidence among the group with a particular exposure to glyphosate by the cumulative incidence among a group without that exposure, a relative risk is calculated. Similar to an OR, a when an RR = 1 then the exposure is not associated with the outcome, if the RR is < 1, there is a lower risk associated with the outcome and if the RR >, there is a higher risk associated with the outcome. Considering the 95%-CI, the same applies as with the OR as discussed above.

With the design of epidemiological studies, several uncertainties should be taken into account. For example, confounding may occur. Confounding means the distortion of the association between the independent and dependent factor because a third factor is independently associated with both. A third factor might be a confounder if it is a) associated with the outcome independent of the exposure—that is, it must be an independent risk factor; and b) associated with the exposure but is not a consequence of it. A method for looking for confounding is to stratify the exposure–outcome association of interest by the third variable suspected to be a confounder[8]. The list of potential confounders should include the known risk factors for the disease of interest (e.g. family history, smoking status) and matching variables (age, sex, social-economic status). If confounding is identified, the next step is to control for or adjust for its distorting effect by using statistical methods. When assessing a study, it should be verified that potential confounding factors are appropriately identified and considered and it should be checked how it has been controlled for these potential confounders.

[8] Reference: https://www.cdc.gov/eis/field-epi-manual/chapters/analyze-Interpret-Data html

Moreover, other types of bias should be considered when assessing the reliability of a study. The main types of bias include selection bias, information bias (including recall bias and interviewer/observer bias) and confounding (already discussed above). Selection bias concerns a systematic error relating to validity that occurs as a result of the procedures and methods used to select subjects into the study, the way that subjects are lost from the study or otherwise influence continuing study participation (EFSA Journal 2017; 15(10):5007). Information bias concerns a systematic error when there are systematic differences in the way information regarding exposure or the health outcome are obtained from the different study groups that result in incorrect or otherwise erroneous information being obtained or measured with respect to one or more covariates being measured in the study. Information bias results in misclassification which in turn leads to incorrect categorisation with respect to either exposure or disease status and thus the potential for bias in any resulting epidemiological effect size measure such as an OR or RR (EFSA Journal 2017; 15(10):5007). Other types of bias may be selective reporting, publication bias and other biases (e.g. conflict of interest).

The RMS has evaluated all submitted epidemiological studies for their reliability using some of the recommendations made in the Scientific Opinion of the PPR Panel on the follow-up of the findings of the External Scientific Report 'Literature review of epidemiological studies linking exposure to pesticides and health effects' (EFSA Journal 2017; 15(10):5007). For each study, an assessment has been made on (refer to Volume 3, CA B6.5.18 section Long-term toxicity – public literature in the RMS commenting box):
- Study design and conduct: Was the study design appropriate to account for the expected distributions of the exposure and outcome, and population at risk? Was the study conducted primarily in a hypothesis generating or a hypothesis-testing mode?
- Population: Did the study sample the individuals of interest from a well-defined population? Did the study have adequate statistical power and precision to detect meaningful differences for outcomes between exposed and unexposed groups? Was there a potential for selection bias?
- Exposure assessment: Were the methods used for assessing exposure valid, reliable and adequate? Was a wide range of exposures examined? Was exposure assessed at quantitative level or in a categorical or dichotomous (e.g. ever vs never) manner? Was exposure assessed prospectively or retrospectively?
- Outcome assessment: Were the methods used for assessing outcomes valid, reliable and adequate? Was a standardised procedure used for collecting data on health outcomes? Were health outcomes ascertained independently from exposure status to avoid information bias?
- Confounder control: were potential confounding factors appropriately identified and considered? How were they controlled for? Were the methods used to document these factors valid, reliable and adequate?
- Statistical analysis: Did the study estimate quantitatively the independent effect of an exposure on a health outcome of interest? Were confounding factors appropriately controlled in the analyses of the data?
- Reporting: Is reporting adequate and transparent? Are key elements of the material and methods and results section are reported in sufficient details?

For each of the above parameters, a reliability score is given as high, moderate and low. Together, these study quality considerations are used for weighting the epidemiological studies and these may be used in the weight-of-evidence approach. Refer to Table 54 above for more detailed information on the studies including the limitations.

2.6.5.1.2.2 Summary of the epidemiological studies on glyphosate

The largest epidemiological study of pesticide exposure and health outcomes in the United States was the Agricultural Health Study (AHS) that also addressed and included glyphosate exposure. The Agricultural Health Study (AHS) is a prospective study of cancer and other health outcomes in a cohort of licensed pesticide applicators and their spouses from Iowa and North Carolina in the United States (https://aghealth.nih.gov). The AHS started in 1993 with the aim to investigate how agricultural, lifestyle and genetic factors affect the health of farming populations. Between 1993 and 1997, 52,394 licensed private pesticide applicators (mostly farmers) enrolled together with 32,345 of their spouses. In addition, the study also included 4,916 commercial pesticide applicators. Dozens of publications have resulted from data generated in this study of approximately 57000 farmers (applicators) and 32000 of their spouses.

De Roos *et al.* (2005, B.6.5.18.16) reported on AHS data evaluating glyphosate use and multiple cancer endpoints. No association was noted for glyphosate with all cancers, including cancer of the lung, oral cavity, colon, rectum, pancreas, kidney, bladder, prostate, melanoma, all lymphohematopoietic cancers, non-Hodgkin lymphoma (NHL) and leukemia. However, the study authors did report a potential association between glyphosate exposure and multiple myeloma (relative risk of 1.1 (0.5-2.4) when adjusted for age and 2.6 (0.7-9.4) when adjusted for multiple confounder (both non-significant) although this was not significant and based on a small number of cases (n=32 for analyses without exposure-day metrics and n=19 for adjusted analyses of exposure-day metrics). Therefore, the authors concluded that the results should be followed up as more cases occur in the AHS.

Blair and Freeman (2009, B.6.5.18.14) provided an overview of cancer endpoints associated with different agricultural chemicals reported in earlier AHS publications. Glyphosate was only mentioned once in the study report that future attention should be given to a possible link between glyphosate exposure and multiple myeloma which is likely based on the report by de Roos *et al.*, 2005.

Sorahan *et al*. (2015, B.6.5.18.12) calculated the relative risk (RR) estimates for exposed and non-exposed applicators using Poisson regression based on the AHS database and, unlike the study by De Roos *et al.*, 2005 subjects with missing data were not excluded from the main analyses. When using the full dataset adjusted for age and gender, the analysis produced a RR for multiple myeloma which was close to unity for ever-use of glyphosate (RR 1.08, 95%-CI 0.48 to 2.41). Additional adjustment for lifestyle factors and use of ten other pesticides had little effect. To conclude, this study found no statistically significant trends of multiple myeloma risk. This was irrespective of whether the analyses had adjustment for a few basic variables (age and gender) or adjustment for many other lifestyle factors or pesticide exposures, as long as data on all available pesticide applicators were used.

Andreotti *et al*. 2018 (B.6.5.18.10) updated the 2005 AHS publication by DeRoos *et al*. (2005), extending cancer incidence follow-up through 2012 in North Carolina and 2013 in Iowa and incorporating additional exposure information from a follow-up questionnaire. The authors also dealt with missing information through imputation and conducted sensitivity analyses to address the potential for various types of bias in their primary analyses. This 2018 publication includes a total of 7,290 cancers, 3.6 times as many as in the earlier publication by De Roos (2005). The median lifetime days of glyphosate use for cohort members who reported glyphosate use (83% of the cohort) was 48 days (interquartile range (IQR) 20 to 166 days). The authors found no evidence of an association between glyphosate use and risk of any solid tumour, non-Hodgkin lymphoma (NHL) (RR 0.87 (95%-CI 0.64-1.20 in the highest intensity weighted exposure quartile, p_{trend}=0.95), or multiple myeloma (RR 0.87, 95%-CI 0.45-1.69 for highest quartile, p_{trend} 0.84). The study did find an elevated RR for acute myeloid leukaemia in the highest quartile of exposure (RR: 2.44, 95%-CI 0.94-6.32, p_{trend}=0.11). The effect was not statistically significant, although the RR was significant for the highest tertile of exposure when a 20-year lag period was taken into account (RR 2.04, 95%-CI 1.05-3.97). It should be noted that a low number of cases was included in this subgroup (n = 15). **As reported by the study authors an association between glyphosate exposure and acute myeloid leukaemia has not been previously reported in other epidemiological studies and merits further evaluation**. Further, also for non-Hodgkin lymphoma of T cell subtype (NHL) an elevated risk ratio was found for the 20-year lagged exposure (NHL: RR of 2.97, 95%-CI 1.2-7.31). However, it should be noted that also for this tumour type a low number of cases was included in this subgroup (n = 9), therefore this finding is of limited value. The lack of statistically significant findings for other cancer types were consistent across different exposure metrics, in various sensitivity analyses, and for lagged exposure analyses meant to address cancer induction-latency.

Overall, the studies based on the AHS data do not provide a clear indication that glyphosate exposure is associated with cancer although the finding in the most recent update (Andreotti, 2018) of a possible association with acute myeloid leukaemia should be looked at carefully in future updates. It should be noted that a high number of cancer sites were analysed so there is the possibility of statistical findings by chance.

Further, it should be noted that NHL is not a specific disease but a broad spectrum of disorders more correctly referred to as lymphocytic lymphomas, each with possible different aetiologies. They are all classified as not being Hodgkin lymphoma, and the terminology has changed over the years - some lymphomas are described differently today compared to previously. This complicates the evaluation of the studies.

Besides the AHS study, also a number of other epidemiological studies are available in literature. These were all case-control studies. Most studies focussed on lymphoid neoplasms (mainly NHL), but also other types of cancer were investigated. The studies on lymphoid neoplasms are presented first, in chronological order. Then the studies investigating other neoplasms are discussed. Please refer to Table 54 above for more detailed information on the studies and the reliability assessment.

Hardell and Eriksson (1999, B.6.5.18.20) investigated in a case-control study the incidence of NHL in relation to pesticide exposure in Sweden. 404 cases and 741 controls have been included. The authors discussed an increased risk for NHL especially for phenoxyacetic acids. Glyphosate was included in the univariate and multi-variate analyses. However, only 7 of 1145 subjects in the study gave exposure histories to this agent. The authors reported a moderately elevated odds ratio (OR) of 2.3 for glyphosate. This OR was not statistically significant and was based on only 4 “exposed” cases and 3 “exposed” controls making the reliability of the study very low.

A further study was submitted by Hardell *et al*. (2002, B.6.5.18.21). This study pools data from the above mentioned publication by Hardell and Eriksson (1999, B.6.5.18.20) with data from a previously submitted publication from Nordström *et al*. (1998, B.6.5.18.25). The authors found increased risks in a univariate analysis for subjects exposed to herbicides, insecticides, fungicides and impregnating agents. Among herbicides, significant associations were found for glyphosate and MCPA. However, in multivariate analyses, the only significantly increased risk was found with a heterogenous category of “other herbicides” and not for glyphosate. A limitation of the study was that no adjustments were made for confounders such as medical history, lifestyle factors (e.g. smoking, use of prescribed

drugs etc.) and exposure to other pesticides. In all, the same limitation of the publication of Hardell and Eriksson (1999) is also applicable to the publication by Hardell *et al*. (2002) as the study only had 8 exposed glyphosate cases and 8 exposed controls.

McDuffie *et al*. (2001, B.6.5.18.23) mentioned a non-significant positive association between self-reported glyphosate exposure and NHL in a Canadian study. The adjusted OR for any reported glyphosate use was not statistically elevated with an OR of 1.2 (95%-CI 0.8-1.7). Analysis for glyphosate use by days of use did show a significantly elevated OR for >2 days of exposure/year with an OR of 2.1 (95%-CI 1.20-3.73). However, it is noted that no adjustment for confounders were made in the latter analysis (except for age and province of residence).

De Roos *et al*. (2003, B.6.5.18.15) reported an association between NHL and glyphosate use (OR first stage logistic regression of 2.1 (95%-CI 1.1-4.0), OR second stage hierarchical regression of 1.6 (95%-CI 0.9-2.8). The study was considered reliable with restriction, however, a main limitation is that cases with data missing information for any of the 47 pesticides were excluded (in contrast to the re-analysis of the data set in Pahwa, 2019). In addition, there was a fairly high number of proxy respondents (40% of cases, 31% of controls).

Fritschi *et al*. (2005, B.6.5.18.19) submitted a case-control study with 694 cases of NHL and 694 controls in Australia. Substantial exposure to any pesticide was associated with an increase of NHL. However, no association between NHL and glyphosate can be made on basis of this study as only combined pesticide exposure was addressed and not specific glyphosate exposure. Therefore, this report is not considered to provide reliable information for glyphosate exposure.

Eriksson *et al*. (2008, B.6.5.18.17) reported a case-control study investigating exposure to pesticides as risk factor for NHL which included 910 cases and 1016 controls living in Sweden. The highest risk was calculated for exposure to MCPA. Glyphosate exposure was reported by 29 cases and 18 controls, and the corresponding odds ratio (OR) was 2.02 (95%-CI 1.10-3.71) when adjusting for age, sex and year of diagnosis/enrollment. However, the multivariate analysis (which adjusted for use of other specific pesticides) resulted in a lower and not significant OR of 1.51 (95%-CI 0.77-2.94), which is indicative of confounding of the glyphosate/NHL association. The association between glyphosate and NHL was stratified by median days of use for controls (≤ 10 days, > 10 days). ORs were 1.69 (95%-CI 0.70-4.07) for 10 days or less and 2.36 (95%-CI 1.04-5.37) for more than 10 days of use. When considering latency periods, higher ORs were observed with a latency period of >10 years (OR of 2.26 with 95%-CI of 1.16-4.40). It is noted that no multivariate analysis (which adjusted for use of other specific pesticides) was conducted on these analyses of glyphosate exposure by days or by latency period. Several other limitations were noted to the study. Cases who enrolled were referred by their physician and therefore referral bias may have occurred. There also appears to be a high likelihood for recall bias in the study as high ORs were observed for virtually all pesticides evaluated. In addition, adjustments for certain confounders appear to be lacking including lifestyle factors, medical history including the occurrence of NHL in first degree relatives and use of other pesticides. This is particular a concern for this study as the unexposed group consisted of subjects unexposed to all pesticides which can be expected to lead to differences between the groups based on other covariates. Due to the potential for recall bias, lack of adjustment for confounders and the limitations noted in the statistical analysis, this study is considered of low reliability.

Orsi *et al.* (2009, B.6.5.18.26) also investigated the effect of pesticides on lymphoid neoplasms. No effect on NHL was observed, while slight elevated but not statistically significant increased ORs were observed for Hodgkin lymphoma (OR 1.7 95%-CI 0.6-5.0) and multiple myeloma (OR 2.4, 95%-CI 0.8-7.3). However, the number of exposed cases were very low (6 for Hodgkin lymphoma and 5 for multiple myeloma). Moreover, there were issues with the exposure assessment conducted in the study and repeat interviews needed to be conducted because the initial information was insufficient. It was also indicated that when information on pesticides exposure was missing expert had to allocate a list of chemicals that might have been used. It is not reported how often this was the case. Based on these limitation the study was concluded to be of low reliability.

Alavanja *et al*. (2013, B.6.5.18.13) reviewed studies on cancer burden among pesticide applicators and others due to pesticide exposure. In this article, the epidemiological, molecular biology, and toxicological evidence emerging from recent literature assessing the link between specific pesticides and several cancers including prostate cancer, NHL, leukaemia, multiple myeloma, and breast cancer were integrated. Glyphosate was reported to be the most commonly used conventional pesticide active substance worldwide. However, the only association between the use of glyphosate and cancer burden mentioned in this review was the observation of Eriksson *et al*. (2008, see above).

Schinasi and Leon (2014, Vol 3 B.6.5.18.28) published the results of a meta-analysis of six epidemiological studies on the relationship between non-Hodgkin lymphoma (NHL) and occupational exposure to pesticides (based on McDuffie *et al.*, 2001, Hardell *et al.*, 2002, DeRoos *et al.*, 2003, Eriksson *et al.*, 2008 and Orsi *et al.*, 2009). Phenoxy

herbicides, carbamate insecticides, organophosphorus insecticides and lindane were positively associated with NHL. For glyphosate, they calculated an increased meta relative risk (mRR) of 1.5 (95%- CI 1.1- 2.0) for one day or more of use in a lifetime. However, there were data extraction errors by Schinasi & Leon that were identified in a subsequent meta-analysis by IARC working groups and by Chang and Delzell (2016)**. For the process under Regulation (EC) No 1107/2009, the applicant is requested to provide the study by Chang and Delzell and an assessment. For the process under the Regulation (EC) No 1272/2008, the applicant is asked to submit the missing information during the public consultation period**. When the calculations were replicated after considering the adjusted estimates of two Swedish studies (Hardell *et al.*, 2002 and Eriksson *et al.*, 2008) in the meta-analysis, a meta-RR of 1.3 (1.03 - 1.65) was identified. The meta-RR - the result of the meta-analysis - appears to show a very moderate effect. Two additional meta-analyses of epidemiological studies are available (Zhang *et al.* (2019)[9] and Leon *et al.* (2019)[10]). The applicant has submitted these studies, however, no extensive study summary has been provided as these were considered supplementary studies by the applicant. **For the process under Regulation (EC) No 1107/2009, the applicant is requested to provide a further assessment of these studies (a more extensive summary) and to include these studies in the overall assessment of epidemiological studies in Volume 1. For the process under the Regulation (EC) No 1272/2008, the applicant is asked to submit the missing information during the public consultation period.**

Pahwa *et al*. (2019, B.6.5.18.8) performed a pooled re-analysis of the data from two published non-Hodgkin lymphoma (NHL) case control studies (McDuffie *et al*. 2001 and DeRoos *et al*. 2003). The re-analysis sought to evaluate associations for glyphosate use and NHL overall and by histological sub-type. In addition, the pooled analysis implemented more extensive control of confounding factors than in the original publications and considered the impact of excluding pesticide information provided by next-of-kin or proxy respondents. The OR for NHL overall for ever using glyphosate was 1.4 (95%-CI 1.1-1.8). After adjustment for use of other pesticides, this OR was reduced to 1.1 (95%-CI 0.8-1.5). For diffuse large B-cell lymphoma (DLBCL) findings were similar. The OR for DLBCL for ever using glyphosate was 1.6 (95%-CI 1.1-2.3), whereas after adjustment for use of other pesticides the association was no longer significant (OR decreased to 1.2 with 95%-CI 0.8-1.9). For other NHL subtypes, consistent patterns of association across exposure metrics were not seen, with the possible exception of small lymphocytic lymphoma (SLL), though SLL findings were not statistically significant. In general, exclusion of proxy respondents reduced ORs to a minor extent with the exception of the analyses for other NHL subtypes, however all associations were not significant. For NHL overall, analyses that considered duration of use per se or lifetime days of use did not show a relationship with glyphosate. **However, there was a moderate association seen, which was borderline significant, between NHL overall cases and glyphosate use for the metric > 2 days/year (OR 1.7, 95%-CI 1.02-2.94). In addition, handling glyphosate during >2 days/year had an excess of diffuse large B-cell lymphoma (DLBCL) (OR 2.14, 95%-CI 1.1–4.3).** It should be noted, however, that the analyses by days per year (≤/> 2 days/year) included only 50% of the pooled population – essentially the Canadian subjects and one of the four US case-control studies. For NHL overall and DLBCL, only 30 and 14 cases were included with >2 days of glyphosate use, respectively. In addition, no trend in ORs were seen when cases with 0 to ≤ 2 days of glyphosate exposure were compared with cases with >2 days of glyphosate were compared (for NHL overall and for DLBCL both the P-value for trend is 0.2). Further, as only a small number of cases is included in this sub-analysis, it is uncertain how representative these results are for the entire pooled population. The results of those analyses should be interpreted accordingly.

With regard to multiple myeloma Presutti *et al*. (2016, B.6.5.18.11) analysed a subset of three NAPP studies (Iowa, Nebraska and Canada) where multiple myeloma (MM) cases were recruited. Self-reported information on pesticide use, farming activities and demographic characteristics was collected and the odds ratios (OR) were calculated for "ever/never" exposure, years of exposure (less or more than three years) and cumulated lifetime days of exposure (less or more than 6 lifetime days of exposure) to glyphosate with and without exclusion of proxy respondents. The result is that no statistically significant increases in risk of multiple myeloma (MM) associated with self-reported exposure to glyphosate were observed. This publication was considered to be acceptable but with restrictions because confounding factors such as exposure to other pesticides, chemical or radiation as well as occurrence of multiple myeloma in first degree relatives were not taken into account.

With regard to glioma, Lee *et al*. (2005, B.6.5.8.22) reported a glyphosate association with primary adult gliomas, with the odds ratio differing between self-respondents (OR = 0.4, 95%-CI 0.1-16) and proxy respondents i.e. spouses

[9] Zhang et al. (2019). Exposure to glyphosate-based herbicides and risk for non-Hodgkin lymphoma: a meta-analysis and supporting evidence. Mutation Research/Reviews in Mutation Research 781:186-206. doi:10.1016/j mrrev.2019.02.001.

[10] Leon et al. (2019). Pesticide use and risk of non-Hodgkin lymphoid malignancies in agricultural cohorts from France, Norway, and the USA: a pooled analysis from the AGRICOH consortium. Int J. Epidemiol. 48(5):1519-1535. doi:10.1093/ije/dyz017

or first-degree relatives (OR = 3.1, 95% CI 1.2-8.2). Glioma is a type of tumour that originates in the glial cells of the brain or the spine. The authors expressed their concern about higher positive associations observed for proxy-respondents with glyphosate and several other pesticides. They suggested perhaps more accurate reporting of proxies for cases and underreporting by proxies for controls. The RMS agrees that this may come from either information bias (knowing status of the self-respondents) or/and exaggerated exposure response in proxy group or/and lower accurate reporting in self-respondent group in comparison to the proxy-group. However, another interpretation could be that reaching proxies may not result in an actual bias but simply their attention was caught and a more accurate reporting from proxy group occurred i.e., whilst self-respondent exposure was genuinely underestimated and proxy reply was correct. In theory, and since this study is reliable and since adjustments seem to have been adequately included study authors should have undertaken further investigation.

With regard to childhood leukaemia Monge *et al*. (2007, B.6.5.18.24) investigated associations between parental pesticide exposures and childhood leukaemia in Costa Rica. Results are not interpretable for glyphosate as exposure was estimated with "other pesticides", including paraquat, chlorothalonil and "others". No association was noted for paternal exposures, but elevated incidence of leukaemias was associated with maternal exposures to "other pesticides" during pregnancy. However, the study showed a high potential for recall bias as the ORs for virtually all pesticide groups were higher than one.

One of the limitations of case-control studies is the potential for recall bias. The purpose of the analysis by Crump (2019, B.6.5.18.5) was to evaluate the evidence for recall bias in the overall pattern of results in five case control studies and two cohort studies that comprise the main part of the glyphosate-NHL literature. In evaluating the case control studies, Crump reasoned that the percentage of odds ratios > 1 for non-glyphosate exposures should be approximately 50% if recall bias was not operative and those exposures did not cause NHL. Yet, it turned out that the percentages of ORs >1 for non-glyphosate exposures were 90% for Hardell *et al*. (2002), 90% for Erikson *et al*. (2008), 93% for McDuffie *et al*. (2001), 76% for Orsi *et al*. (2009), and 53% for DeRoos *et al*. (2003). These extreme departures from 50% for 4 of the 5 case control studies is consistent with recall bias, perhaps augmented by a type of selection bias in the analyses by Hardell *et al*. (2002) and Eriksson *et al*. (2008). In contrast, in the most recent publication from the Agricultural Health Study (Andreotti *et al*. 2018), only 48% of the relative risks (RR) calculated were >1 – a percentage in the range expected with a true probability of 50%. While the evaluation of Andreotti *et al*. (2018) concerned glyphosate and other cancer sites and not to other exposures and NHL, the principle is the same: under the null hypothesis the proportion of ORs or RRs > 1 should be roughly 50% if bias is absent. Based on the high percentage of ORs above 1 it seems that recall bias may have played a factor in a number of the case-control studies.

The following epidemiological studies **did not reveal an association** between glyphosate and specific cancer types. Brief summaries are provided for these publications in Volume 3 CA Section B.6.5.18.27.

- Alavanja *et al*. (2003) reported on prostate cancer associations with specific pesticide exposures in the AHS prospective cohort study; glyphosate did not demonstrate a significant exposure-response association with prostate cancer.
- Multigner *et al*. (2008) also reported a lack of association between glyphosate use and prostate cancer. This data appears to have also been reported by Ndong *et al*. (2009).
- The lack of association between glyphosate use and prostate cancer was also shown in an epidemiological study in farmers in British Columbia, Canada, by Band *et al*. (2011).
- Koutros *et al*. (2011) also studied associations between pesticide and prostate cancer. No statistically significant positive association between pesticides and prostate cancer were observed. There was suggestive evidence on an increased risk (OR>1.0) with an increasing number of days of use of petroleum oil/petroleum distillate used as herbicide, terbufos, fonofos, phorate and methyl bromide. However, no increased risk (OR>1.0) was observed for glyphosate.
- Lee *et al*. (2004) reported a lack of association between glyphosate use and stomach and oesophageal adenocarcinomas.
- El-Zaemey and Heyworth (2013) reported a case control study on the association between pesticide spray drift from agricultural pesticide application areas and breast cancer in Western Australia. The findings support the hypothesis that woman who ever noticed spray drift or who first noticed spray drift at a younger age had increased risk of breast cancer. However, it was not possible to examine whether the observed associations are the result of a particular class of pesticides and therefore no conclusion can be made on the basis of this study for glyphosate.
- Engel *et al*. (2005) reported AHS data on breast cancer incidence among farmers' wives, with no

association between breast cancer and glyphosate. There was no difference in incidence of breast cancer for women who reported ever applying glyphosate (odds ratio 0.9 (95%-CI 0.7-1.1) and also not for women who never used glyphosate but whose husband had used (no information on duration of use) with an odds ratio of 1.3 (95%-CI 0.8-1.9).

- Flower *et al*. (2004) reported AHS cohort data on parental use of specific pesticides and subsequent childhood cancer risk among 17,280 children, with no association between childhood cancer and glyphosate. The reported ORs for glyphosate were 0.61 (95%-CI 0.32-1.16) for maternal use and 0.84 (95%-CI 0.35-2.34) for paternal use. It is noted that in the evaluation of this study by IARC it was quoted that: "For all the children of the pesticide applicators, risk was increased for all childhood cancers combined, for all lymphomas combined, and for Hodgkin lymphoma, compared with the general population." However, this citation refers to risk for children of all pesticide applicators and not for glyphosate specifically. According to this study, there was an increased odds ratio in result of application of pesticides aldrin, dichlorvos and ethyl dipropylthiocarbamate, but the results for glyphosate did not demonstrate any risk for childhood cancer (refer to ORs stated above). Therefore, the statement in the IARC evaluation is not relevant for the assessment of glyphosate.
- Andreotti *et al*. (2009) reported on a case-control study within the AHS data where glyphosate was not associated with pancreatic cancer. The odds ratio for ever- versus never-exposure to glyphosate was 1.1 (95%-CI 0.6-1.7) while the odds ratio for the highest category of level of intensity-weighted lifetime days was 1.2 (95%-CI 0.6-2.6).
- Pahwa *et al*. (2011) investigated the putative association of specific pesticides with soft-tissue sarcoma (STS). A Canadian population-based case-control study conducted in six provinces was used on this analysis. The incidence of STS was associated with insecticides aldrin and diazinon after adjustment for other independent predictors. However, no statistically significant association between STS and exposure to glyphosate or other herbicides was observed. The fully adjusted odds ratio for glyphosate was 0.90 (95%-CI 0.58-1.40).
- Carreon *et al*. (2005) reported epidemiological data on gliomas and farm pesticide exposure in women; glyphosate had no association with gliomas.
- Landgren *et al*. (2009) reported AHS data on monoclonal gammopathy of undetermined significance (MGUS, a condition that is sometimes a precursor to multiple myeloma), showing no association with glyphosate use. The prevalence OR for MGUS for glyphosate users versus non-users, adjusted for age and education level, was 0.5 (95% CI 0.2–1.0).
- Karunanayake *et al*. (2011) reported a lack of association between glyphosate and Hodgkin lymphoma. Based on 38 cases exposed to glyphosate, the odds ratios were 1.14 (95%-CI 0.74-1.76) adjusted for age and province, and 0.99 (95%-CI 0.62-1.56) when additionally adjusted for medical history variables.
- Kachuri *et al*. (2013) investigated an association between lifetime use of multiple pesticides and multiple myeloma in Canadian men. Excess risks of multiple myeloma were observed among men reported using at least one carbamate pesticide, one phenoxy herbicide and ≥ 3 organochlorines. However, no excess risk was observed for ever us of glyphosate (OR 1.1, 95%-CI 0.66-1.86) although a nearly significant association was reported when considering >2 days/year of glyphosate use (OR 2.11, 95%-CI 0.95-4.70). It is noted this was based on a fairly low number of exposed cases (n=10).
- Pahwa *et al*. (2012) analysed the same study base applying slightly different analyses and reported a lack of association between glyphosate and multiple myeloma. The difference between to two studies are that Kachuri *et al.* 2013 excluded 10% of controls who did not have an age match, adjusted the ORs for smoking and provided a separate analysis for proxy respondents.
- Cocco *et al*. (2013) investigated the role of occupational exposure to agrochemicals in the aetiology of lymphoma overall, B cell lymphoma and its most prevalent subtypes. No statistically increased risk for B-cell lymphoma was observed in relation to glyphosate. However, it is noted that the number of exposed subjects were very low (4 exposed cases and 2 exposed controls) and therefore the study was concluded to be of low reliability by the RMS.

The following review studies are available:

- Alavanja and Bonner (2012) reviewed studies on occupational pesticide exposure and cancer risk. Twenty one pesticides showed significant exposure-response associations in studies of specific cancers.

No significant association was observed for glyphosate although the study authors did conclude that for NHL inconsistent results were observed and that additional epidemiological studies would be required.

In a comprehensive review of the AHS publications and data, Weichenthal *et al*. (2010) noted that increased rates in the following cancers were not associated with glyphosate use: overall cancer incidence, lung cancer, pancreatic cancer, colon or rectal cancer, lymphohematopoietic cancers, leukaemia, NHL, multiple myeloma, bladder cancer, prostate cancer, melanoma, kidney cancer, childhood cancer, oral cavity cancers, stomach cancer, oesophagus cancer and thyroid cancer. However, it is noted by the RMS that this review is now dated as new studies are available from the AHS.

Mink *et al*. (2012) submitted a comprehensive review of epidemiologic studies of glyphosate and cancer. To examine potential cancer risks in humans they reviewed the epidemiologic literature to evaluate whether exposure to glyphosate is associated causally with cancer risk in humans. They also reviewed relevant methodological and biomonitoring studies of glyphosate. The review found no consistent pattern of positive associations indicating a causal relationship between total cancer (in adults or in children) or any site-specific cancer and exposure to glyphosate. However, this review is dated as more recent publications are now available. It should also be noted that this review paper was funded by Industry.

2.6.5.1.2.3 Overall conclusion on epidemiological studies

Refer to Section 2.6.5.2.

2.6.5.1.3 Other public literature

A literature search for the active substance glyphosate was performed in accordance to the provisions of the EFSA Guidance "Submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) 1107/2009" and updated Appendix to this Guidance document. Besides the studies presented above the following publications were found relevant and reliable for this section and the summaries are thus presented below (and are summarized in Table 55 above).

Wozniak *et al.*, 2020 investigated the effect of glyphosate on methylation in the promotor regions of certain tumour suppressors. Glyphosate changed methylation pattern of the P21 and TP53 suppressor gene promoters, but in case of other analysed genes: P16, BCL2 and CCND1 they did not identify any statistically significant changes. Gene expression was decreased for P16 and TP53 and increased for BC12, CCND1 and P21. It was noted that in most cases no clear concentration-response was observed for these effects. Moreover, it is difficult to correlate the tested *in vitro* concentrations to *in vivo* doses and therefore an adverse outcome *in vivo*. As the authors also indicated changes in the DNA methylation profile were minimally correlated with gene expression level and that further and more global analysis (genome-wide based) are necessary to give a clear answer about epigenetic-transcriptomic changes induced by glyphosate.

Biserni *et al.*, 2019 investigated the effect of several pesticides, including glyphosate, on adipogenesis in 3T3-L1 adipocytes. In this study glyphosate did not affect lipid accumulation in this cell line.

Duforestel *et al.*, 2019 evaluated the effect of glyphosate on DNA methylation and tumorigenesis in non-neoplastic MCF10A cells. The study concluded that glyphosate is not oncogenic by itself, but it acts as an oncogenic hit factor that, combined with another oncogenic hit, promotes the development of mammary tumours. In the study, the cells were exposed to glyphosate *in vitro* at one very low dose of 10^{-11} M every three to four days over 21 days, whereas control cultures were treated with vehicle DMSO. As a control, the MCF10A cells were exposed to the carcinogenic UP peptide (0.5 μM) which is previously described to promote global DNA hypomethylation. Both exposure to glyphosate and to UP peptide resulted in DNA hypomethylation which is potentially tumorigenic according to the study authors. The DNA hypomethylation mediated by glyphosate was associated with TET3 overexpression instead of the DNMT1 pathway (which is the major pathway for UP peptide) and glyphosate exposure also resulted in a lower degree of DNA hypomethylation than UP peptide. In a second experiment, MCF10A cells exposed to glyphosate (same exposure as in the first experiment) were injected subcutaneously in 7- to 8-week old Swiss nude mice. No tumours developed, whereas the control experiment with MCF10A cells exposed to the UP peptide led to visible tumour growth within 21 days in 100% of the mice. To investigate the possibility of a two factor hit oncogenic impact with glyphosate, in a subsequent experiment six microRNAs (miRs) associated with a poor prognosis of breast cancer [miR-182-5p, miR-27a, miR-500a-5p, miR-30a, miR-495, and miR-146a] were transfected individually in MCF10A cells. These cells were exposed to glyphosate (same exposure as in the first experiment) and injected subcutaneously in Swiss nude mice. Tumour nodules were observed in two out of the four mice with subcutaneous injection of glyphosate-exposed MCF10A overexpressing miR-182-5p, whereas none

of the other five miRs were associated with tumour formation. The authors concluded that work shows that two epigenetic events (global DNA hypomethylation and overexpression of a miR) cooperate to promote breast cancer. However, due to the nature of this study design with only one very low level of glyphosate in an experimental setting, it is difficult to relate the exposure levels to *in vivo* situations. In addition, as only was dose was tested, no information on any dose-response is available. Overall, while the study provides relevant information on *in vitro* effects of glyphosate it cannot be directly correlated to an adverse *in vivo* outcome.

Hao et al. 2019 evaluated the *in vitro* autophagic effect of Roundup, glyphosate and polyethoxylated tallow amine (POEA) on human A549 cells. In the study glyphosate did not induce autophagic effects while Roundup and POEA did. Since tallow amine has been banned as co-formulant in the EU the outcome of this study is not considered to impact the evaluation of glyphosate.

Wang et al. 2019 evaluated the effect of glyphosate on multiple myeloma (MM) in Vk*MYC and wildtype (WT) mice. Glyphosate exposure resulted in reduced survival, increased spleen weight, chance in splenocyte number. Glyphosate induced benign monoclonal gammopathy (mouse equivalent to monoclonal gammopathy of undetermined significance (MGUS) in human) in WT mice and promotes MM progression in Vk*MYC mice. In Vk*MYC mice, glyphosate causes haematological abnormalities like anaemia and multiple organ dysfunction like lytic bone lesions and renal damage, which are hallmarks of human MM. Some limitations were noted for the study, including a low number of animals in the sub-acute study (n=5) and an unclear number of animals for the chronic study although it appeared to be 10 per group based on the individual data points in the result figures. It is also noted that the dose level used in the chronic study of 1 g/L correlates to 90 mg/kg bw/day based on default values for water consumption. This dose level is very low compared to the guideline toxicity studies so it is surprising that findings such as effects on haematological parameters were observed in WT C57BL/6 mice while the NOAELs in the guideline chronic studies are far higher. Considering the low number of animals and the remarkably low dose levels in which effects are observed there uncertainties regarding the reliability of the study. Therefore the study is not considered to directly impact the overall assessment of glyphosate.

2.6.5.2 Comparison with the CLP criteria regarding carcinogenicity

Table 56: Compilation of factors to be taken into consideration in the hazard assessment

Species and strain	Tumour type and background incidence	Multi-site responses	Progression of lesions to malignancy	Reduced tumour latency	Responses in single or both sexes	Confounding effect by excessive toxicity?	Route of exposure	MoA and relevance to humans
Refer to assessment above at section 2.6.5.1.1.3 (Rat and mouse studies – overall consideration of tumour incidences)								

Long-term toxicity and carcinogenicity studies in rats and mice

As already pointed out in the previous CLH report (2016) for glyphosate, a large quantity of animal data regarding carcinogenicity was submitted and some of these studies are also available from published scientific literature. Seven acceptable chronic toxicity and carcinogenicity studies in rats and five acceptable carcinogenicity studies in mice have been evaluated in the current assessment. Therefore, all available data including published literature were considered together using a weight of evidence approach with consideration of the biological significance, dose response, relationship of the highest doses used to the maximum tolerated dose and the consistency of the neoplastic findings among the studies. In addition, the previous findings by IARC, the previous assessment and the public literature assessment by Portier (2020) were taken into account. For each tumour type, the conclusions are summarized as follows (refer to the assessment above in section 2.6.5.1.1.3 for a complete overview):

1) Testes interstitial cell tumours in rats. This type of tumours was already discussed during the previous evaluation (CLH 2016 and RAC 2017) and highlighted in the publication by Portier (2020). Although there are no new findings (except updated historical control data), an assessment of the relevance of this tumour type was presented in order to provide a complete picture. A statistically significant increase in incidence of interstitial testicular tumours in rats was observed in one of the seven rat studies. However, this study is no longer considered acceptable if current standards are applied [redacted] 1981). Although the incidence was above (very limited) historical control data, this tumour type was considered incidental, a conclusion that was confirmed in six more recent, guideline-compliant studies. This conclusion is in line with the previous EU evaluation.

2) Pancreatic islet cell tumours in rats. This type of tumours was already discussed during the previous evaluation (CLH 2016 and RAC 2017) and highlighted in the publication by Portier (2020). Although there are no new findings, an assessment of the relevance of this tumour type was presented here again in order to provide a complete picture. Two studies ([redacted], 1990 and [redacted], 1981) showed an increased incidence of pancreatic islet cell tumours in low dose males, which were both statistically significant by pairwise comparison. No significant differences were observed for the mid and high dose males compared with controls. There was no dose-response relationship observed in the two studies as indicated by the lack of a statistically significant trend. A trend was observed for carcinomas in the study by [redacted], although it should be noted this was based on a single incidence in high dose males compared to none in the control, low and mid dose groups. In the five remaining carcinogenicity studies in the rat with even higher dose levels clearly no effect of pancreatic islet cell adenomas was observed. There was no increase in pancreatic tumours in the females. The observed findings are therefore concluded to be incidental and not treatment related. This conclusion is in line with the previous EU evaluation.

3) Thyroid C-cell tumours in rats. This type of tumours was already discussed during the previous evaluation (CLH 2016 and RAC 2017) and highlighted in the publication by Portier (2020). Although there are no new findings (except updated historical control data), an assessment of the relevance of this tumour type was presented here again in order to provide a complete picture. In one study ([redacted]), a statistically significant increase in thyroid C-cell adenomas in females was observed (not significant by pair-wise comparison but was weakly positive in a Cochran-Armitage trend test). The observed incidence was slightly outside historical control data. There was no progression to carcinomas. Further, no effect on non-neoplastic precursors was observed in the study. In fact, the thyroid does not appear to be a target organ for glyphosate in any of the repeated dose toxicity studies in rats. No effect on thyroid C-cell adenoma was observed in any of the other studies. Although the reported incidence is above historical control range, still the conclusions of the previous evaluation remain that the increased incidences are considered incidental and not treatment-related as in the other studies no thyroid C-cell adenomas. This conclusion is in line with the previous EU evaluation.

4) Hepatocellular adenoma in rats. This type of tumours was already discussed during the previous evaluation (CLH 2016 and RAC 2017), however, only one study was considered ([redacted], study report no. [redacted]-10495). In the current assessment also a second study is taken into account in which an apparent increase is seen ([redacted], 2001) as highlighted by Portier (2020). In the study by [redacted] ([redacted]-10495), high dose males showed a statistically significant increase in hepatocellular adenomas (8/60 versus 3/60 in controls; Cochran-Armitage trend test (p=0.0171). The incidence in high dose males was slightly above HCD mean but clearly within the HCD range. No statistical trend was observed when the incidences of adenoma and carcinoma were combined (p=0.0752). The incidence of carcinomas in this study was 3/60, 2/60, 1/60 and 2/60 for the control, low, mid and high dose, respectively, which results in a combined incidence of 6/60, 4/60, 4/60 and 10/60. In addition, no non-neoplastic precursors were observed in the liver.

In the other study by [redacted] ([redacted]/PR1111), hepatocellular adenoma was observed in 5 out of 64 animals (7.8%) compared to zero incidences in controls, which was statistically significant using the Peto-test for trend. The incidence in high dose males was slightly outside historical control data range. However, although a statistical trend is observed, no clear-dose response is seen. In this study, no progression to carcinomas is shown. In addition, no non-neoplastic precursors were observed in the liver in both studies. In the study by [redacted], 2001, an increased incidence in hepatitis was noted in top dose males. However, as the background incidence of hepatitis is highly variable and as the incidence at the top dose is within HCD range, the relation to treatment is doubted. The other four carcinogenicity studies in rats did not show an effect on hepatocellular adenomas. In addition, no effect in females was observed in any of the studies. Therefore, the majority of the carcinogenicity studies in the rat did not show a treatment-related effect on hepatocellular adenoma. Based on the argumentation above, the observed increase in hepatocellular adenomas is considered incidental and not related to treatment. This conclusion is in line with the previous EU evaluation.

5) Pituitary adenoma in rats. The publication by Portier (2020) highlighted a statistically significant trend in the incidence of pituitary adenomas in male and female rats in the study by [redacted], 2009 (study report no. 2060-0012). This finding has not been previously discussed at EU level. First of all, the RMS doubts whether a trend-test is appropriate in this case as not all animals were investigated in the low and mid dose, which might have led to an distorted association. When considering the results from the available carcinogenicity studies in the rat together it is clear that pituitary adenomas are very common in rats and that no increases in incidence were seen in any of the other studies. No progression to carcinomas was observed and no effect on concomitant non-neoplastic findings were observed. Therefore, it is concluded that glyphosate has no effect on pituitary adenomas.

6) Skin basal cell tumours in rats. The publication by Portier (2020) highlighted a statistically significant trend in these types of tumours in male rats. These findings have not been previously discussed at EU level.
A positive trend for skin basal cell tumours in male Sprague-Dawley rats was reported for the [redacted] study (study

report no. [redacted] 94-0150). This trend was confirmed by an external statistician upon request by AGG. In this study, an incidence of 3 benign adenomas and 1 malignant carcinoma was reported at the top dose. The incidence was above very limited historical control data. The other studies reported no skin basal cell tumours in rats, except the study by [redacted] in which one carcinoma in the mid dose groups was observed. Considering that the carcinoma was observed in the mid-dose only, thus lacking dose-response and that no carcinomas were observed in any of the other five studies, the single carcinoma is considered a chance finding by the RMS.
For the skin basal cell adenomas reported in the study by [redacted], the effect was confined to one study at the top dose in males (accompanied by follicular hyperkeratosis), whereas no effect was observed in the other five studies for which a similar dosing regime was applied. As very limited historical control data is available for this type of tumours (only two studies) it is difficult to put this finding into perspective. Moreover, no effect was observed in females nor in other species. Further, there is no plausible mechanism as no clear effects on skin upon systemic exposure to glyphosate were reported in the whole database (except for the follicular hyperkeratosis). Therefore this finding is considered of equivocal relevance and not sufficient for classification.

7) Skin keratoacanthomas in rats. This tumour type was not discussed during the previous EU renewal of glyphosate as, based on the statistical analysis in the study reports, the incidences were not significantly increased and were therefore previously not further considered in an overall weight-of-evidence approach. The publication by Portier (2020), however, highlighted increased incidences of skin keratoacanthomas in male rats as evidence for carcinogenicity of glyphosate. Therefore, this tumour type was further considered in a relevance assessment.
In three studies in Sprague-Dawley rats ([redacted] (1997), [redacted] (1990) and [redacted] (1993) increased incidences of skin keratoacanthomas in males were observed at the top dose which were above historical control data in Sprague-Dawley rats. However, these incidences were not significantly increased based on a pairwise comparison and also not by a stratified Cochran-Armitage trend test (both 2-sided testing). For Wistar rats, three studies are available of which in one study an apparent increase is seen at the top dose ([redacted], 2009), however, historical control data from concurrently performed studies are not available anymore. Also for this study, the incidence at the top dose was not significantly increased based on a pairwise comparison or a trend test (2-sided testing).

For the discussion on the relevance of the increased incidence of skin keratoacanthomas, it is important to note that skin keratoacanthomas are benign tumours, which is rather common in aged male rats. In general, this tumour type involves both the dermis and epidermis and is commonly seen with hyperkeratosis of the squamous epithelium. However, nor in the long-term studies nor in the other studies epithelial hyperkeratosis is reported. Further, the keratoacanthomas were only observed at very high dose rates, which slightly exceeded the maximum recommend dose rate according to the OECD GD. Whereas in another study in Wistar rats at the same dose level (1214 mg/kg bw/day) no increase in skin keratoacanthomas was seen. In the Weight of Evidence approach, it was further taken into account that the skin keratoacanthomas were only observed in one species (rat) of one sex (males). Moreover, in the available studies no malignant squamous cell carcinomas were reported.

Overall, when considering that:
- The increased incidence in skin keratoacanthomas were observed at very high dose rates, which slightly exceeded the maximum recommend dose rate of 1000 mg/kg bw/day according to the OECD guideline. The only exception is the study by [redacted] (1990) in which an apparent dose-response is seen which, but not linear with the three-fold increase in dose levels.
- Even at this high dose rate (≥ 1000 mg/kg bw/day), it is still a relatively rare tumour with 6/51 (12%) as the highest incidence;
- In one study in Wistar rats ([redacted], 2001) at the same high lose level (1214 mg/kg bw/day) no increase in skin keratoacanthomas was seen;
- Although the incidences exceeded the background incidence (for which limited information is available for most of the studies), no statistically significant differences were observed (either by pairwise comparison or by trend analysis; 2-sided testing);
Together with the following factors:
- The skin keratoacanthomas were only observed in one species (rat) of one sex (males);
- The tumour is a benign tumour, which is rather common in aged male rats;
- No non-neoplastic precursor effects were observed; and
- No malignant squamous cell carcinomas were reported;
the RMS considers that the apparent increase in **skin keratoacanthomas is not of sufficient relevance for classification and labelling**.

Mice

8) Malignant lymphoma in mice. In the previous assessment, this tumour type observed in mice was extensively discussed (CLH 2016 and RAC 2017). These tumours were also highlighted in the publication by Portier (2020). For the current evaluation, a new statistical analysis was available (a Peto-analysis) and updated historical control data has been provided. In three mouse studies, a slightly higher incidences in the rather common malignant lymphoma in males was seen ([redacted], 1997, report no [redacted] 94-0151); [redacted], 2001, report no Toxi 1559.CARCI-M and [redacted] 2009, report no 2060-0011), This finding was already extensively discussed during the previous EU evaluation (CLH report 2016) and by RAC (2017).
In the study by [redacted] (2001) in Swiss mice, no significant trend was observed (2-sided testing) and together with the high variability in the background incidence, the apparent increase in malignant lymphomas is not considered treatment-related. In two studies in CD-1 mice an increase in malignant lymphomas was noted in males ([redacted], 2009 and [redacted], 1997) and in one study in females ([redacted] 1999). The latter study was not evaluated by the RMS as no study report is available. Only limited tumour incidence data is available. All effects were only significant by trend-analysis, but not by pairwise comparison. Only for one of the three studies HCD is available, showing that the incidence at the top dose level was within HCD range ([redacted], 1997). Two studies showed negative results ([redacted] 1993 and [redacted], 1983). Importantly, a high variability in background incidence was shown based on the (limited) historical control data. The increases in malignant lymphoma incidences appeared to be confined to the high dose groups in the CD-1 mice which were around or above the OECD limit dose of 1000 mg/kg bw/day. However, no clear dose-effect concordance between studies was observed. In the studies with CD-1 mice by [redacted] (2009) and [redacted] (1993), comparable top doses were administered and a similar incidence of malignant lymphoma was reported in high dose males (5/51 and 6/50 for at a dose level of 810 or 1000 mg/kg bw/day, respectively). However, the control group incidences were clearly different (0/51 vs. 4/50) resulting in a positive trend test for the study by [redacted], but not for the [redacted] study. In the study by [redacted] (1997), which was also performed in CD-1 mice, a top dose of 4348 mg/kg bw/day was applied in males. The incidence in malignant lymphomas of 6/50 at the top dose level was similar to what was seen in the two studies mentioned before even though the applied dose was by four to five times higher. This is surprising since a further increase would be expected if it was a treatment-related effect. Whereas a fourth study in CD-1 mice by [redacted] (1983) in which an even higher top dose of 4841 mg/kg bw/day was fed without any increase in lymphoreticular tumours in general. The incidence of malignant lymphomas is known to be related to the age of the animals. However, significant associations between exposure to glyphosate and induction of malignant lymphomas were not observed in the 24-month studies. Furthermore, there was no reduction in overall survival in the exposed groups. No parallel increases were observed in female CD-1 mice. It is known that female CD-1 mice are usually more prone to develop spontaneous malignant lymphoma than male mice. The lymphoma incidences were generally higher in females than in males, but no glyphosate related increases were seen in female CD-1 mice. The study by [redacted] (1999; study report not available to RMS) in which an increased incidence in females was noted, is considered of limited relevance as the increase is only slight and as this finding occurred at a very high dose level, which is 8- to 9-fold higher than the maximum recommended dose level of 1000 mg/kg bw/day according to the OECD TG 453 (2009). Therefore, the previous conclusion that no parallel increases were reported in females remains. Further, no increase in treatment-related non-neoplastic findings in lymph nodes were reported, thus supporting the conclusion that the tumours were of a spontaneous nature.
Considering all the above arguments, the increased incidences malignant lymphomas in male CD-1 mice are not considered to be treatment-related when a weight of evidence approach was applied. This conclusion is in agreement with the previous assessment. The very different dose levels in all the studies and the dose-specific incidences were taken into account as well as the high variability in spontaneous occurrence of this tumour type together with the statistical uncertainties. In addition, the current assessment did not find any new information that would change the outcome of the previous evaluation.

9) Renal tubule tumours in male mice. Also this type of tumour has been extensively discussed during the previous assessment (CLH 2016 and RAC 2017). These tumours in mice were also highlighted in the publication by Portier (2020). Although there are no new findings (except updated historical control data), an assessment of the relevance of this tumour type was presented above in order to provide a complete picture.
Low, but elevated incidences of renal tumours were reported at the high doses exposures in three of the five mouse carcinogenicity studies. The effects occurred at very high dose levels above the OECD-recommended limit of 1000 mg/kg bw/day and seem to be near and possible beyond the maximum tolerable dose (MTD). The increases in renal tumours were not statistically significant in pairwise comparisons (Fisher's exact test), but only for the Cochran-Armitage trend-test. All kidney tumours were observed at termination. No increase was reported in related preneoplastic lesions (renal tubular hyperplasia or necrosis) in male mice. Moreover, no clear pre-neoplastic kidney lesions (such as renal tubular hyperplasia or necrosis) were observed. In two of the five studies, no renal tumours were reported at the two highest doses and in two studies, adenomas/carcinomas were reported in the control groups. Furthermore, no increase in renal tumours was reported in female mice. There was a positive trend in male mice, but the findings were not consistent across all studies. In agreement with the previous assessment (CLH 2016 and RAC 2017), the human relevance of the renal tumours at very high doses is considered to be low and the overall

evidence for the increase in renal tumours having been caused by glyphosate is considered insufficient for classification.

10) Haemangiosarcoma in male mice and haemangioma in female mice.
The haemangiosarcomas in male mice have been extensively discussed during the previous assessment (CLH 2016 and RAC 2017). These tumours in mice were also highlighted in the publication by Portier (2020). Although there are no new findings, an assessment of the relevance of this tumour type was presented above in order to provide a complete picture. For the haemangiosarcomas in male mice, two of the mice studies ([redacted], 1993; study report no 7793 and [redacted], 1997, study report no 94-0151) reported an increased incidence in high dose males. It is considered that the effect in males is unlikely to be treatment-related as the effect observed in the two studies were within historical control data, no effect was observed in the other two studies with CD-1 mice nor in the study with Swiss mice and no effect was seen in females. As already stated before, this conclusion is in line with the previous EU evaluation and RAC opinion (2017).
The mesenteric lymph node haemangioma observed in female mice in one study, have not been discussed before, but were highlighted by the publication from Portier (2020). In females, a statistically significant trend for mesenteric lymph node haemangioma was seen in the study with Swiss mice ([redacted], 2001; report number Toxi 1559.CARCI-M) with 4/50 incidences at the top dose against 1/50 in the control group and none in the low and middle dose. This trend was also highlighted in the publication by Portier (2020). However, this apparent effect was not seen in any of the other carcinogenicity studies in female mice. As the increase in haemangioma at the top dose was only seen in females in one study, this finding is considered incidental and not treatment-related.

Epidemiological studies

Several epidemiological studies exist investigating the relation between glyphosate exposure and cancer, this includes both case-control and cohort studies. In addition, reviews, statistical re-analyses, systematic reviews and meta-analyses of already published data are available and were considered for the hazard assessment within the current assessment.

One general concern with the epidemiological studies is that no accurate exposure assessment occurs and all exposure assessments are based on questionnaires instead of e.g. biomonitoring data. It should also be noted that the epidemiological studies concern exposure to formulations which makes it difficult in general to establish a direct link between exposure to a specific active substance and an adverse outcome.

As already mentioned a large number of epidemiological studies are available for evaluation. These are case-control studies and studies resulting from the data collected in the context of the Agricultural Health Study (AHS) from the US. In this latest study, data was prospectively collected from more than 57,000 farmers (users of crop protection products). Most studies have already been included in the previous review (CLH 2016, RAC 2017). In addition, since the previous RMS (Germany) has already conducted an extensive review of the differences in assessment between IARC and the EU review at that time, this has not been repeated in the current evaluation.

In the current review, all studies have been (re-)assessed and an extensive relevance/reliability check has been performed based on the EFSA recommendations[11] (of which the outcome is shown in Table 54 of Volume 1). New compared to the previous review is the recent study by Pahwa (2019), which combines data from two case-control studies. The use of glyphosate in general has not been associated with Non-Hodgkin Lymphoma (NHL). However, a weak association was found for a subgroup with those who worked > 2 days per year with glyphosate and the occurrence of Non-Hodgkin Lymphoma (NHL) and Diffuse large B-cell lymphoma (DLBCL) (odds ratios of 1.7 (95%-CI 1.0-2.9) and 2.1 (95%-CI 1.1-4.3, respectively). However, it should be noted that this only concerns a very small research population of n=30 and n=14 cases, respectively. As only a small number of cases was included in this sub-analysis, it is uncertain how representative these results are for the entire pooled population. The results of those analyses should be interpreted accordingly.

Another new study (Andreotti, 2018) shows that, based on the data from the Agricultural Health Study, no overall effect was reported. However, a weak association can be seen for persons with a relatively high exposure (third tertile) and acute myeloid leukaemia and Non-Hodgkin Lymphoma after a 20-year lag time (the so-called time between exposure and tumour development). Again, it only concerns a very small research population of n=15 and n=8 cases, respectively, and therefore these findings are considered of questionable value. Given the limitations (mainly the small study sub-populations), there is currently no reason to classify on the basis of these findings. The finding in the most recent update (Andreotti, 2018) of a possible association with acute myeloid leukaemia should be looked at carefully in future updates on the AHS data. It should, however, be noted that a high number of cancer

[11] Scientific Opinion of the PPR Panel on the follow-up of the findings of the External Scientific Report 'Literature review of epidemiological studies linking exposure to pesticides and health effects' (EFSA Journal 2017 ; 15(10):5007

sites were analysed so there is the possibility of statistical findings by chance and this effect was not observed in any of the other epidemiological studies with glyphosate.

As already reported in the previous evaluation (CLH 2016, RAC 2017) some of the case-control studies reported slightly increased ORs for certain tumours. However, most of these studies had limitations such as a lack of adjustment for confounders such as other pesticide exposure or lifestyle factors, were based on a very low number of exposed cases and/or had a high proportion of proxy responders. Adjusting for confounding factors such as exposure to other pesticides was shown to lower the ORs in most of the studies where such an exercise was conducted. Proxy responders were also found to lead to higher ORs than self-responders (e.g. Lee et al. 2005). Besides these limitations there is a concern for recall bias for the case-control studies and it is worth noting that the observed effects were not replicated in the prospective study. Further, considering NHL as an outcome parameter, it should be noted that this is not a specific disease but a broad spectrum of disorders more correctly referred to as lymphocytic lymphomas, each with possible different aetiologies. They are all classified as not being Hodgkin lymphoma, and the terminology has changed over the years - some lymphomas are described differently today compared to previously. This complicates the evaluation of the studies.

Overall, it is concluded that the results reported in the epidemiological studies do not warrant classification and labelling of glyphosate.

Comparison with the CLP criteria

The database for the evaluation of glyphosate carcinogenicity is extensive and the assessment is based on data from human epidemiological studies and a large number of carcinogenicity studies. There are seven rat studies (including one study that was not acceptable) and five mouse studies (plus one additional study for which limited data is available). The exposure route was oral in all studies and the doses used were sufficiently high in all but one of the evaluated studies. The database includes studies of sufficient reliability and relevance to allow a robust evaluation following the requirements of CLP.

Criteria for classification as carcinogen according to Table 3.6.1 of CLP Regulation, Annex I

Category 1 (H350): Known or presumed human carcinogens

A substance is classified in category 1 for carcinogenicity on the basis of epidemiological and/or animal data. A substance may be further distinguished as:

Category 1A:

Category 1A, known to have carcinogenic potential for humans, classification is largely based on human evidence.

Category 1B:

Category 1B, presumed to have carcinogenic potential for humans, classification is largely based on animal evidence.

The classification in category 1A and 1B is based on strength of evidence together with additional considerations (see section 3.6.2.2). Such evidence may be derived from:

- Human studies that establish a causal relationship between human exposure to a substance and the development of cancer (known human carcinogen); or

- Animal experiments for which there is sufficient evidence to demonstrate animal carcinogenicity (presumed human carcinogen).

In addition, on a case-by-case basis, scientific judgement may warrant a decision of presumed human carcinogenicity derived from studies showing limited evidence of carcinogenicity in humans together with limited evidence of carcinogenicity in experimental animals.

As was already indicated in the previous CLH report (2016), one important remark is that for the majority of chemical substances evaluated under the CLP Regulation, normally one study addressing each endpoint is required and this is usually considered sufficient for classification and labelling purposes. In the case of glyphosate, a large quantity of animal data is available (as discussed in the previous paragraph). Therefore, the criteria of the CLP

Regulation may not be applicable directly to the available information for glyphosate as several studies are available per endpoint. In line with the previous assessment, all available data should be considered together using a weight of evidence approach with consideration of the biological significance, relationship of the applied doses to the maximum tolerated dose and the consistency of the neoplastic findings. And therefore, no conclusions were based only on the statistical significance of an increased tumour incidence identified in a single study. The current assessment is continued in the same line.

Category 1A:
As stated above, classification in category 1A concerns substances known to have carcinogenic potential for humans and is largely based on human evidence. Although a few of the available epidemiological cohort and case-control studies show weak statistically significant associations between exposure to glyphosate based formulations and findings of cancer (NHL or a subtype and acute myeloid leukemia), chance, bias and confounding factors could not be ruled out. This has been extensively discussed in the previous sections. Therefore, a causal relationship to cancer following exposure to glyphosate based formulations is not proven. Hence, classification of glyphosate in category Carc.1A is not justified. The detailed reasoning has been provided above.

Category 1B:
As listed above, category 1B concerns substances presumed to have carcinogenic potential for humans. Classification is largely based on animal evidence. Following the extensive overall evaluation of the human evidence and the tumour data from multiple carcinogenicity studies in mice and rats (refer to sections above), it is concluded that there is not sufficient evidence for carcinogenicity and a classification of glyphosate in category 1B is thus not warranted. Furthermore, the active substance glyphosate is devoid of genotoxic potential.

Criteria for classification as carcinogen according to Table 3.6.1 of CLP Regulation, Annex I

Category 2 (H351): Suspected human carcinogens

The placing of a substance in Category 2 is done on the basis of evidence obtained from human and/or animal studies, but which is not sufficiently convincing to place the substance in Category 1A or 1B, based on strength of evidence together with additional considerations (see section 3.6.2.2, Specific considerations for classification of substances as carcinogens, see below). Such evidence may be derived either from limited evidence of carcinogenicity in human studies or from limited evidence of carcinogenicity in animal studies.

Regulation (EC) No 1272/2008 (CLP Regulation), Annex I

3.6.2.2. Specific considerations of substances as carcinogens (see below)

Strength of evidence involves the enumeration of tumours in human and animal studies and determination of their level of statistical significance.

Sufficient human evidence demonstrates causality between human exposure and the development of cancer, whereas sufficient evidence in animals shows a causal relationship between the substance and an increased incidence of tumours. Limited evidence in humans is demonstrated by a positive association between exposure and cancer, but a causal relationship cannot be stated. Limited evidence in animals is provided when data suggest a carcinogenic effect, but are less than sufficient. The terms ‘sufficient’ and ‘limited’ have been used here [in the CLP regulation, red.] as they have been defined by the International Agency for Research on Cancer (IARC) and read as follows:

(a) Carcinogenicity in humans

The evidence relevant to carcinogenicity from studies in humans is classified into one of the following categories:

- sufficient evidence of carcinogenicity: a causal relationship has been established between exposure to the agent and human cancer. That is, a positive relationship has been observed between the exposure and cancer in studies in which chance, bias and confounding could be ruled out with reasonable confidence;

- limited evidence of carcinogenicity: a positive association has been observed between exposure to the agent and cancer for which a causal interpretation is considered to be credible, but chance, bias or confounding could not be ruled out with reasonable confidence.

(b) Carcinogenicity in experimental animals

Carcinogenicity in experimental animals can be evaluated using conventional bioassays, bioassays that employ

genetically modified animals, and other in-vivo bioassays that focus on one or more of the critical stages of carcinogenesis. In the absence of data from conventional long-term bioassays or from assays with neoplasia as the end-point, consistently positive results in several models that address several stages in the multistage process of carcinogenesis should be considered in evaluating the degree of evidence of carcinogenicity in experimental animals. The evidence relevant to carcinogenicity in experimental animals is classified into one of the following categories:

- sufficient evidence of carcinogenicity: a causal relationship has been established between the agent and an increased incidence of malignant neoplasms or of an appropriate combination of benign and malignant neoplasms in (a) two or more species of animals or (b) two or more independent studies in one species carried out at different times or in different laboratories or under different protocols. An increased incidence of tumours in both sexes of a single species in a well-conducted study, ideally conducted under Good Laboratory Practices, can also provide sufficient evidence. A single study in one species and sex might be considered to provide sufficient evidence of carcinogenicity when malignant neoplasms occur to an unusual degree with regard to incidence, site, type of tumour or age at onset, or when there are strong findings of tumours at multiple sites;

- limited evidence of carcinogenicity: the data suggest a carcinogenic effect but are limited for making a definitive evaluation because, e.g. (a) the evidence of carcinogenicity is restricted to a single experiment; (b) there are unresolved questions regarding the adequacy of the design, conduct or interpretation of the studies; (c) the agent increases the incidence only of benign neoplasms or lesions of uncertain neoplastic potential; or (d) the evidence of carcinogenicity is restricted to studies that demonstrate only promoting activity in a narrow range of tissues or organs.

3.6.2.2.4. Additional considerations (as part of the weight of evidence approach)).

Beyond the determination of the strength of evidence for carcinogenicity, a number of other factors need to be considered that influence the overall likelihood that a substance poses a carcinogenic hazard in humans. The full list of factors that influence this determination would be very lengthy, but some of the more important ones are considered here.

3.6.2.2.5. The factors can be viewed as either increasing or decreasing the level of concern for human carcinogenicity.

The relative emphasis accorded to each factor depends upon the amount and coherence of evidence bearing on each. Generally there is a requirement for more complete information to decrease than to increase the level of concern. Additional considerations should be used in evaluating the tumour findings and the other factors in a case-by-case manner.

3.6.2.2.6. Some important factors which may be taken into consideration, when assessing the overall level of concern are:
(a) tumour type and background incidence;
(b) multi-site responses;
(c) progression of lesions to malignancy;
(d) reduced tumour latency;
(e) whether responses are in single or both sexes;
(f) whether responses are in a single species or several species;
(g) structural similarity to a substance(s) for which there is good evidence of carcinogenicity;
(h) routes of exposure;
(i) comparison of absorption, distribution, metabolism and excretion between test animals and humans;
(j) the possibility of a confounding effect of excessive toxicity at test doses;
(k) mode of action and its relevance for humans, such as cytotoxicity with growth stimulation, mitogenesis, immunosuppression, mutagenicity.
Regarding mutagenicity, it is recognised that genetic events are central in the overall process of cancer development. Therefore, evidence of mutagenic activity in vivo may indicate that a substance has a potential for carcinogenic effects.

Category 2:
As listed above, category 2 substances are suspected human carcinogens. Classification is based on evidence obtained from human and/or animal studies, but which is not sufficiently convincing to place the substance in Category 1A or 1B, based on strength of evidence together with additional considerations.

Animal data

In the rat and mice, tumours were only occasionally seen. The types of tumours that were observed in rats were not seen in mice, and *vice versa*. In addition, the tumours occurred in only one sex (except for pituitary tumours). Seven types of tumours have already been discussed extensively during the previous renewal (CLH 2016 and RAC 2017) and were re-assessed in the current assessment. In response to a recent publication by Portier (2020), three additional tumour types are included in the assessment (pituitary adenomas in rats, skin basal cell tumours in rats and skin keratoacanthomas in rats). The most important comment on Portier's publication is that only statistics were considered and not the biological plausibility of the results. In addition, statistical testing was one-sided with a significance level of 0.05, whereas in the original study reports and the AGG analysis two-sided testing is applied with a significance level of 0.05. Also, no adjustment was made for chance findings by performing multiple testing.

The tumours in the **testis, pancreas** and **thyroid gland** in rats and **kidney tumours** and **heamongiosarcomas** in male mice have already been extensively examined in the previous review (CLH 2016, RAC 2017) in response to the findings in the IARC analysis. The tumours occur in one or a few studies and, based on a weight-of-evidence approach, are considered incidental and not treatment-related. Compared to the previous assessment, there were no major differences, except that historical control data has been added or updated. The conclusions regarding these tumour types have not changed from the previous review. For further details please refer to the sections above.

The assessment of **liver tumours** in rats has also been extensively reviewed (CLH 2016, RAC 2017) in response to the findings in the IARC analysis. The current assessment of liver tumours in rats includes a second study in which liver tumours were observed following the publication of Portier. Based on the weight-of-evidence approach, the conclusion remains that these findings are not related to glyphosate exposure. For further details please refer to the sections above.

The previous review (CLH 2016, RAC 2017) also extensively discussed **malignant lymphomas** in mice. These tumours have also been included in the current assessment. A higher incidence of this tumour type has been reported in several studies in mice, however, often without statistical significance (2-sided testing). In addition, the weight-of-evidence approach takes into account that there is no dose-effect concordance between studies. Further, the background incidence of this type of tumours is relatively high and variable. As described in detail above and in line with the earlier conclusion, these tumours are not considered to be related to exposure to glyphosate. For further details please refer to the sections above.

The analysis of **tumours in the pituitary gland** in rats is new. As highlighted in the study by Portier (2020), in one study a statistically significant positive trend was observed in males and females. However, as it concerns a possible increase in only one of the six studies and the fact that these tumour types are very common in rats, it has been concluded on the basis of the weight-of-evidence approach that these are not related to exposure to glyphosate. For further details please refer to the sections above.

In addition, compared to the previous review (CLH 2016 and RAC 2017), it is also new that two types of skin tumors have been included, namely **skin basal cell tumors and skin keratoacanthomas**. These were included as a result of the publication of Portier (2020) in which statistically significant positive trends were found for a number of studies in male rats. For skin basal cell tumours, it is noted that these have only been found in one rat study and not in other studies at comparable doses, that they have only been found in males and not in females and that they have not been found in mice. However, in one study, an increased incidence of follicular hyperkeratosis was observed, which may be a precursor effect. But when taken together in a weight-of-evidence approach, there is insufficient evidence that these tumours are related to glyphosate exposure. For further details please refer to the sections above.

For **skin keratoacanthomas**, three studies in Sprague-Dawley rats found an increased incidence in male rats and one of the three studies in Wistar rats. For the studies for which historical control data is available, the incidences were above the natural background. However, it is noted that only very limited historical control data is available. The incidences were not significantly increased, either on pairwise comparison or trend analysis (2-sided testing). In the weight-of-evidence approach, it is taken into account that these skin tumours were only found in males and also only in rats and not in mice. The increased incidences were only seen at the highest doses, which were (slightly) above the maximum recommended test dose of 1000 mg/kg bw/day (according to OECD TG 453 (2009)). In addition, the tumour is benign and no non-neoplastic precursors have been found. Nor have malignant variants of this type of tumours been seen. Taken together, based on the weight of evidence approach and taking into account the aforementioned aspects, the increase in skin keratoacanthomas is of insufficient relevance for classification for Category 2 carcinogenicity.

Epidemiological studies

No overall association between exposure to glyphosate and cancer was found in the AHS, which is the only prospective cohort study available. However, a weak association can be seen for subjects with a relatively high exposure (third tertile) and acute myeloid leukaemia and Non-Hodgkin Lymphoma after a 20-year lag time. As it only concerns a very small research population of n=15 and n=8 cases, respectively, these findings are considered of questionable value. Further, some weak positive association has been observed in some case-control studies or meta-analysis of these studies between exposure to glyphosate and cancer-outcomes. However, no causal relationship could be established as chance, bias, and confounding factors could not be ruled out in these studies. In line with the previous assessment (CLH 2016, RAC 2017), there is insufficient evidence from epidemiological studies to demonstrate carcinogenicity in humans. The increased risk observed in some case-control studies was not consistently observed among the available case-control studies nor in the data from the only cohort study available. When all available epidemiology data is taken into consideration, it is concluded that the criteria for classification for Cat 2 carcinogenicity are not fulfilled.

2.6.5.3 Conclusion on classification and labelling for carcinogenicity

Based on the epidemiological data as well as on data from long-term studies in rats and mice, taking a weight of evidence approach, no hazard classification for carcinogenicity is warranted for glyphosate according to the CLP criteria.

2.6.6 Summary of reproductive toxicity [equivalent to section 10.10 of the CLH report template]

2.6.6.1 Adverse effects on sexual function and fertility – generational studies [equivalent to section 10.10.1 of the CLH report template]

Table 57: Summary table of animal studies on adverse effects on sexual function and fertility – generational studies

Method, guideline, deviations[1] if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results** **- NOAEL/LOAEL (for sexual function and fertility, parents)** **- target tissue/organ** **- critical effects at the LOAEL** **Adverse effects in bold text**	**Reference** **New data for renewal: Yes/No**
Two generation reproduction study (dietary) OECD TG 416 (2001) Rat Sprague-Dawley Crl:CD (SD) IGS BR M, F 28/sex/group GLP: Yes Acceptable No deviations from OECD TG 416 (2001)	Glyphosate technical Purity: 95.7% (w/w) Lot/Batch#: H05H016A 0, 1500, 5000, 15000 ppm (equivalent to mean achieved dose levels of 0, 104, 351 and 1063 mg/kg bw/day for males, and 0, 162, 530 and 1634 mg/kg bw/day for females) Administration: daily by dietary admixture throughout the treatment period.	F0 and F1 adults: 1500 ppm: No treatment-related effects 5000 ppm: -one F0 female in extremis due to suspected prolonged parturition (not considered attributable to the administration of test substance according to study author) 15000 ppm: -one F0 female found dead on day 97 possibly due to complications during parturition (not considered attributable to the administration of glyphosate according to study author) ↑ **liver weight** (F0 females: abs weight: 13%, rel weight: 8%; F1 females: absolute weight: 10%, relative weight: 8%) ↑ **kidney weight** (F0 females: abs. weight: 11%, rel. weight: 7%) **-changes in sperm parameters** (↓ number of homogenisation resistant spermatid in cauda epididymis) (309	[redacted] (2007) CA 5.6.1/001 CA 5.6.1/002 CA 5.6.1/003 Report No. 2060/0013 New data for renewal: No

Method, guideline, deviations[1] if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results - NOAEL/LOAEL (for sexual function and fertility, parents) - target tissue/organ - critical effects at the LOAEL Adverse effects in bold text	Reference New data for renewal: Yes/No
	Control animals were treated in an identical manner with untreated laboratory diet. Exposure of F0 began at approximately 8 weeks of age. After 10 weeks of exposure F0 rats were mated. The appropriate experimental diet was fed throughout the study, to F0 and F1 parents and offspring until termination.	million/gram compared to 400 million/gram in control) (F0 generation) <u>Offsprings (F0-F1 and F1-F2 generations):</u> <u>1500 ppm:</u> No treatment-related effects <u>5000 ppm:</u> No treatment-related effects <u>15000 ppm:</u> **-delayed sexual maturation** (delayed preputial separation, Days at completion: 45.9 compared to 43.0 in control) (F1 generation) NOAEL for parental, offspring and reproductive toxicity: 5000 ppm (351 mg/kg bw/day).	
Two generation reproduction study (dietary) OECD TG 416 Rat Alpk:APfSD (Wistar-derived) M, F 26/sex/group GLP: Yes Acceptable The study was checked for compliance with current OECD TG 416 (2001) and following deviations were observed: (i) no individual animal data presented in study report (ii) anogenital distance not	Glyphosate acid Purity: 97.6% (w/w) Lot/Batch#: Y04707/082 0, 1000, 3000, 10000 ppm equivalent to mean achieved dose levels of: F0: 0, 99.4, 292.6, 984.7 mg/kg bw/day for males and 0, 104.4, 322.8, 1054.3 mg/kg bw/day for females during pre-mating period F1: 0, 116.5, 351 and 1161 mg/kg bw/day for males, and 0, 123.3, 370.8 and 1218.1 mg/kg bw/day for females, during the premating period	<u>F0 and F1 adults:</u> <u>1000 ppm:</u> No treatment related effects <u>3000 ppm:</u> No treatment related effects <u>10000 ppm:</u> ↓ bw for selected F1 parent males during the pre-mating period (up to 5% reduction compared to control group) <u>Offsprings:</u> <u>1000 ppm:</u> No treatment related effects <u>3000 ppm:</u> No treatment related effects <u>10000 ppm:</u> ↓ **bw** (10%) (F1A) NOAEL for parental toxicity: 10000 ppm (985 mg/kg bw/day, mean daily intake of glyphosate during pre-mating phase in F0 males of 10000 ppm group) NOAEL for offspring toxicity: 3000 ppm (293 mg/kg bw/day, mean daily intake of glyphosate during pre-mating phase in F0 males of 3000 ppm group)	[redacted] (2000) CA 5.6.1/004 Report No.: [redacted]/P/6332 New data for renewal: No

Method, guideline, deviations[1] if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results - NOAEL/LOAEL (for sexual function and fertility, parents) - target tissue/organ - critical effects at the LOAEL Adverse effects in bold text	Reference New data for renewal: Yes/No
examined as no treatment-related differences in sex ratio and sexual maturation were observed (iii) the thyroid was not weighed (iv) preimplantation loss not determined (v) pup development investigations restricted to body weight, vaginal opening and preputial separation	Administration: daily by dietary admixture throughout the treatment period. Control animals were treated in an identical manner with untreated laboratory diet. Exposure of F0 began at approximately 5 weeks of age. After 10 weeks of exposure F0 rats were mated. The appropriate experimental diet was fed throughout the study, to F0 and F1 parents and offspring until termination.	NOAEL for reproductive toxicity: 10000 ppm (985 mg/kg bw/day, mean daily intake of glyphosate during pre-mating phase in F0 males of 10000 ppm group)	
Two generation reproduction study (dietary) OECD TG 416 Rat Sprague Dawley Crj:CD (SD) M, F 24/sex/group GLP: Yes Acceptable The study was checked for compliance with current OECD TG 416 (2001) and following deviations were observed:	Glyphosate technical Purity: 94.61 % (w/w) Lot No.: T-950308 0, 1200, 6000, 30000 ppm Equivalent to: F0: 0, 83.6, 417, 2151 and 0, 96.9, 485, 2532 mg/kg bw/day in males and females, respectively F1: 0, 91.7, 458, 2411 and 0, 104.8, 530, 2760 mg/kg bw/day in males and females, respectively)	<u>F0 and F1 adults:</u> <u>1200 ppm:</u> No treatment-related effects <u>6000 ppm:</u> No treatment related effects <u>30000 ppm:</u> **-clinical signs** (loose stool) (F0, F1, both sexes) ↓bw (F0 males: 8%; F1 males: 7%) **-organ weight changes** (liver: F1 males: abs weight: ↑13%; F1 females: abs weight: ↑ 22%, rel weight: ↑ 20%; kidney: F0 males: rel weight: ↑11%; F1 males: rel weight: ↑14%; F1 females: abs weight: ↑17%, rel weight: ↑15%; prostate: F1 males abs and rel weight:↓32% **-lower fertility indices** of F1 females (not statistically significant) (79.2% compared to 95.8% in control) **-distension of caecum** (F0, F1) (both sexes) <u>Offsprings (F1 and F2):</u>	[redacted] (1997) CA 5.6.1/005 Report No.: [redacted] 96-0031 New data for renewal: No

Method, guideline, deviations[1] if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results - NOAEL/LOAEL (for sexual function and fertility, parents) - target tissue/organ - critical effects at the LOAEL Adverse effects in bold text	Reference New data for renewal: Yes/No
(i) testes were not used for enumeration of homogenisation-resistant spermatids but cauda epididymal sperm was enumerated (the guideline recommends both testes and epididymides to be used for enumeration of homogenisation-resistant spermatids and cauda epididymides sperm reserves, respectively) (ii) thyroid and spleen not weighed (iii) vaginal opening and preputial separation not examined (iv) anogenital distance not determined (v) no organ weighed for pups (the guideline recommends brain, speen and thymus to be weighed) (vi) pre-and post-implantation loss not reported (vii) number of corpora lutea not given. (viii) time to mating not reported (ix) number of animals used are	Administration: daily by dietary admixture throughout the treatment period. Control animals were treated in an identical manner with untreated laboratory diet. Exposure of F0 began at approximately 5 weeks of age. After 10 weeks of exposure F0 rats were mated. The appropriate experimental diet was fed throughout the study, to F0 and F1 parents and offspring until termination. Duration of administration for both F0 and F1 parental animals: a total of approximately 18 weeks (for a part of F1 parental animals duration of administration extended to approximately 22 weeks due to a 4-week extension for reciprocal crosses with untreated animals)	<u>1200 ppm:</u> No treatment-related effects <u>6000 ppm:</u> No treatment-related effects <u>30000 ppm:</u> ↓ **pup weights** (F1 males:14%, F1 females: 13%; F2 males: 9%, F2 females: 8%) **-distension of caecum** (F1 and F2 litters) NOAEL for parental, offspring and reproductive toxicity: 6000 ppm (417 mg/kg bw/day)	

Method, guideline, deviations[1] if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results - NOAEL/LOAEL (for sexual function and fertility, parents) - target tissue/organ - critical effects at the LOAEL Adverse effects in bold text	Reference New data for renewal: Yes/No
not in line with the recommendation by the guideline			
Two generation reproduction study (dietary) OECD TG 416 (1983) Rat Wistar rats (Random bred) M, F 30/sex/group GLP: Yes Supplementary only (effect dose lacking, limited parameters investigated in study) The study was checked for compliance with current OECD TG 416 (2001) and following deviations were observed: (i) the highest dose level was too low (the guideline recommends that the highest dose level should be chosen with the aim to induce toxicity but not death or severe suffering (ii) details of achieved concentrations not presented	Glyphosate technical Purity: 96.8 % (w/w) Batch No.: 60 0, 100, 1000, 10000 ppm Dietary level would correspond to a mean daily compound intake of 0, 7.7, 77 and 770 mg/kg bw/day. [The mean daily intake was not reported for all dietary levels, but for the low level of 100 ppm a corresponding average value of 7.7 mg/kg bw/d was given in the original report]. Administration by dietary admixture. Control animals were treated in an identical manner with untreated laboratory diet. P0 generation: Treatment commenced at 8th week age of parental generation and continued throughout the experimental period until P1	<u>F0 and F1 adults:</u> <u>100 ppm:</u> No treatment-related effects <u>1000 ppm:</u> No treatment-related effects <u>10000 ppm:</u> No treatment-related effects <u>Offsprings (F1 and F2):</u> <u>100 ppm:</u> No treatment-related effects <u>1000 ppm:</u> No treatment-related effects <u>10000 ppm:</u> No treatment-related effects NOAEL for parental, offspring and reproductive toxicity: 10000 ppm (would correspond to a mean daily compound intake of 700-800 mg/kg bw/d)	[redacted] (1993) CA 5.6.1/006 Report No.: TOXI 885-RP-G2 New data for renewal: No

Method, guideline, deviations[1] if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results - NOAEL/LOAEL (for sexual function and fertility, parents) - target tissue/organ - critical effects at the LOAEL Adverse effects in bold text	Reference New data for renewal: Yes/No
(iii) Parental animals were dosed at least 8 weeks before the mating period (the guideline recommend that dosing shall be continued for at least 10 weeks before the mating period) (iv) estrous cycle monitoring not performed (v) pre-coital interval not recorded (vi) data for gestation length not presented (vii) sperm analysis not performed (viii) monitoring of physical and sexual offspring development not performed (ix) organ weights not determined (x) a quantitative evaluation of primordial follicles not conducted (xi) no histopathology (only for organs found abnormal in the macroscopical investigation)	generation was weaned P1 generation: Treatment commenced from weanling and continued throughout the experimental period until F2 litters were weaned and parents and pups were sacrificed		
Two generation reproduction study (dietary) OECD TG 416 (1983) Rat	Glyphosate technical Purity: 99.2 % (w/w) Batch No.: 206-JaK-119-1	<u>F0 and F1 adults:</u> <u>1000 ppm:</u> No treatment-related effects <u>3000 ppm:</u> **-histopathological changes in salivary gland** (minimal hypertrophy of acinar cells with prominent granular cytoplasm) (Parotid, males: F0: 3/28, F1: 4/23; Parotid	[redacted] (1992) CA 5.6.1/007 CA 5.6.1/008 Report No.: [redacted] 47/911129 New data for renewal: No

Method, guideline, deviations[1] if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results - NOAEL/LOAEL (for sexual function and fertility, parents) - target tissue/organ - critical effects at the LOAEL Adverse effects in bold text	Reference New data for renewal: Yes/No
Sprague-Dawley Crl:CD (SD) BR VAF/Plus M, F 28/sex/group GLP: Yes Acceptable The study was checked for compliance with current OECD TG 416 (2001) and following deviations were observed: (i) relative humidity in experimental animal room was 46%±24% (the guideline recommends the relative humidity to be at least 30%) (ii) pre-mating oestrous cycles not determined (iii) pre- and post-implantation loss were not reported (iv) sperm analysis not performed (v) uterus, spleen and thyroid of parental animals not weighed (vi) brain, spleen and thymus of pups not weighed	0, 1000, 3000, 10000 ppm Equivalent to: <u>F0:</u> 0, 66, 197, 668 and 0, 75, 226, 752 mg/kg bw/day in males and females, respectively <u>F1:</u> 0, 76, 230, 771 and 0, 82, 245, 841 mg/kg bw/day in males and females, respectively) Administration: daily by dietary admixture throughout the treatment period. Control animals were treated in an identical manner with untreated laboratory diet. Exposure of F0 began at approximately 6 weeks of age. After 10 weeks of exposure F0 rats were mated. The appropriate experimental diet was fed throughout the study, to F0 and F1 parents and offspring until termination. F0 animals were treated from 6 weeks of age for up to 29 weeks, F1 animals were treated up to 37 weeks of age	females: F0: 5/28, F1: 4/24; Submaxillary, females: F0: 4/28; F1: 0/24) <u>10000 ppm:</u> ↑water consumption (F1 females, 17%) ↑food intake (F1 females) (3%) ↓ mean bw (F1 males, 1-7%) **-histopathological changes in salivary gland** (increased incidence of minimal hypertrophy of acinar cells with prominent granular cytoplasm) (Parotid, males: F0: 12/26, F1: 11/23; Parotid females: F0: 17/28, F1: 9/23; Submaxillary, females: F0: 14/28; F1: 3/23) <u>Offsprings (F1 and F2):</u> <u>1000 ppm:</u> No treatment-related effects <u>3000 ppm:</u> No treatment-related effects <u>10000 ppm:</u> No treatment-related effects The NOAEL for parental toxicity was set at 1000 ppm (66 mg/kg bw/day) The NOAEL for offspring and reproductive toxicity was set at 10000 ppm (668 mg/kg bw/day).	
One-generation range finding study (dietary)	Glyphosate technical	*Note: No statistically analyses conducted in this study*	[redacted] (1991) CA 5.6.1/009

Method, guideline, deviations[1] if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results - NOAEL/LOAEL (for sexual function and fertility, parents) - target tissue/organ - critical effects at the LOAEL Adverse effects in bold text	Reference New data for renewal: Yes/No
No guideline Rat Sprague-Dawley Crl:CD (SD) BR VAF/Plus M, F F0 females: 10 time mated/group F1 generation: 10/sex/group GLP: No Supplementary only (low number of animals, limited parameters investigated, no statistics) The study was checked for compliance with OECD TG 416 (2001) and following deviations were observed: (i) only 10 females/group were used (the guideline recommends 20 pregnant females/group) (ii) the F0 females were time-mated (the guideline recommends a mating procedure) (iii) duration of study was only 10 weeks. F0 exposed from	Purity: 98.6 % (w/w) Lot/Batch No.: 206-Jak-25-1 0, 3000, 10000, 30000 ppm Equivalent to: <u>F0 females:</u> 0, 236-311, 799-1010 and 2515-2789 mg/kg bw/day <u>F1 offspring:</u> 0, 368-390, 1291-1335 and 3918-4453 mg/kg/day for males and 355-402, 1191-1271 and 3961-4397 mg/kg/day for females Administration: daily by dietary admixture throughout the treatment period. Control animals were treated in an identical manner with untreated laboratory diet. Time-mated F0 females were used. Exposure of F0 females began at Day 3 of pregnancy and was continued through pregnancy to termination of the study. The study duration was 10 weeks	<u>Adults (F0):</u> <u>3000 ppm:</u> ↓bw gain by Day 14 of pregnancy (F0 females: 2% compared to control) -macroscopic salivary gland changes (enlarged/firm/congested/swollen) (F0 females: 2/9) -macroscopic gastro-intestinal changes (content watery and/or dark: F0 females (2/9); stomach distended and/or congested: F0 females (2/9)) -microscopical changes in salivary gland (minimal granular basophilic cytoplasm of acinar cells with minimal hypertrophy of acinar cells) (F0 females: 2/9) <u>10000 ppm:</u> **-clinical signs** (soft faeces and yellow stained sawdust) (F0 females) ↓ bw gain by Day 14 of pregnancy (F0 females: 3% compared to control) **-macroscopic gastrointestinal changes** (content watery and/or dark: F0 females (7/10); stomach distended and/or congested: F0 females (5/10) **-macroscopic salivary gland changes** (enlarged/firm/congested/swollen) (F0 females 6/10) **-microscopical changes** in salivary gland (moderate/marked granular basophilic cytoplasm of acinar cells and minimal/moderate hypertrophy of acinar cells (F0 females: 10/10) <u>30000 ppm:</u> **-mortality** (one F0 animal died on Day 21 *post partum*, cause of death not known) **-clinical signs** (soft faeces and yellow stained sawdust) (F0 females) -increased water consumption towards the end of pregnancy (F0 females: 11%) ↓ **bw gain** F0 females: Gestation Day 6 (23%), 14 (22%), 20 (11%); Lactation Day 7 (19%), 14 (39%), 21 (47%) ↓ bw F0 females: Gestation day 6 (2%), 14 (7%), 20 (5%); Lactation Day 7 (5%), 14 (13%), 21 (14%) **-macroscopic gastrointestinal changes** (content watery and/or dark: F0 females (8/9); stomach distended and/or congested: F0 females (4/9); distended caecum (4/9) **-macroscopic salivary gland changes**	Report No. [redacted] 42/90619 New data for renewal: No

Method, guideline, deviations[1] if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results - NOAEL/LOAEL (for sexual function and fertility, parents) - target tissue/organ - critical effects at the LOAEL Adverse effects in bold text	Reference New data for renewal: Yes/No
Day 3 of pregnancy through the termination of the study, females were allowed to litter and rear their young to weaning, when 10 males and 10 female offspring per group were selected and reared on their respective diets to six weeks of age (the guideline recommends that F0 animals are dosed at least 10 weeks before mating period, dosing continued in both sexes during the 2 week mating period and continued throughout pregnancy and up to the weaning of the F1 offspring. The same procedure for the F1 offspring to produce the F2 generation) (iv) oestrous cycle not evaluated (v) litter parameters limited (vi) sperm parameters not evaluated (vii) sexual maturation not investigated (viii) no organ weighed		(enlarged/firm/congested/swollen) F0 females (8/9) **-microscopical changes** in salivary gland (marked granular basophilic cytoplasm of acinar cells and moderate hypertrophy of acinar cells (9/9) and prominent mitoses in acinar cells (2/9)) <u>Pups to F0 generation:</u> <u>3000 ppm:</u> ↓ **mean pup weight** (Day 21 *post partum*: 9%) -macroscopical changes in salivary gland (congested) (one pup) (significance unclear) <u>10000 ppm:</u> ↓ **mean pup weight** (Day 21 *post partum*: 13%) -macroscopical changes in salivary gland (congested) (4 pups) (significance unclear) <u>30000 ppm:</u> ↓ **mean pup weight** (at birth: 4.5%, Day 21 *post partum*: 38%) <u>Offspring from birth to 6 weeks of age (F1 generation):</u> <u>3000 ppm:</u> -macroscopical changes in parotid salivary gland (enlarged/swollen) (1/10 male) <u>10000 ppm:</u> No treatment-related effects <u>30000 ppm:</u> **-clinical signs** (soft faeces) ↓ **bw gain** (Day 42 *post partum*: males: 25%; females: 15%) ↓ food during Weeks 5 (22%) and 6 (12%) (males only) **-macroscopical changes in parotid salivary gland** (enlarged/swollen) (males 5/10, females 2/10) **-macroscopic gastrointestinal changes** (soft content: males (7/10), females (9/10)) The study is not suitable for NOAEL setting. Study acceptable as dose range	

Method, guideline, deviations[1] if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results - NOAEL/LOAEL (for sexual function and fertility, parents) - target tissue/organ - critical effects at the LOAEL Adverse effects in bold text	Reference New data for renewal: Yes/No
(ix) histopathology limited (salivary glands investigated only) (x) statistical analyses not performed		finding study only (low number of animals, limited parameters investigated, no statistics)	
Two generation reproduction study (dietary) No guideline Rat Sprague-Dawley M, F 30/sex/group GLP: Yes Acceptable The study was checked for compliance with current OECD TG 416 (2001) and following deviations were observed: (i) minor deviations in housing conditions: Temperature was 18-26°C (the guideline recommends that the temperature in the experimental animal room should be 22 ± 3°C) (ii) no data on food efficiency (iii) no details on fertility indices, number of live	Glyphosate Purity: 97.67 % (w/w) Lot No.: XLI-203 0, 2000, 10000, 30000 ppm Corresponding to 132-140, 666-711, 1983-2230 mg/kg bw/day for males and 160-163, 777-804, 2322-2536 mg/kg bw/day for females) (calculated for F0 and F1A adults) F0 generation rats (30/sex/group) were administered daily by dietary admixture for approximately 11 weeks and then mated to produce the F1a generation; 30 rats/sex/group from the F1 a generation were similarly exposed (approximately 14 weeks) and mated twice, to produce the F2a and F2b generations	Adults: 2000 ppm: No treatment-related effects 10000 ppm: No treatment-related effects 30000 ppm: **-clinical signs (soft stool)** **↓bw** (Terminal bw: F0 males: 8%, F1 males: 13%, F1 females: 10%; Maternal bw during gestation: Day 1: F0 females: 7%, F1 females first mating: 12%, F1 females second mating: 13%; Day 21: F0 females: 7%, F1 females first mating: 8%, F1 females second mating: 8%) **↓ litter size** (F0 dams: 13%) Pups: 2000 ppm: No treatment-related effects 10000 ppm: No treatment related effects 30000 ppm: **↓ pup weight** (Day 21: F0 males: 13%, F0 females: 11%; F1A males and females: 14%; F1B males: 19%, F1B females: 13%) NOAEL for parental, offspring and reproductive toxicity: 10000 ppm (666-711 mg/kg bw/day for males and 777-804 mg/kg bw/day for females)	[redacted] (1990) CA 5.6.1/010 Report No. [redacted]-10387 New data for renewal: No

Method, guideline, deviations[1] if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results - NOAEL/LOAEL (for sexual function and fertility, parents) - target tissue/organ - critical effects at the LOAEL Adverse effects in bold text	Reference New data for renewal: Yes/No
births and pre- and post-implantation loss (iv) no determination of oestrus cycle length (v) sperm analysis not performed (vi) no determination of physical or sexual development landmarks (vii) parental animals: only ovaries and testes with epididymides weighed (uterus, prostate, seminal vesicles, brain, liver, kidneys, spleen, pituitary, thyroid and adrenal glands not weighed) (viii) brain, spleen and thymus of pups not weighed (ix) coagulating gland and cervix not included in the histopathological examination (x) no details on number of pups with grossly visible abnormalities			
One generation reproduction study Oral (gavage) No guideline Rat	Glyphosate technical Purity: not reported Lot No.: not reported	5 and 10 mg/kg bw/day: No treatment related effects	[redacted] (1988)[1] CA 5.6.1/011 Report No.: not specified New data for renewal: No

Method, guideline, deviations[1] if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results - NOAEL/LOAEL (for sexual function and fertility, parents) - target tissue/organ - critical effects at the LOAEL Adverse effects in bold text	Reference New data for renewal: Yes/No
Wistar M, F Groups of 10 males and 30 females GLP: No Study not acceptable (dose levels tested much too low and other major deviations from OECD TG 416 (2001)) The study was checked for compliance with current OECD TG 416 (2001) and following deviations were observed: (i) purity not specified (ii) group size too small (12-13 dams/group) (the guideline recommends at least 20 dams/group) (iii) parental animals were dosed 60 days (males) or 14 days (females) before the mating period (the guideline recommends that dosing shall be continued for at least 10 weeks before the mating period) (iv) animals were mated in a sex ratio of 1	0, 5, 10 mg/kg bw/day Vehicle: corn oil Administration prior to mating (males 60 days and females 14 days before mating), during pregnancy and up to day 21 of lactation		

Method, guideline, deviations[1] if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results - NOAEL/LOAEL (for sexual function and fertility, parents) - target tissue/organ - critical effects at the LOAEL Adverse effects in bold text	Reference New data for renewal: Yes/No
male:3 females (the guideline recommends that each female shall be placed with a single male from the same dose level (1:1 mating)) (v) only two dose levels were used (the guideline recommends three dose levels) (vi) dose levels tested too low (the guideline recommends that the highest dose level should be chosen with the aim to induce toxicity but not death or severe suffering) (vii) body weights for females during premating period and until Day14 of gestation missing (viii) oestrous cycle monitoring not performed (ix) pre-coital interval not recorded (x) duration of gestation not evaluated (xi) no sperm analyses (xii) a quantitative evaluation of primordial follicles not conducted (xiii) sexual maturation not investigated			

Method, guideline, deviations[1] if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results - NOAEL/LOAEL (for sexual function and fertility, parents) - target tissue/organ - critical effects at the LOAEL Adverse effects in bold text	Reference New data for renewal: Yes/No
(xiv) no organ weighed (xv) histopathology not performed except for testis (xvi) statistics not reported			
Three-generation reproduction study (dietary) No guideline Rat Wistar M, F Groups of 8 males and 16 females GLP: No Study not acceptable (dose levels tested much too low and other major deviations from the OECD TG 416 (2001)) The study was checked for compliance with current OECD TG 416 (2001) and following deviations were observed: (i) purity not specified (ii) parental animals were dosed 60 days (males) or 14 days (females) before the mating period (the guideline	Glyphosate technical Purity: not reported Lot No.: not reported 0, 75, 150, 300 ppm (using a conversion factor of 20, this concentration would correspond to an approximate daily intake of 0, 3.75, 7.5 and 15 mg/kg bw/day) F0 generation rats were administered daily by dietary admixture for 60 days (males) or 14 days (females). Animals were kept on this diet until weanling of the F3b generation	75, 150 and 300 ppm: No treatment related effects	[redacted] (1988)[1] CA 5.6.1/012 Report No.: not specified New data for renewal: No

Method, guideline, deviations[1] if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results - NOAEL/LOAEL (for sexual function and fertility, parents) - target tissue/organ - critical effects at the LOAEL Adverse effects in bold text	Reference New data for renewal: Yes/No
recommends that dosing shall be continued for at least 10 weeks before the mating period) (iii) animals were mated in a sex ratio of 1 male:2 females to produce the F1 litters (the guideline recommends that each female shall be placed with a single male from the same dose level (1:1 mating)) (iv) Age of F0 animals at initiating of dosing was 14 weeks (the guideline recommends the animals to be 5 to 9 weeks old at the start of dosing) (v) group size too small (12-16 dams/group) (the guideline recommends at least 20 dams/group) (vi) dose levels tested too low (the guideline recommends that the highest dose level should be chosen with the aim to induce toxicity but not death or severe suffering) (vii) no information on pre-mating dosing period			

Method, guideline, deviations[1] if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results - NOAEL/LOAEL (for sexual function and fertility, parents) - target tissue/organ - critical effects at the LOAEL Adverse effects in bold text	Reference New data for renewal: Yes/No
(viii) body weights during gestation not recorded (ix) oestrous cycle monitoring not performed (x) pre-coital interval not recorded (xi) duration of gestation not evaluated (xii) no sperm analyses (xiii) a quantitative evaluation of primordial follicles not conducted (xiv) physical and sexual maturation not investigated in offsprings (xv) no organ weighed (xvi) vagina, cervix, epididymides, prostate, coagulating gland not included in the histopathological examination for adults (xvii) statistics not reported			
Three-generation reproduction study (dietary) No guideline Rat Wistar MD M, F F0 generation: 6/sex/dose	Glyphosate Purity: not reported Lot No.: not reported 0, 200, 1000, 5000 ppm (0, 17.8, 92.2 and 462.2 mg/kg bw/day in males	200 ppm **-histopathological findings in stomach** (widened gastric mucosa and fundus mucosa (F1B males and females); increased incidences of small bleedings in the glandular stomach of the antrum (F1B males and females)) **-histopathological findings in pancreas** (fatty degeneration) (F0, F1B, F2B males and females) 1000 ppm **-reduced lactation index** (F3B; 31%)	[redacted] (1985)[2] CA 5.6.1/013 Report No.: not specified New data for renewal: No

Method, guideline, deviations[1] if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results - NOAEL/LOAEL (for sexual function and fertility, parents) - target tissue/organ - critical effects at the LOAEL Adverse effects in bold text	Reference New data for renewal: Yes/No
F1B generation: 12/sex/dose F2B generation: 24/sex/dose F3C generation: 10/sex/dose GLP: No Study not acceptable (reporting deficiencies and major deviations from the OECD TG 416 (2001)) The study was checked for compliance with current OECD TG 416 (2001) and following deviations were observed: (i) purity not specified (ii) lot/batch number not specified (iii) no analytical determinations on stability and homogeneity of the test material (iv) mating period was 6 consecutive days (the guideline recommends a 2-week mating period) (v) during mating, males changed daily so that each female cohabited with different males (the guideline recommends for each mating	and 0, 19.2, 106.0 and 502.0 mg/kg bw/day in females) Rats were administered the test substance in their diet for 12-week period before first mating. Males and females were mated in a sex ratio of 1:1. In the F1 and F2 generation, there were two mating. In contrast, three litters were produced in the F3 generations. The number of paired animals varied between the generations. In the F0 generation, 6 males and 6 females were used. The respective numbers were 12 and 24 for the F1B and F2B generations. These animals were sacrificed on day 28 after parturition. Glyphosate administration was continued until this day. In the F3C generation, 10 animals per sex and dose group were selected by randomisation and examined following an 8-week dosing period and a subsequent 4-	**-histopathological findings in stomach** (widened gastric mucosa and fundus mucosa (F1B and F2B males and females); increased incidences of small bleedings in the glandular stomach of the antrum (F1B and F2B males and females)) **-histopathological findings in pancreas** (fatty degeneration) (F0, F1B, F2B males and females) <u>5000 ppm</u> **-reduced fertility index** (F1A: 67% compared to 83% in control; F1B: 67% compared to 100% in control) **-reduced lactation index** (F3B; 30%) **-macroscopical changes in thymus** involution, F1B males) -macroscopical changes in liver (clay-coloured, F1B males) **-histopathological findings in thymus** (involution, F1B) **-histopathological findings in stomach** (widened gastric mucosa and fundus mucosa (F1B and F2B males and females) **-histopathological findings in pancreas** (fatty degeneration) (F0, F1B, F2B males and females) The study is not suitable for NOAEL setting. Study not acceptable due to reporting deficiencies and major deviations from OECD TG 416)	

Method, guideline, deviations[1] if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results - NOAEL/LOAEL (for sexual function and fertility, parents) - target tissue/organ - critical effects at the LOAEL Adverse effects in bold text	Reference New data for renewal: Yes/No
each female should be placed with a single male from the same dose level (1:1 mate) until copulation occurs 2 weeks have elapsed). (vi) age of F0 animals at initiating of dosing was 28 days (the guideline recommends the animals to be 5 to 9 weeks old at the start of dosing) (vii) few animals were used. Six F0 animals/sex/dose group were used for production of the F1 generations, F1A and F1B generations. Twelve animals/sex/dose groups of the F1B generation were mated to get F2 generations (the guideline recommends each test and control group should contain a sufficient number of animals to yield preferable not less than 20 pregnant females at or near parturition) (viii) oestrous cycle monitoring not performed	week recovery phase.		

Method, guideline, deviations[1] if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results - NOAEL/LOAEL (for sexual function and fertility, parents) - target tissue/organ - critical effects at the LOAEL Adverse effects in bold text	Reference New data for renewal: Yes/No
(ix) pre-coital interval not recorded (x) no sperm analyses (xi) a quantitative evaluation of primordial follicles not conducted (xii) sexual maturation not investigated in offsprings (xiii) uterus, ovaries, seminal vesicles with coagulating glands, pituitary and thyroid not weighed (xiv) vagina, uterus with cervix, ovaries, testis, epididymidis, prostate, seminal vesicles, coagulating gland not included in the histopathological examination for adults			
Three generation reproduction study (dietary) No guideline Rat CD® (Sprague Dawley derived) M, F Groups of 12 males and 24 females GLP: No	Glyphosate Purity: considered 100% active ingredient for dosing preparations Lot No.: XHJ-64 0, 3, 10, 30 mg/kg bw/day Rats were administered glyphosate oral vid diet continuously for	3, 10 and 30 mg/kg bw/day: No treatment related effects	[redacted] (1981)[1] CA 5.6.1/014 Report No.: 77-2063 New data for renewal: No

Method, guideline, deviations[1] if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results - NOAEL/LOAEL (for sexual function and fertility, parents) - target tissue/organ - critical effects at the LOAEL Adverse effects in bold text	Reference New data for renewal: Yes/No
Study not acceptable (dose levels tested much too low) The study was checked for compliance with current OECD TG 416 (2001) and following deviations were observed: (i) parental animals were dosed 63 days before the mating period (the guideline recommends that dosing shall be continued for at least 10 weeks before the mating period) (ii) animals were mated in a sex ratio of 1 male:2 females to produce the F1 litters (the guideline recommends that each female shall be placed with a single male from the same dose level (1:1 mating)) (iii) dose levels tested much too low (the guideline recommends that the highest dose level should be chosen with the aim to induce toxicity but not death or severe suffering) (iv) no information on	three successive generations. Parental animals were dosed 63 days prior to mating.		

Method, guideline, deviations[1] if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results - NOAEL/LOAEL (for sexual function and fertility, parents) - target tissue/organ - critical effects at the LOAEL Adverse effects in bold text	Reference New data for renewal: Yes/No
pre-mating dosing period (ix) oestrous cycle monitoring not performed (x) pre-coital interval not recorded (xii) no sperm analyses (xiii) a quantitative evaluation of primordial follicles not conducted (xiv) physical and sexual maturation not investigated in offsprings (xv) thymus of pups not weighed			

[1] Study not considered acceptable. The scientific value of this study was limited mainly because the dose levels tested were much too low for the identification of harmful effects of glyphosate administration on reproduction. The study was presented in the table but not considered further in this report.
[2] Study not considered acceptable. The scientific value of this study was limited due to several deviations and reporting deficiencies. The study was presented in the table but not considered further in this report.

The potential of glyphosate to cause effects on sexual function and fertility was examined in several generational studies in the rat (Table 57). All studies listed in Table 57 were submitted and evaluated in previous EU evaluations (RAR 2013 and/or DAR1998). There are no new standard toxicity studies (generational studies) submitted for this report.

In three studies (one study by [redacted] (1981) and two studies by [redacted] (1988)), the top dose levels were considered much too low to reveal any toxic effect. Thus, these studies were not acceptable (studies not suitable for the purpose of classification and labelling). Also, the study by [redacted] (1985) was considered not acceptable. This study was limited due to several deviations and reporting deficiencies). For more details about all these unacceptable studies, please see Vol. 3-B.6 (AS), section B.6.6.1 (Generational studies).

Acceptable and supplementary studies are shortly summarised in text (below):

Two-generation reproductive toxicity study in the rat ([redacted] *et al.* (2007), Report No. 2060/0013)

In this study, groups of 28 male and 28 female (F0) parents Sprague-Dawley Crl:CD (SD) IGS BR strain rats were fed diet containing 0, 1500, 5000 or 15000 ppm glyphosate technical (equivalent to mean achieved dose levels of 0, 104, 351 and 1063 mg/kg bw/day for males, and 0, 162, 530 and 1634 mg/kg bw/day for females). After 10 weeks, the animals were mated and allowed to rear the F1 litters to weaning. The regime was repeated with the F1 parents (24 males and 24 females for each group). Pregnant F1 females were allowed to give birth and maintain their offspring on the appropriate diet until Day 21 *post partum*.

Increased liver weights were observed in adult females of both generations at 15000 ppm (F0 females: absolute weight: ↑13%, relative weight: ↑8%; F1 females: absolute weight: ↑10%, relative weight: ↑8%), and increased kidney weights were observed in adult F0 females at 15000 ppm (absolute weight: ↑11%, relative weight: ↑7%).

Lower number of homogenisation resistant spermatid present in the cauda epididymis was observed in F0 generation males at 15000 ppm (309 million/gram compared to 400 million/gram in control). No sperm changes were seen in testis, and histopathological examinations did not reveal any changes in the testis or epididymis. Offspring toxicity was confined to delayed preputial separation observed at F1 males at 15000 ppm (Days at completion: 45.9 compared to 43.0 in control). Although, the later onset of preputial separation in male offspring at 15000 ppm had no impact on reproductive performance in week 29, a treatment related effect on sexual maturation at high dose level cannot be excluded.

The NOAEL for parental toxicity was set at 5000 ppm based on increased liver and kidney weights observed in females of both generations at 15000 ppm. The NOAEL for offspring was set at 5000 ppm based on delayed sexual maturation (preputial separation) observed in F1 male offspring at 15000 ppm. The NOAEL for reproductive toxicity was set at 5000 ppm based on reduced number of homogenisation resistant spermatid in cauda epididymis observed in F0 generation males at 15000 ppm. The study is acceptable. The study is conducted in accordance with GLP and follows OECD TG 416 (2001).

Table 2.6.6.1-1: Liver, kidney and thyroid weights (relative and absolute) of females (group mean values)

Dietary concentration (ppm)	No. of animals		Organ weight (g)					
			Liver		Kidney		Thyroid	
			Absolute	Relative	Absolute	Relative	Absolute	Relative
			F0 Generation					
0 (Control)	26	mean	15.0328	4.3103	2.4315	0.6977	0.0475	0.0137
		sd	1.0493	0.2864	0.1706	0.0548	0.0087	0.0023
1500	27	mean	15.1465	4.3027	2.5395	0.7233	0.0504	0.0143
		sd	1.4948	0.3435	0.1602	0.0560	0.0136	0.0036
5000	27	mean	15.8791	4.3570	2.5654* (5%)	0.7062	0.0579* (22%)	0.0159
		sd	1.7649	0.2810	0.2361	0.0592	0.0099	0.0026
15000	26	mean	16.9704*** (13%)	4.6806*** (8%)	2.7096*** (11%)	0.7490** (7%)	0.0563 (19%)	0.0156
		sd	1.7620	0.2977	0.2203	0.0521	0.0085	0.0021
			F1 Generation					
0 (Control)	22	mean	16.4887	4.5970	2.6792	0.7483	0.1024	0.0288
		sd	2.0275	0.4038	0.4137	0.1070	0.0212	0.0064
1500	23	mean	16.3848	4.6047	2.5777	0.7257	0.0948	0.0267
		sd	1.7744	0.2858	0.2776	0.0647	0.0192	0.0051
5000	24	mean	17.2591	4.6543	2.8124	0.7585	0.1063	0.0289
		sd	2.0969	0.3628	0.5326	0.1229	0.0164	0.0055
15000	23	mean	18.0724* (10%)	4.9591** (8%)	2.7660	0.7578	0.0937	0.0258
		sd	1.2434	0.3130	0.2616	0.0517	0.0120	0.0039

sd standard deviation
* significantly different from control group $p < 0.05$
** significantly different from control group $p < 0.01$
*** significantly different from control group $p < 0.001$

Table 2.6.6.1-2: Relative organ weights for F0 males -selected (group mean values)

Dietary Concentration (ppm)	Number of Animals		Bodyweight (g) at Terminal Kill	Relative Organ Weight (%)							
				Adrenals	Brain	Epididymides Left	Epididymides Right	Kidneys	Left Cauda	Liver	Pituitary
0 (Control)	28	mean	596	0.0098	0.3668	0.1283	0.1346	0.6694	0.0568	3.1481	0.0020
		sd	60	0.0027	0.0313	0.0214	0.0168	0.0635	0.0110	0.1846	0.0007
1500	27	mean	614	0.0090	0.3505	0.1253	0.1290	0.6514	0.0575	3.0595	0.0021
		sd	59	0.0021	0.0339	0.0180	0.0158	0.0556	0.0098	0.2771	0.0003
5000	28	mean	617	0.0096	0.3484	0.1307	0.1315	0.6603	0.0602	3.0996	0.0019
		sd	50	0.0028	0.0335	0.0176	0.0151	0.0696	0.0114	0.1918	0.0004
15000	28	mean	591	0.0104	0.3657	0.1296	0.1346	0.6961	0.0605	3.2645	0.0023
		sd	73	0.0025	0.0392	0.0144	0.0188	0.0736	0.0092	0.2908	0.0005

Dietary Concentration (ppm)	Number of Animals		Bodyweight (g) at Terminal Kill	Relative Organ Weight (%)						
				Prostate	Seminal Vesicles	Spleen	Testes Left	Testes Right	Thymus	Thyroid
0 (Control)	28	mean	596	0.1219	0.4925	0.1501	0.3085	0.3145	0.0672	0.0250
		sd	60	0.0302	0.0948	0.0252	0.0322	0.0344	0.0182	0.0101
1500	27	mean	614	0.1131	0.4712	0.1423	0.2973	0.2992	0.0694	**0.0196
		sd	59	0.0308	0.0826	0.0188	0.0338	0.0333	0.0173	0.0069
5000	28	mean	617	0.1156	0.4954	0.1460	0.2983	0.3040	0.0692	*0.0189
		sd	50	0.0411	0.0958	0.0276	0.0293	0.0286	0.0142	0.0044
15000	28	mean	591	0.1214	0.5170	0.1563	0.3178	0.3126	0.0700	0.0206
		sd	73	0.0296	0.1021	0.0810	0.0402	0.0454	0.0162	0.0045

sd=standard deviation
*=significantly different from control group p<0.05
**= significantly different from control group p<0.01

Table 2.6.6.1-3: Absolute organ weights for F1 males-selected (group mean values)

Dietary Concentration (ppm)	Number of Animals		Bodyweight (g) at Terminal Kill	Organ Weight (g)							
				Adrenals	Brain	Epididymides Left	Epididymides Right	Kidneys	Left Cauda	Liver	Pituitary
0 (Control)	24	mean	599	0.0896	2.1963	0.7363	0.7594	4.0604	0.3122	19.7293	0.0148
		sd	51	0.1051	0.1172	0.0764	0.0847	0.4264	0.0480	2.1994	0.0045
1500	24	mean	596	0.0607	2.1255	0.8273	0.8585	4.0099	0.4029	19.5341	0.0128
		sd	66	0.0171	0.1023	0.1011	0.1084	0.3983	0.1239	2.6825	0.0042
5000	24	mean	611	0.0600	2.1254	0.8912	0.8412	4.2958	0.3567	20.1228	0.0150
		sd	61	0.0161	0.1253	0.2685	0.0894	0.6573	0.0661	2.2770	0.0040
15000	24	mean	580	0.0525	**2.0975	0.7205	0.8001	4.1505	0.3172	19.3033	0.0175
		sd	45	0.0140	0.0995	0.1422	0.0985	0.3138	0.0331	2.0957	0.0231

Dietary Concentration (ppm)	Number of Animals		Bodyweight (g) at Terminal Kill	Organ Weight (g)						
				Prostate	Seminal Vesicles	Spleen	Testes Left	Testes Right	Thymus	Thyroid
0 (Control)	24	mean	599	0.7789	3.0146	0.8983	1.7672	1.7750	0.4314	0.1277
		sd	51	0.2084	0.4849	0.1428	0.2843	0.2622	0.1038	0.0336
1500	24	mean	596	0.6900	3.0679	0.8890	1.8950	1.8998	0.4878	0.1286
		sd	66	0.1480	0.4526	0.1222	0.1711	0.1767	0.1653	0.0195
5000	24	mean	611	0.7865	2.9590	0.9413	1.9209	1.9091	0.4738	0.1239
		sd	61	0.1679	0.5543	0.1915	0.1683	0.1290	0.0998	0.0255
15000	24	mean	580	0.6826	3.1101	*0.7852	1.8330	1.8717	0.4100	0.1270
		sd	45	0.1465	0.4335	0.1842	0.1477	0.1533	0.1127	0.0225

sd=standard deviation
*=significantly different from control group p<0.05
**=significantly different from control group p<0.01

Table 2.6.6.1-4: Relative organ weights for F1 males- selected (group mean values)

Dietary Concentration (ppm)	Number of Animals		Bodyweight (g) at Terminal Kill	Relative Organ Weight (%)							
				Adrenals	Brain	Epididymides Left	Epididymides Right	Kidneys	Left Cauda	Liver	Pituitary
0 (Control)	24	mean	599	0.0153	0.3687	0.1240	0.1278	0.6824	0.0528	3.2962	0.0025
		sd	51	0.0191	0.0269	0.0178	0.0182	0.0875	0.0104	0.2172	0.0007
1500	24	mean	596	0.0102	0.3601	0.1397	0.1450	0.6748	0.0683	3.2699	0.0021
		sd	66	0.0027	0.0383	0.0188	0.0201	0.0481	0.0229	0.1797	0.0006
5000	24	mean	611	0.0099	0.3512	0.1468	0.1389	0.7080	0.0590	3.2955	0.0025
		sd	61	0.0026	0.0389	0.0445	0.0188	0.1202	0.0127	0.1898	0.0007
15000	24	mean	580	0.0092	0.3632	0.1253	0.1386	0.7177	0.0550	3.3295	0.0029
		sd	45	0.0026	0.0225	0.0259	0.0184	0.0499	0.0071	0.2438	0.0036

Dietary Concentration (ppm)	Number of Animals		Bodyweight (g) at Terminal Kill	Relative Organ Weight (%)						
				Prostate	Seminal Vesicles	Spleen	Testes Left	Testes Right	Thymus	Thyroid
0 (Control)	24	mean	599	0.1303	0.5091	0.1506	0.2978	0.2991	0.0725	0.0214
		sd	51	0.0336	0.1034	0.0243	0.0549	0.0521	0.0185	0.0056
1500	24	mean	596	0.1162	0.5168	0.1491	0.3204	0.3213	0.0806	0.0216
		sd	66	0.0248	0.0712	0.0114	0.0376	0.0392	0.0229	0.0028
5000	24	mean	611	0.1297	0.4889	0.1544	0.3172	0.3156	0.0777	0.0205
		sd	61	0.0323	0.1050	0.0288	0.0378	0.0373	0.0151	0.0047
15000	24	mean	580	0.1190	0.5397	0.1351	0.3182	0.3248	0.0705	0.0220
		sd	45	0.0296	0.0853	0.0304	0.0365	0.0364	0.0176	0.0042

sd=standard deviation

Table 2.6.6.1-5: Absolute organ weights for F0 females- selected (group mean values)

Dietary Concentration (ppm)	Number of Animals		Bodyweight (g) at Terminal Kill	Organ Weight (g)				
				Adrenals	Brain	Kidneys	Liver	Ovaries
0 (Control)	26	mean	349	0.0821	1.9629	2.4315	15.0328	0.1292
		sd	20	0.0139	0.0930	0.1706	1.0493	0.0263
1500	27	mean	352	0.0806	1.9566	2.5395	15.1465	0.1328
		sd	27	0.0154	0.0807	0.1602	1.4948	0.0283
5000	27	mean	364	0.0800	1.9614	*2.5654	15.8791	0.1331
		sd	26	0.0132	0.0800	0.2361	1.7649	0.0212
15000	26	mean	362	0.0827	1.9745	***2.7096	***16.9704	0.1231
		sd	28	0.0107	0.0689	0.2203	1.7620	0.0213

Dietary Concentration (ppm)	Number of Animals		Bodyweight (g) at Terminal Kill	Organ Weight (g)				
				Pituitary	Spleen	Thymus	Thyroid	Uterus
0 (Control)	26	mean	349	0.0150	0.5991	0.1622	0.0475	0.9071
		sd	20	0.0030	0.0777	0.0539	0.0087	0.4754
1500	27	mean	352	0.0139	0.6063	0.1789	0.0504	0.8579
		sd	27	0.0035	0.0961	0.0679	0.0136	0.4507
5000	27	mean	364	0.0151	0.6037	0.1810	*0.0579	0.9378
		sd	26	0.0034	0.1028	0.0451	0.0099	0.4743
15000	26	mean	362	0.0151	0.5824	0.1585	0.0563	0.7508
		sd	28	0.0028	0.0755	0.0544	0.0085	0.5229

sd=standard deviation
*=significantly different from control group p<0.05
***=significantly different from control group p<0.001

Table 2.6.6.1-6: Absolute organ weights for F1 females- selected (group mean values)

Dietary Concentration (ppm)	Number of Animals		Bodyweight (g) at Terminal Kill	Organ Weight (g) Adrenals	Brain	Kidneys	Liver	Ovaries
0 (Control)	22	mean	358	0.0903	1.9433	2.6792	16.4887	0.1277
		sd	24	0.0117	0.0720	0.4137	2.0275	0.0262
1500	23	mean	356	0.0917	1.9114	2.5777	16.3848	0.1394
		sd	27	0.0261	0.0780	0.2776	1.7744	0.0288
5000	24	mean	370	0.1366	1.9233	2.8124	17.2591	0.1423
		sd	26	0.1607	0.1107	0.5326	2.0969	0.0236
15000	23	mean	365	0.0903	1.8950	2.7660	*18.0724	0.1358
		sd	24	0.0124	0.2331	0.2616	1.2434	0.0359

Dietary Concentration (ppm)	Number of Animals		Bodyweight (g) at Terminal Kill	Organ Weight (g) Pituitary	Spleen	Thymus	Thyroid	Uterus
0 (Control)	22	mean	358	0.0121	0.6922	0.1853	0.1024	0.6166
		sd	24	0.0035	0.1051	0.0642	0.0212	0.1765
1500	23	mean	356	0.0137	0.6453	0.1803	0.0948	0.6166
		sd	27	0.0039	0.0892	0.0448	0.0192	0.1427
5000	24	mean	370	0.0136	0.7240	0.1876	0.1063	0.5778
		sd	26	0.0045	0.1511	0.0601	0.0164	0.1694
15000	23	mean	365	0.0131	0.6509	0.1651	0.0937	0.5225
		sd	24	0.0028	0.0886	0.0582	0.0120	0.1291

sd=standard deviation
*=significantly different from control group p<0.05

Table 2.6.6.1-7: Relative organ weights for F0 females- selected (group mean values)

Dietary Concentration (ppm)	Number of Animals		Bodyweight (g) at Terminal Kill	Relative Organ Weight (%) Adrenals	Brain	Kidneys	Liver	Ovaries
0 (Control)	26	mean	349	0.0235	0.5634	0.6977	4.3103	0.0372
		sd	20	0.0039	0.0365	0.0548	0.2864	0.0084
1500	27	mean	352	0.0230	0.5581	0.7233	4.3027	0.0378
		sd	27	0.0045	0.0457	0.0560	0.3435	0.0082
5000	27	mean	364	0.0220	0.5414	0.7062	4.3570	0.0367
		sd	26	0.0032	0.0428	0.0592	0.2810	0.0062
15000	26	mean	362	0.0228	0.5483	**0.7490	***4.6806	0.0340
		sd	28	0.0027	0.0522	0.0521	0.2977	0.0057

Dietary Concentration (ppm)	Number of Animals		Bodyweight (g) at Terminal Kill	Relative Organ Weight (%) Pituitary	Spleen	Thymus	Thyroid	Uterus
0 (Control)	26	mean	349	0.0043	0.1718	0.0465	0.0137	0.2601
		sd	20	0.0010	0.0222	0.0154	0.0023	0.1361
1500	27	mean	352	0.0040	0.1721	0.0508	0.0143	0.2468
		sd	27	0.0011	0.0241	0.0190	0.0036	0.1365
5000	27	mean	364	0.0042	0.1656	0.0500	0.0159	0.2585
		sd	26	0.0009	0.0237	0.0131	0.0026	0.1309
15000	26	mean	362	0.0042	0.1614	0.0436	0.0156	0.2107
		sd	28	0.0008	0.0228	0.0146	0.0021	0.1517

sd=standard deviation
**=significantly different from control group p<0.01
***=significantly different from control group p<0.001

Table 2.6.6.1-8: Relative organ weights for F1 females- selected (group mean values)

Dietary Concentration (ppm)	Number of Animals		Bodyweight (g) at Terminal Kill	Relative Organ Weight (%)				
				Adrenals	Brain	Kidneys	Liver	Ovaries
0 (Control)	22	mean	358	0.0253	0.5441	0.7483	4.5970	0.0356
		sd	24	0.0031	0.0283	0.1070	0.4038	0.0071
1500	23	mean	356	0.0258	0.5401	0.7257	4.6047	0.0394
		sd	27	0.0072	0.0396	0.0647	0.2858	0.0081
5000	24	mean	370	0.0372	0.5214	0.7585	4.6543	0.0387
		sd	26	0.0443	0.0416	0.1229	0.3628	0.0078
15000	23	mean	365	0.0249	0.5198	0.7578	**4.9591	0.0371
		sd	24	0.0038	0.0646	0.0517	0.3130	0.0092

Dietary Concentration (ppm)	Number of Animals		Bodyweight (g) at Terminal Kill	Relative Organ Weight (%)				
				Pituitary	Spleen	Thymus	Thyroid	Uterus
0 (Control)	22	mean	358	0.0034	0.1930	0.0518	0.0288	0.1728
		sd	24	0.0010	0.0248	0.0178	0.0064	0.0513
1500	23	mean	356	0.0039	0.1814	0.0506	0.0267	0.1751
		sd	27	0.0012	0.0204	0.0115	0.0051	0.0447
5000	24	mean	370	0.0037	0.1950	0.0509	0.0289	0.1570
		sd	26	0.0012	0.0349	0.0161	0.0055	0.0495
15000	23	mean	365	0.0036	0.1789	0.0454	0.0258	0.1440
		sd	24	0.0007	0.0254	0.0164	0.0039	0.0374

sd=standard deviation
**=significantly different from control group p<0.01

Table 2.6.6.1-9: Sperm assessment and morphology- group mean values (F0)

Dose (ppm)		**0**		**1500**		**5000**		**15000**	
#Animals (No. of males used for sperm concentration only)		28(24)		27(23)		28(27)		28(22)	
Concentration (M/mL)		1145.8±561.6		1288.5±549.0		1124.5±475.5		1291.4±551.7	
Motile sperm (%)		77±12		81±11		77±11		82±13	
Progressively motile sperm (%)		3±3		4±4		3±3		5±5	
Cauda epididymis	Sperm count (10^6/g	399.9±151.2		-		-		309*±162.6 (23%)	
Testis	Sperm count (10^6/g)	42.1±12.9						41.3±11.2	
Sperm morphology		#	%	#	%	#	%	#	%
	Normal	200	100%	200	100	200	100	200	100
	Decapitate	0	0	0	0	0	0	0	0
	Abnormal	0	0	0	0	0	0	0	0

*Significantly different from the control group p<0.05

Table 2.6.6.1-10: Sperm assessment and morphology- group mean values (F1)

Dose (ppm)		**0**		**1500**		**5000**		**15000**	
#Animals (No. of males used for sperm concentration only)		24(23)		24		24		24	
Concentration (M/mL)		873.8±469.6		1008.3±472.7		1229.7±573.2		872.3±598.5	
Motile sperm (%)		59±20		69±14		70±14		59±23	
Progressively motile sperm (%)		2±2		3±2		3±3		2±2	
Cauda epididymis	Sperm count (10^6/g	445.9±213.1		-		-		417.0±190.5 (6%)	
Testis	Sperm count (10^6/g)	39±13.6						40.2±5.4	
Sperm morphology		#	%	#	%	#	%	#	%
	Normal	200	100	200	100	200	100	200	100
	Decapitate	0	0	0	0	0	0	0	0

	Abnormal	0	0	0	0	0	0	0	0

Table 2.6.6.1-11: Balano-preputial separation of F1 males

Dietary concentration (ppm)	No. of animals		Age (days) at completion	Body weight (g) at attainment
0 (Control)	24	mean SD	43.0 2.3	210 23
1500	24	mean SD	43.3 1.6	216 22
5000	24	mean SD	43.5 2.3	219 22
15000	24	mean SD	45.9** 3.1	230** 28

SD standard deviation
** significantly different from control group $p<0.01$

Table 2.6.6.1-12: Separation of the labia of F1 females

Dietary concentration (ppm)	No. of animals		Age (days) at completion	Body weight (g) at attainment
0 (Control)	24	mean SD	34.0 1.8	116 13
1500	24	mean SD	33.5 2.1	115 12
5000	24	mean SD	33.6 2.3	118 14
15000	24	mean SD	34.1 1.9	119 14

SD=standard deviation

Table 2.6.6.1-13: Ano-genital distance of F2 generation (group mean litter values)

Dietary concentration (ppm)	No. of animals		Ano-Genital Distance (mm) on Day 1 *post partum*	
			Males	Females
0 (Control)	22	mean SD	4.62 0.50	2.50 0.47
1500	23	mean SD	4.34 0.39	2.38 0.41
5000	24	mean SD	4.55 0.52	2.73 0.51
15000	23	mean SD	4.70 0.49	2.69 0.41

SD=standard deviation

Two-generation reproductive toxicity study in the rat ([redacted] (2000), Report No.: [redacted]/P/6332)

In this study, groups of 26 male and 26 female (F0) parents Alpk:APfSD (Wistar-derived) rats were fed diet containing 0, 1000, 3000 or 10000 ppm glyphosate acid. After 10 weeks, the animals were mated and allowed to rear the F1 litters to weaning. The regime was repeated with the F1 parents (26 males and 26 females for each group). F1A and F1B litters were weaned to day 29 *post partum*. No effect on sexual performance and fertility were observed in this study, and there was no indication of a teratogenic effect of the test substance at any of these concentrations.

Treatment was associated with a reduction (10%) in the body weight of the F1A pups in the 10000 ppm group with a subsequent reduction in body weight (up to 5%) of the selected F1 parent males for the duration of the pre-mating period.

The NOAEL for parental toxicity was set at 10000 ppm (985 mg/kg bw/day, mean daily intake of glyphosate during pre-mating phase in F0 males) (highest dose level)

The NOAEL for offspring toxicity was set at 3000 ppm (293 mg/kg bw/day, mean daily intake of glyphosate during pre-mating phase in F0 males) based on reduction in the body weight of the F1A pups in the 10000 ppm group.

The NOAEL for reproductive toxicity was set at 10000 ppm (985 mg/kg bw/d, mean daily intake of glyphosate during pre-mating phase in F0 males) (highest dose level)

The study is acceptable. The study is conducted in accordance with GLP and follows OECD TG 416 (2001) with exception of some deviations which do not invalidate the study (see Table 57).

Table 2.6.6.1-14: Mean pup body weights (g) – F1A

ppm	0	1000	3000	10000	0	1000	3000	10000
Sex	Males				Females			
Age (d)								
1	5.8 ± 0.6	6.1 ± 0.6	6.0 ± 0.8	6.1 ± 0.6	5.4 ± 0.6	5.8 ± 0.6	5.6 ± 0.6	5.7 ± 0.6
N	23	22	22	24	23	22	22	24
5	8.8 ± 1.6	9.2 ± 1.7	9.0 ± 1.4	8.6 ± 1.2	8.7 ± 1.1	8.9 ± 1.8	8.3 ± 1.4	8.1 ± 1.4
Adj. mean	9.2	9.1	8.9	8.5	9.0	8.5	8.4	8.1** (7%)
N	22	22	21	23	21	21	21	24
8	13.3 ± 1.6	13.5 ± 2.7	13.3 ± 1.9	12.8 ± 1.6	13.0 ± 1.6	13.2 ± 2.4	12.4 ± 2.1	12.2 ± 1.5
Adj. mean	13.8	13.4	13.2	12.6* (9%)	13.3	12.8	12.4	12.1** (9%)
N	21	22	21	23	21	20	21	23
15	26.3 ± 2.6	26.2 ± 5.1	25.9 ± 3.6	24.9 ± 2.8	25.7 ± 2.6	25.8 ± 4.4	24.3 ± 3.6	24.0 ± 2.5
Adj. mean	26.8	26.1	25.8	24.6*(8%)	26.1	25.2	24.5	23.8* (9%)
N	21	22	21	23	21	20	21	23
22	42.5 ± 4.8	42.7 ± 8.1	41.7 ± 6.2	39.7 ± 5.6	41.3 ± 4.8	41.4 ± 7.2	39.1 ± 5.9	38.3 ± 5.3
Adj. mean	43.4	42.4	41.4	39.2* 10%)	41.9	40.3	39.4	37.7* (10%)
N	21	22	21	23	21	20	21	23
29	80.3 ± 8.3	79.9 ± 12.1	79.8 ± 10.5	75.4 ± 8.1	76.1 ± 8.0	75.6 ± 9.9	73.5 ± 8.8	70.8 ± 7.4
Adj. mean	81.7	79.5	79.6	74.6* (9%)	77.1	74.0	74.1	69.9** (9%)
N	21	22	21	23	21	20	21	23

Values expressed as group mean ± SD
* statistically significant difference from control group p<0.05
** statistically significant difference from control group p<0.01

Table 2.6.6.1-15: Body weight during the pre-mating period-F1 generation (Group mean values)

		body weight (g)							
		Control		Low		Mid		High	
F1 generation		(0 ppm)		(1000 ppm)		(3000ppm)		(10000 ppm)	
Week		♂ (n=25)	♀ (n=26)	♂ (n=26)	♀ (n=26)	♂ (n=26)	♀ (n=26)	♂ (n=26)	♀ (n=26)
1		80.2	74	81.1	75.2	78.1	74.2	75.3	73.4
2		130.1	115.4	132.3	115.7	128.6	114.7	127.6* (2%)	115.2
3		188.5	152.6	190.7	154.7	186.5	151.2	183.3* (3%)	152.3
4		246.2	178.3	247.6	180.2	242.8	176.5	237.3** (4%)	179.4
5		300.3	201	304.1	202.7	296.5	199.7	289.5** (4%)	202.1
6		345	219.8	347.5	224.1	334.5*	217.2	328.7** (5%)	218.4
7		377.2	231.7	382.4	237.1	369	228.3	360.5** (4%)	234.4
8		403.6	241.9	410.1	245.1	395.3	237.2	387.0* (4%)	245.6
9		425	250.3	433.3	253.6	416.3	245.1	411.8	252.5
10		443.4	259.7	453.1	263.8	435.1	251.7	431.6	258.1
11		461.7	265.7	471.3	271.2	455.5	258.8	449.7	266.9

Two-generation reproductive toxicity study in the rat ([redacted] (1997), Report No.: [redacted] 96-0031)

In this study groups of 24 male and 24 females Sprague Dawley Crj:CD (SD) rats were fed diets containing glyphosate technical at concentrations of 0, 1200, 6000 or 30000 ppm for two successive generations.

Findings in parental animals consisted of clinical signs (loose stool) observed at 30000 ppm (F0, F1, both sexes), reduced body weights observed in males at 30000 ppm (F0 males: 8%; F1 males: 7%), increased liver weight (13-22%) observed at 30000 ppm (F1 males and females), increased kidney weight (up to 17%) observed at 30000 ppm (F0, F1, both sexes), decreased prostate weight (32%) observed at 30000 ppm (F1 generation males), and distension of caecum in parental animals (F0, F1, both sexes). Lower gestation indices were observed at high dose level, however without statistical significance. Indeed, most of the F1 animals were proved to have normal reproductive performance after re-mating with untreated animals, but this is not in accordance with current test guidelines: re-mating should be performed with treated males of the same dose group.

Offspring toxicity was observed in F1 and F2 litter animals at the top dose level (30000 ppm) and confined to reduced body weight (8-14%) and caecum distention.

The NOAEL for parental toxicity was set at 6000 ppm (equivalent to 417-458/485-530 mg/kg bw/day for males and females, respectively) based on clinical signs (loose stool, both sexes), reduced body weights (males), lower fertility indices of F1 females, organ weight changes (increased liver and kidney weights, both sexes, reduced prostate weight) and histopathological changes (distension of caecum) observed in males and females of both generations at 30000 ppm. The NOAEL for offspring was set at 6000 ppm (417 mg/kg bw/day) based on reduced pup weights and distension of caecum observed in both F1 and F2 litters at 30000 ppm. The NOAEL for reproductive toxicity was set at 6000 ppm (417 mg/kg bw/day) based on lower fertility indices of F1 females at high dose level.

The study is acceptable. The study is performed in accordance with GLP and follows OECD TG 416 (2001) with exception of some deviations which do not invalidate the study (see Table 57).

Table 2.6.6.1-16: Observed clinical signs in male rats (F0 and F1 generation)

Clinical sign	Number of male rats affected in dose group (ppm)							
	Pre-mating growth period				Breeding period			
	0	1200	6000	30000	0	1200	6000	30000
F0								
No. of animals examined	24	24	24	24	23	24	24	24
Swelling of the right auricle	0	0	0	0	0	1	0	1
Red sebum	1	0	0	0	0	0	0	0
Lacrima	0	0	0	1	0	0	0	1
Malocculusion	1	0	0	0	0	0	0	0
Wound: head, neck or back	2	0	0	0	2	0	0	0
Hair loss: head, neck, back, etc.	2	1	1	0	6	1*	0**	0**
Soiled fur perianal region	0	0	0	2	0	0	0	1
Loose stool	0	0	0	3	0	0	0	2
Killed in extremis	1	0	0	0	0	0	0	0
F1								
No. of animals examined	24	24	24	24	23	24	23	24
Soiled fur perinasal region	1	1	1	0	0	1	1	0
Red sebum	1	1	2	0	0	2	1	0
Malocculusion	1	1	1	0	0	1	0	0
Distention of the abdomen	0	0	1	0	0	0	0	0
Hair loss: head, neck, back, etc.	1	1	1	0	1	0	2	0
Soiled fur perianal region	0	0	0	4	0	0	0	1
Erosion in the perianal region	0	0	0	2	0	0	0	0
Loose stool	0	0	0	13***	0	0	0	0
Killed in extremis	1	0	1	0	0	0	0	0

* Significantly different from control at $p<0.05$
** Significantly different from control at $p<0.01$
*** Significantly different from control at $p<0.001$

Table 2.6.6.1-17: Observed clinical signs in female rats (F0 and F1 generation)

Clinical sign	Number of female rats affected in dose group (ppm)							
	Pre-mating growth period				Mating/gestation and Lactation/post-weaning period			
	0	1200	6000	30000	0	1200	6000	30000
F0								
No. of animals examined	24	24	24	24	24	24	24	24
Red sebum	0	0	0	0	0	0	1	0
Hair loss: head, neck, back, etc.	2	0	0	1	2/2	0	0	3/3
Mass on the chest	0	0	0	0	0	0	0	1
Scab on the chest	0	0	0	0	0	0	0	1
Loose stool	0	0	0	1	0	0	0	6*
F1								
No. of animals examined	24	24	24	24	23	23	21	19
Red sebum	0	0	0	1	0	1	0	0
Hair loss: head, neck, back, etc.	1	1	1	0	1/2	1/1	1	0
Mass on the chest	0	0	0	0	0	1/1	0	0
Soiled fur perianal region	0	0	0	1	0	0	0	0
Erosion in the perianal region	0	0	0	0	0	0	0	1
Loose stool	0	0	0	4	0	0	0	2

* Significantly different from control at $p<0.05$

Table 2.6.6.1-18: Selected body weights throughout treatment period – F0 and F1 males (group mean values)

Dietary concentration (ppm)	No. of animals				Body weight (g) at Week				
					0	5	10	14	18
					F0 Generation				
0 (Control)	24[1]			mean	140	366	454	498	531
				SD	4	34	52	60	64
1200	24			mean	140	373	463	516	549
				SD	4	31	44	53	58
6000	24			mean	140	363	456	501	532
				SD	4	24	37	40	44
30000	24			mean	140	341* (7%)	417* (8%)	457* (8%)	486
				SD	4	25	35	39	44
					F1 Generation				
0 (Control)	24[2]			mean	71	351	484	528	558
				SD	6	29	44	46	51
1200	24			mean	73	355	485	532	567
				SD	7	32	54	70	72
6000	24			mean	71	349	487	540	578
				SD	7	23	43	52	55
30000	24			mean	67** (6%)	326** (7%)	464	511	554
				SD	6	24	39	45	46

1 initial group size, reduced to 23 from week 4 onwards
2 initial groups size, reduced to 23 from week 8 onwards
SD standard deviation
* significantly different from control at $p\leq0.05$
** significantly different from control at $p\leq0.01$

Table 2.6.6.1-19: Mean pup body weights (F1)

ppm	0	1200	6000	30000	0	1200	6000	30000
Sex	Males				Females			
Age (d)								
0	6.7 ± 0.6	6.8 ± 0.5	6.7 ± 0.4	7.2* ± 0.7	6.3 ± 0.6	6.4 ± 0.5	6.4 ± 0.5	6.8* ± 0.6
N	24	24	23	24	24	24	23	24

ppm	0	1200	6000	30000	0	1200	6000	30000
Sex	Males				Females			
4	11.6 ± 1.2	11.6 ± 1.2	11.7 ± 1.0	11.6 ± 1.2	11.1 ± 1.2	11.2 ± 1.1	11.3 ± 0.9	11.3 ± 1.2
N	23	24	24	24	23	24	24	24
7	19.5 ± 1.7	19.1 ± 2.0	19.5 ± 1.6	19.3 ± 1.2	18.6 ± 1.8	18.4 ± 1.9	18.8 ± 1.5	18.3 ± 1.6
N	23	24	24	24	23	24	24	24
14	39.5 ± 3.2	39.4 ± 2.6	39.3 ± 2.6	36.6** ± 2.6 (7%)	38.4 ± 3.6	37.9 ± 2.6	38.2 ± 2.2	35.4** ± 2.6 (8%)
N	23	24	24	24	23	24	24	24
21	63.9 ± 4.4	63.8 ± 4.1	62.4 ± 3.7	55.1 ± 3.5*** (14%)	61.0 ± 4.8	60.6 ± 3.9	59.8 ± 3.1	53.2*** ± 4.0 (13%)
N	23	24	24	24	23	24	24	24

Values expressed in group mean ± SD
* statistically significant difference from control group p <0.05
** statistically significant difference from control group p <0.01
*** statistically significant difference from control group p <0.001

Table 2.6.6.1-20: Mean pup body weights (F2)

ppm	0		1200	6000	30000	0	1200	6000	30000
Sex			Males			Females			
Age (d)									
0	7.0 ± 0.5		6.9 ± 0.6	7.3 ± 0.7	7.1 ± 0.5	6.6 ± 0.5	6.6 ± 0.7	6.8 ± 0.6	6.8 ± 0.6
N	23		23	21	19	23	23	21	19
4	12.0 ± 1.2		12.1 ± 1.5	12.5 ± 1.5	12.5 ± 1.3	11.6 ± 1.2	11.5 ± 1.6	12.0 ± 1.5	12.1 ± 1.1
N	23		23	21	19	23	23	21	19
7	19.8 ± 1.5		20.0 ± 1.9	20.4 ± 2.2	20.6 ± 1.7	18.9 ± 2.0	19.1 ± 2.1	19.6 ± 2.2	19.9 ± 1.4
N	23		23	21	19	23	23	21	19
14	40.1 ± 3.0		39.0 ± 2.8	38.7 ± 2.9	39.1 ± 2.8	38.7 ± 3.5	38.0 ± 2.2	37.5 ± 2.9	38.1 ± 2.9
N	23		23	21	19	23	23	21	19
21	58.6 ± 5.1		59.4 ± 4.4	58.3 ± 4.3	53.1** ± 4.4 (9%)	56.4 ± 5.5	57.1 ± 4.4	56.2 ± 4.5	51.8* ± 4.2 (8%)
N	23		23	21	19	23	23	21	19

Values expressed in group mean ± SD
* statistically significant difference from control group p<0.05
** statistically significant difference from control group p<0.01

Table 2.6.6.1-21: Selected organ weights of males

Dietary conc. (ppm)		bw		Organ weight (absolute weights in mg$)							
				Brain		Kidney		Liver		Prostate	
		(g)		absolute	relative	absolute	relative	absolute	relative	absolute	relative
				F0 Generation							
0	mean	538		2197	0.411	1656	0.308	16900	3.13	642	0.1204
	SD	54		111	0.030	184	0.021	2382	0.20	208	0.0389
1200	mean	533		2154	0.406	1631	0.307	16351	3.08	606	0.1141
	SD	43		68	0.025	115	0.028	1094	0.24	159	0.0287
6000	mean	538		2179	0.409	1690	0.316	16568	3.07	707	0.1332
	SD	55		59	0.040	120	0.033	2335	0.19	126	0.0305
30000	mean	473*		2123	0.451*	1617	0.342*	15617	3.29	569	0.1204
	SD	45		142	0.035	178	0.021 (11%)	2410	0.26	94	0.0184
				F1 Generation							
0	mean	569		2219	0.393	1717	0.303	18163	3.19	662	0.1178

Dietary conc. (ppm)		bw		Organ weight (absolute weights in mg$)							
				Brain		Kidney		Liver		Prostate	
		(g)		absolute	relative	absolute	relative	absolute	relative	absolute	relative
	SD	50		46	0.035	150	0.023	2136	0.18	185	0.0355
1200	mean	532		2170	0.413	1694	0.318	17638	3.28	493	0.0931
	SD	66		81	0.049	288	0.035	3655	0.33	85	0.0158
6000	mean	559		2261	0.406	1796	0.322	17509	3.12	582	0.1039
	SD	47		114	0.031	180	0.019	2380	0.22	196	0.0319
30000	mean	567		2217	0.393	1942	0.344*	20523	3.62**	450*	0.0797*
	SD	50		131	0.029	175	0.038 (14%)	2252	0.23 (13%)	153 (32%)	0.0296 (32%)

$ Relative organ weights to body weights are shown as percent of body weights (considering 10 rats in every group)
SD standard deviation
* significantly different from control group p≤0.05
** significantly different from control group p≤0.01

Table 2.6.6.1-22: Selected organ weights of females

Dietary concentration (ppm)		bw	Organ weight (absolute weights in mg$)							
			Brain		Kidney		Liver		Ovaries	
		(g)	absolute	relative	absolute	relative	absolute	relative	absolute	relative
			F0 Generation							
0	mean	306	2014	0.658	1147	0.374	13491	4.39	61.1	0.0199
	SD	18	110	0.031	120	0.030	1909	0.41	7.3	0.0021
1200	mean	304	1979	0.652	1143	0.376	13219	4.36	66.6	0.0219
	SD	10	66	0.028	74	0.025	1053	0.36	10.3	0.0030
6000	mean	301	1967	0.655	1110	0.369	13090	4.36	60.0	0.0200
	SD	12	82	0.033	84	0.026	1005	0.29	5.9	0.0022
30000	mean	313	2013	0.646	1202	0.385	14009	4.47	64.6	0.0207
	SD	21	73	0.041	125	0.031	1761	0.38	6.3	0.0014
			F1 Generation							
0	mean	314	2035	0.650	1114	0.355	13436	4.28	68.4	0.0218
	SD	19	112	0.050	125	0.028	1598	0.41	7.0	0.0025
1200	mean	326	2026	0.624	1211	0.372	14611	4.49	61.3	0.0189*
	SD	19	64	0.034	99	0.020	1240	0.32	7.6	0.0027
6000	mean	327	2038	0.628	1242*	0.381	15528	4.73	68.0	0.0209
	SD	28	71	0.056	102 (11%)	0.025	2830	0.53	7.6	0.0023
30000	mean	319	2073	0.652	1304	0.409***	16394**	5.13***	69.5	0.0218
	SD	16	94	0.051	108** (17%)	0.030 (15%)	1835 (22%)	0.44 (20%)	7.6	0.0027

$ Relative organ weights to body weights are shown as percent of body weights (considering 10 rats in every group)
SD standard deviation
* significantly different from control group p≤0.05
** significantly different from control group p≤0.01
*** significantly different from control group p≤0.001

Table 2.6.6.1-23: Reproductive parameters and litter data (F0)

	ppm	0	1200	6000	30000
Oestrous cycle – prior to mating (F0)	Regular 4 or 5d cycles	24/24 (100 %)	23/24 (95.8 %)	24/24 (100 %)	24/24 (100 %)
Mating Index	Males	23/23 (100 %)	24/24 (100 %)	24/24 (100 %)	24/24 (100 %)
	Females	24/24 (100 %)	24/24 (100 %)	24/24 (100 %)	24/24 (100 %)
	Fertility Index	24/24 (100 %)	24/24 (100 %)	24/24 (100 %)	24/24 (100 %)
	Duration of gestation (days)	22.4	22.3	22.1	22.3
	Number of implantation sites	14.9 ± 1.6	14.9 ± 1.6	15.1 ± 1.1	14.8 ± 2.6
	# of pups delivered (mean ± SD)	13.8 ± 1.6	13.2 ± 1.8	14.1 ± 1.4	13.6 ± 2.6
	Sex ratio	0.459	0.491	0.499	0.446
Viability Index on lactation day	0	97.5	97.9	95.9	97.4

	ppm	0	1200	6000	30000
	4	95.5	99.7	97.8	98.9
	21	100.0	98.4	99.5	99.5

Table 2.6.6.1-24: Reproductive parameters and litter data (F1)

	ppm	0	1200	6000	30000
Oestrous cycle – prior to mating (F1)	Regular 4 or 5d cycles	24/24 (100 %)	24/24 (100 %)	24/24 (100 %)	24/24 (100 %)
Mating Index	Males	23/23 (100 %)	24/24 (100 %)	23/23 (100 %)	24/24 (100 %)
	Females	24/24 (100 %)	24/24 (100 %)	24/24 (100 %)	24/24 (100 %)
Fertility Index		23/24 (95.8 %)	23/24 (95.8 %)	21/24 (87.5 %)	19/24 (79.2 %)
Duration of gestation (days)		22.2	22.4	22.2	22.2
Number of implantation sites		13.9 ± 2.1	15.7 ±1.4*	13.6 ±2.5	14.5 ± 2.1
# of pups delivered (mean ± SD)		12.8 ± 2.1	13.7 ± 2.3	13.0 ± 2.7	13.1 ± 2.7
Sex ratio		0.481	0.514	0.551	0.500
Viability Index on lactation day	0	98.8	98.9	98.9	98.1
	4	99.3	99.7	98.7	99.7
	21	100.0	100.0	100.0	99.3

* Significantly different from control at p<0.05

Table 2.6.6.1-25: Incidence of distension of the caecum in F0 and F1 parental male rats

	0, 1200, 6000 ppm			30000 ppm		
	Terminal kill	Unscheduled death (control group only investigated)	Total	Terminal kill	Unscheduled death	Total
F0 males						
Large intestine: distension of cecum	0	0 (control)	0	21	-	21***
F1 males						
Large intestine: distension of cecum	0	0 (control)	0	19	-	19***

Table 2.6.6.1-26: Incidence of distension of the caecum in F0 and F1 parental female rats

	0, 1200, 6000 ppm			30000 ppm		
	Terminal kill	Unscheduled death	Total	Terminal kill	Unscheduled death	Total
F0 females						
Large intestine: distension of cecum	0	-	0	24	-	24***
F1 females						
Large intestine: distension of cecum	0	-	0	17	-	17***

** statistically significant difference from control group p<0.01
Gross pathological examination of weanlings was performed at 21-26 days of age
Figures in parentheses represent the number of pups found dead during lactation days 5-21 and/or post weaning period

Two-generation reproductive toxicity study in the rat ([redacted] (1993), Report No.: TOXI: 885-RP-G2)

In this study groups of 30 males and 30 females Wistar (Random bred) rats were fed diets containing glyphosate technical at concentrations of 0, 100, 1000, and 10000 ppm for two successive generations. No treatment-related effects were observed. However, it could be noted that the study was limited (effect dose lacking, no sperm analyses, sexual offspring development not investigated, limited histopathology).

NOAEL for parental, reproductive and offspring toxicity was set at 10000 ppm. This dietary level would correspond to a mean daily compound intake of 700-800 mg/kg bw/d. [The mean daily intake was not reported for all dietary

levels, but for the low level of 100 ppm a corresponding average value of 7.7 mg/kg bw/d was given in the original report].

The study is supplementary only (effect dose lacking, limited parameters investigated in study). The study is conducted in accordance with GLP. The study was checked for compliance with OECD TG 416 (2001) and several deviations were observed as presented in Table 57.

Two-generation reproductive toxicity study in the rat ([redacted] (1992), Report No.: [redacted] 47/911129)

In this study, groups of 28 male and 28 female (F0) Sprague-Dawley Crl:CD (SD) BR VAF/Plus rats were fed diets containing 0, 1000, 3000, and 10000 ppm glyphosate technical. F0 animals were treated (from 6 weeks of age) for 10 weeks prior to their first of their two mating (mated at 16 and 26 weeks of age) and through to termination. The F1 generation (24/sex/group) was selected from the F1A litters, reared to maturity, and mated at 16 and 27 weeks of age.

Findings in parental animals consisted of increased water consumption (17%) observed F1 females at 10000 ppm, increased food intake (3%) observed in F1 females at 10000 ppm during the latter stage of the first pre-mating period), reduced mean body weight (1-7%) observed in F1 males at 10000 ppm, and histopathological findings in salivary glands (parotid and submaxillary glands) observed in parental animals at ≥3000 ppm, manifested as minimal hypertrophy of acinar cells with prominent granular cytoplasm. The findings in the parotid gland were observed in male and female animals of both generations at 3000 ppm (F0 males 3/28, F0 females 5/28, F1 males 4/23, F1 females 4/24) and 10000 ppm (F0 males 12/26, F0 females 17/28, F1 males 11/23, F1 females 9/23). The findings in the submaxillary gland were observed in F0 females at 3000 ppm (F0 females 4/28), and in F0 and F1 females at 10000 ppm (F0 females 14/28, F1 females 3/23).

Trend analysis conducted by RMS showed that the dose-related increase was statistically significant.

No treatment-related effects were observed in the offspring

The NOAEL for parental toxicity is proposed to be set at 1000 ppm (66 mg/kg bw/day) based on changes observed in salivary gland at ≥3000 ppm (in previous RAR the NOAEL for parental toxicity was set at 3000 ppm).

The NOAEL for, offspring toxicity is proposed to be set at 10000 ppm (668 mg/kg bw/day) (highest dose level) (in previous RAR the NOAEL for offspring toxicity was set at 3000 ppm).

The NOAEL for reproductive toxicity is set at 10000 ppm (668 mg/kg bw/day).

The study is acceptable. The study is performed in accordance with GLP and follows OECD TG 416 (2001) with exception of some deviations which do not invalidate the study (see Table 57).

Table 2.6.6.1-27: Water consumption- group mean weekly values (g/rat/week) F1 generation

Group:	1	2	3	4
Compound:	Control		Glyphosate	
Dietary inclusion (ppm):	-	1000	3000	10000

Week	Group							
	1♂	2♂	3♂	4♂	1♀	2♀	3♀	4♀
5	159.3	150.4	157.3	156.2	143.2	141.7	141.6	152.9
6	198.9	200.4	199.6	201.2	175.8	176.6	178.4	192.4
15	230.5	249.2	237.8	239.3	184.3	188.6	188.2	204.6
16	227.0	242.3	236.3	239.8	175.7	182.9	182.3	206.3**
Cumulative 5 - 16	815.8	842.3	831.0	836.5	679.0	689.8	690.6	756.2
% of control	-	103	102	103	-	102	102	111

Statistical analysis

Analysis of variance followed by intergroup comparison with the controls (Williams' test), significant at; ** P<0.01

Table 2.6.6.1-28: Group mean body weights (g) - F1 generation

Week	Males				Females			
	Dietary concentration (ppm)				Dietary concentration (ppm)			
	0	1000	3000	10000	0	1000	3000	10000
4	108	104	106	100 (7%)	95	93	98	93
10	425	429	423	412 (3%)	266	265	270	267
15	526	542	521	512 (3%)	316	313	325	319
16[1]	540	555	538	528 (2%)	324	321	335	326
21	605	619	600	591 (2%)	373	370	373	381
26	659	677	659	649 (1.5%)	367	368	380	370
27[2]	673	692	671	663 (1%)	374	372	390	376
32	710	727	709	699 (1.5%)	413	424	436	424
37	762	781	746	742 (3%)	415	410	424	417

[1] First mating commenced
[2] Second mating commenced

Table 2.6.6.1-29: Incidence of salivary gland findings

Observation	Dietary concentration (ppm)							
	Males				Females			
	0	1000	3000	10000	0	1000	3000	10000
F0 Generation								
Animals examined	27	28	28	26	28	27	28	28
Hypertrophy of acinar cells with prominent granular cytoplasm (minimal)								
parotid	2	2	3	12	0	2	5	17
submaxillary	0	-	-	0	0	1	4	14
F1 Generation								
Animals examined	24	24	23	23	24	23	24	23
Hypertrophy of acinar cells with prominent granular cytoplasm (minimal)								
parotid	1	0	4	11	0	0	4	9
submaxillary	0	-	-	0	0	0	0	3

- = not examined

Trend analysis using (Cohran-Armitage Trend Test, exact permutation; StatXact-6):

Males		
F0-Parotid	p-value one sided: 0.0002 p-value two-sided: 0.0004	NOAEL: 3000 ppm LOAEL: 10000 ppm
F1-Parotid	p-value one sided: 6.7E-6 p-value two-sided: 1.3 E-5	NOAEL: 3000 ppm LOAEL: 10000 ppm
Females		
F0-Parotid	p-value one sided: 0.014 p-value two-sided: 0.027	NOAEL: 1000 ppm LOAEL: 3000 ppm
F1-Parotid	p-value one sided: 0.011 p-value two-sided: 0.022	NOAEL: 1000 ppm LOAEL: 3000 ppm
F0-submaxillary	p-value one-sided: 0.022 p-value two-sided: 0.045	NOAEL: 1000 ppm LOAEL: 3000 ppm
F1-submaxillary	p-value one sided: 0.013 p-value two-sided: 0.026	NOAEL: 3000 ppm LOAEL: 10000 ppm

One-generation range finding study ([redacted] (1991), Report No. [redacted] 42/90619)

In this preliminary assessment for a subsequent two-generation reproductive toxicity study, groups of 10 time-mated Sprague-Dawley rats received daily dietary doses of 0, 3000, 10000 and 30000 ppm glyphosate from Day 3 of gestation through gestation and lactation to termination at the end of lactation. Control animals received the base diet alone. All females were allowed to litter and rear their young to weaning, when 10 male and 10 female offspring per group were selected and reared on their respective diets to six weeks of age. No adverse effects on reproduction parameters nor on survival of pups through weaning were observed in this study.

Finding in parental animals consisted of mortality observed at 30000 ppm (one F0 female died on Day 21, cause of death not identified), clinical signs (soft faeces and yellow stained sawdust) observed in F0 females at ≥10000 ppm, increased water consumption observed in F0 females at 30000 ppm (towards the end of pregnancy: 11%), reduced bodyweight gain observed in F0 females at 30000 ppm (Gestation: up to 23%; Lactation: up to 47%), reduced bw observed in F0 females at 30000 ppm (Gestation: up to 7%; Lactation up to 14%), macroscopical changes in gastro-intestinal tract observed in F0 at ≥3000 ppm, and macroscopical and histopathological changes in salivary gland observed in F0 females at ≥3000 ppm. The macroscopical findings in the gastro-intestinal tract consisted of gastrointestinal disturbances such as watery and/or dark contents, distended and/or congested stomach observed in all treated groups, and distended caecum observed in F0 females at 30000 ppm. Macroscopical (enlarged/firm/congestion/swollen) and histopathologic changes in salivary glands were recorded in all treatment groups. The microscopical changes in salivary glands consisted of granular basophilic cytoplasm of acinar cells and hypertrophy of acinar cells observed in F0 females at all dose levels and prominent mitoses in acinar cells observed in F0 females at 30000 ppm. The acinar cells hypertrophy was moderate at 10000 ppm and marked at 30000 ppm.

Findings in pups consisted of reduced pup weights observed at ≥3000 ppm (Day 21 *post partum*: reduction of 9%, 13% and 38% for the low-, mid-, and high dose group, respectively compared to control). Furthermore, macroscopic changes in salivary gland (congested) were observed in one pup at 3000 ppm and in four pups at 10000 ppm, but the significance of this finding was not clear since this effect did not occur in the highest dose group (30000 ppm).

In offspring retained to six weeks of age, clinical signs (soft faeces), reduced weight gain (Day 42: males: 25%, females: 15%) and reduced food consumption were observed at 30000 ppm. Furthermore, macroscopic changes in parotid salivary gland (enlarged/swollen) were observed in all treated groups, and macroscopic gastro-intestinal changes (soft content) were observed at 30000 ppm.

The study is acceptable as supplementary data only. The study is not suitable for NOAEL setting (few animals used, limited parameters investigated, no statistical analyses conducted).

Table 2.6.6.1-30: F0 females - body weights and body weight changes during gestation (Group mean values)

Dietary concentration (ppm)	No. of animals	Gestation Day						
		1	3	6	10	14	17	20
	Body weights (g) at Day							
0 (Control)	9	187.7	215.2	241.6	280.1	318.4	352.4	406.9
3000	9	186.8	216.4	246.0	277.2	311.2 (2%)	343.6	393.9
10000	10	185.4	214.5	241.1	277.8	309.9 (3%)	346.4	404.6
30000	9	187.0	215.8	236.0 (2%)	266.0 (5%)	296.7 (7%)	330.0 (6%)	385.6 (5%)
		Bodyweight change (g) relative to gestation Day 3						
0 (Control)	9	-27.6	0.0	26.3	64.9	103.2	137.2	191.7
3000	9	-29.7	0.0	29.6	60.8	94.8 (8%)	127.1	177.4
10000	10	-29.1	0.0	26.6	63.3	95.4 (8%)	131.9	190.1
30000	9	-28.8	0.0.	20.2 (23%)	50.2 (23%)	80.9 (22%)	114.2 (17%)	169.8 (11%)

Table 2.6.6.1-31: F1 females - body weights and body weight changes during lactation (Group mean values)

Dietary concentration (ppm)	No. of animals	Lactation Day			
		0	7	14	21
		Body weights (g)			
0 (Control)	9	315.4	339.1	344.6	335.0
3000	9	299.3	329.2	326.7	311.1
10000	10	309.8	338.0	330.1	322.5
30000	8+	292.5 (7%)	321.0 (5%)	300.0 (13%)	286.9 (14%)
		Body weight change (g) relative to gestation Day 3			
0 (Control)	9	100.2	123.9	129.3	119.8
3000	9	82.9	112.8	110.2	94.7
10000	10	95.3	123.5	115.6	108.0
30000	8+	72.4 (28%)	100.4 (19%)	79.1 (39%)	62.9 (47%)

Table 2.6.6.1-32: Litter data of animals rearing young to weaning- group mean values- F0 generation

	Control (0 ppm)	Low (3000 ppm)	Mid (10000 ppm)	High (30000 ppm)
Mean litter size				
at birth	13.2	14.2	14.1	14.6
at day 4 *post partum*	12.2	13.8	14.0	14.1
at day 8 *post partum*	12.1	13.7	13.6	13.8
at day 21 *post partum*	12.1	13.6	13.5	13.2
Mean pup weight (g)				
at birth	6.6	6.6	6.6	6.3 (4.5%)
at day 4 *post partum*	10.1	9.9	9.9	9.3 (8%)
at day 8 *post partum*	17.6	16.3	16.6	14.8 (16%)
at day 21 *post partum*	49.3	45.1 (9%)	43.1 (13%)	30.8 (38%)
Percentage male pups (%)				
at birth	46.7	50.0	60.5	47.4
At day 21 *post partum*	48.0	50.2	61.5	47.5

Table 2.6.6.1-33: F0 females - necropsy findings

Clinical sign	Control (0 ppm)	Low (3000 ppm)	Mid (10000 ppm)	High (30000 ppm)
Necropsy findings				
Fur, ventral surface: yellow stained	-	-	1	-
Gasto-intestinal tract: contents watery and/or dark	-	2	7	8
Stomach: distended and/or congested	-	2	5	4
Stomach: contents watery	1	2	-	1
Caecum: distended	-	-	-	4
Caecum: contents watery	1	-	-	1
Salivary glands: enlarged /firm/congested/swollen	-	2	6	8
Lungs: multiple pale subpleural foci	1	3	3	1
Uterus: severe fluid distension	1*	-	-	-

* animal not pregnant

Table 2.6.6.1-34: F0 females - microscopic findings

Microscopic findings	Control (0 ppm)	Low (3000 ppm)	Mid (10000 ppm)	High (30000 ppm)
No. of animals examined	9	9	10	9
Parotid salivary glands				
Granular basophilic cytoplasm of acinar cells:				
Minimal	-	2	-	-
Moderate	-	-	2	-
Marked	-	-	8	9
Hypertrophy of acinar cells:				
Minimal	-	2	2	-
Moderate	-	-	8	9
Prominent mitosis in acinar cells	-	-	-	2

Two-generation reproductive toxicity study in the rat [redacted] (1990), Report No.: [redacted]-10387)

In this study, 30 Sprague-Dawley rats/sex/dose group (F0 and F1a generation) were fed daily with glyphosate at concentrations of 2000, 10000 and 30000 ppm through two generations for approximately 11 (F0-generation) and 14 weeks (F1a-generation), respectively. Animals of the F1a generation were mated twice to produce the F2a and F2b-generations.

Findings in parental animals consisted of clinical signs (soft stool) observed in adult animals at 30000 ppm, reduced body weight observed in adult animals at 30000 ppm. Terminal bw: F0 males: 8%, F1 males: 13%, F1 females: 10%; Maternal bw during gestation: Day 1: F0 females: 7%, F1 females first mating: 12%, F1 females second mating: 13%; Day 21: F0 females: 7%, F1 females first mating: 8%, F1 females second mating: 8%), and reduced litter size observed at 30000 ppm (F0 dams, and to a lesser degree the F1 dams). The slight reduction in the average litter size observed in the F0 dams of the 30000 ppm dose group was non statistically significant and not noted when F1 animals were re-mated, and treatment-relation was considered to be equivocal. A reduction in litter size was not confirmed in the study by [redacted] (1997) (Report No.: [redacted] 96-0031), where the same dietary concentrations of glyphosate were tested.

Findings in pups consisted of reduced pup weights observed at 30000 ppm (F0 males: 13%, F0 females: 11%; F1A males and females: 14%; F1B males: 19%, F1B females: 13%). Decreases in pup weights at the 10000 ppm dose level (666-711 and 777-804 mg/kg bw/day in males and females, respectively) did not occur consistently in both sexes from all generations and were considered of unclear toxicological significance. This finding at the 10000 ppm level, was not confirmed in the study by [redacted] (2007), where dose levels up to 15000 ppm (1063 and 1634 mg/kg bw/day) were used or in the study by [redacted] (1992), where dose level up to 10000 ppm (F0: 668 and 752 mg/kg bw/day in males and females, respectively; F1: 771 and 841 mg/kg bw/day in males and females, respectively) were used. The same strain of rat was used in the studies.

NOAEL for parental toxicity was set at 10000 ppm (666-711 mg/kg bw/day for males and 777-804 mg/kg bw/day for females) based on clinical signs of soft stools and reduced body weight (>10%) observed in males and females of both generations at 30000 ppm. NOAEL for offspring was set at 10000 ppm (666-711 mg/kg bw/day for males and 777-804 mg/kg bw/day for females) based on reduced pup weights observed in males and females of both generations at 30000 ppm. NOAEL for reproductive toxicity was set at 10000 ppm (666-711 mg/kg bw/day for males and 777-804 mg/kg bw/day for females) based on equivocal reduction in litter size observed in F0 dams at 30000 ppm.

The study is acceptable. The study is performed in accordance with GLP and follows OECD TG 416 (2001) with exception of deviations (see Table 57) which do not invalidate the study.

Table 2.6.6.1-35: Selected mean group body weights (adults)

Dietary concentration (ppm)	No. of animals		Mean group body weight (g) at Day					
			0	72	T#	0	72	T#
			F0 Generation					
			Males			Females		
0 (Control)	30	mean	187.9	494.6	549.56	150.5	276.7	296.31
		SD	11.65	34.86	46.76	6.86	23.85	23.63
2000	30	mean	188.1	497.6	550.19	150.5	272.6	290.64
		SD	11.35	49.87	80.72	7.03	22.86	19.50
10000	30	mean	188.1	484.4	539	150.2	273	290.71
		SD	11.57	42.13	58.13	7.04	27.92	25.35
30000	30	mean	188	455.8**	503.51**	150.3	253.8**	265.91
		SD	11.56	46.46 (8%)	45.66 (8%)	7.06	18.46 (8%)	15.44
			F1 Generation					
			129	219	T#	128	219	T#
0 (Control)	30	mean	118.3	534.7	625.04	99.8	285.8	316.21
		SD	26.11	38.84	53.11	17.44	27.63	37.37
2000	30	mean	115.2	540.3	632.14	96.7	282.1	313.74
		SD	16.2	44.9	74.57	11.47	24.5	30.53
10000	30	mean	114.8	514.1	590.98	97.1	275.9	312.36
		SD	17.42	58.31	70.06	14.18	20.55	26.71
30000	30	mean	104.9*	483.4**	543.40**	88.8*	253.7**	284.72**
		SD	19.79 (11%)	41.32 (10%)	58.12 (13%)	16.32 (11%)	19.56 (11%)	18.04 (10%)

*: Dunnett´s test (two-tailed) indicates statistically significant difference ($p<0.05$)
**: Dunnett´s test (two-tailed) indicates statistically significant difference ($p<0.01$)
#T: Termination

Table 2.6.6.1-36: Litter size

	Dietary concentration (ppm)			
	0	2000	10000	30000
F0 generation				
# of females	24	29	28	28
Litter size (live)	13.3	12.5	12.7	11.5 (13%)
Dead pups per litter	0.0	0.2	0.1	0.1
F1A (first mating)				
# of females	28	24	24	26
Litter size (live)	12.0	12.3	11.5	10.8 (10%)
Dead pups per litter	0.1	0.3	0.2	0.0
F1A (second mating)				
# of females	16	20	19	25
Litter size (live)	11.9	10.9	13.2	10.7
Dead pups per litter	0.1	0.2	0.2	0.2

Table 2.6.6.1-37: Mean pup weights

Dietary concentration (ppm)	No. of litters[c]		Mean group body weight (g) at Day			
			0	21	0	21
			Males		Females	
			F0 Generation			
0 (Control)	24	mean	6.28	53.39	6.96	50.80
		SD	0.49	3.90	0.52	4.39
2000	29	mean	6.27	51.82	6.91	49.47
		SD	0.48	5.26	0.48	5.05
10000	28	mean	6.43	50.42*	6.15	49.16
		SD	0.47	3.66 (6%)	0.50	3.12
30000	28	mean	6.47	46.30**	6.12	44.99**
		SD	0.62	4.09 (13%)	0.59	4.34 (11%)
			F1A Generation (First Mating)			
0 (Control)	28	mean	6.33	55.11	5.95	51.93
		SD	0.60	5.64	0.55	5.07
2000	23	mean	6.20	52.47	5.90	51.42
		SD	0.76	9.15	0.70	4.08
10000	22	mean	6.32	51.53*	5.98	48.49*
		SD	0.74	7.35 (6%)	0.64	5.93 (7%)
30000	26	mean	6.50	47.29**	6.05	44.41**
		SD	0.84	4.62 (14%)	0.74	4.90 (14%)
			F1B Generation (Second Mating)			
0 (Control)	16	mean	6.48	55.03	6.04	49.35
		SD	0.75	6.38	0.63	10.96
2000	18	mean	6.17	52.74	5.86	50.73
		SD	0.74	6.12	0.83	5.91
10000	17	mean	6.36	52.29	5.92	49.48
		SD	0.52	3.35	0.47	2.52
30000	24	mean	6.51	44.43**	6.04	43.10**
		SD	0.63	6.86 (19%)	0.55	3.81 (13%)

c: Combined sexes

Table 58: Summary table of human data on adverse effects on sexual function and fertility

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data				

A literature search for the active substance glyphosate was performed by the applicant in accordance to the provisions of the EFSA Guidance "Submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) 1107/2009" and updated Appendix to this Guidance document.

The publications identified by the applicant as relevant and reliable for this endpoint are briefly summarised in Table 59. All these studies were considered by RMS as reliable with restrictions. The overall relevance of the studies was further discussed in section 2.6.6.1.1.

For more detailed study summaries, please see Vol. 3-B.6 (AS), section B.6.6.3 (Reproductive toxicity- Information from public literature).

There are also published literature identified by the applicant considered as supplementary after detailed assessment of full-text articles (Category B studies). The test substance used in these studies were glyphosate-based formulations and effects caused by co-formulants cannot be excluded. These studies were considered by RMS as supplementary and reliable with restrictions, and not given further significance for this endpoint. The studies are briefly summarised in Table 2.6.6.1-38. For more detailed study summaries, please see Vol. 3-B.6 (AS) section B.6.6.3 (Reproductive toxicity – Information from public literature).

Table 59: Summary table of other studies relevant for toxicity on sexual function and fertility

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
in vitro Sertoli cell cultures (Sprague-Dawley rat) No guideline No GLP 10-100 ppm The study is reliable with restrictions as the test material identity, in particular purity, is not specified; only 1 or 2 concentration levels were tested; and a positive control was missing.	Glyphosate and Glyphosate formulation Roundup Full II containing 54% w/v acid glyphosate.	The aim of the study was to analyse whether glyphosate and/or glyphosate-based formulation (Roundup) can affect Sertoli cell functions, such as energy metabolism and blood-testis barrier integrity, which are essential to maintain spermatogenesis.	<u>At 100 ppm (glyphosate and Roundup Full II):</u> Altered Sertoli cell junction barrier permeability and decreased testosterone-stimulated TER; redistribution of claudin11	CA 5.6.1/015 Gorga *et al.* (2020). Toxicology *in vitro*, Volume 62, February 2020, 104682
13-week (dose range finding study) Oral (drinking water) Rat (Sprague-Dawley) 24 female animals (17 weeks old) No guideline No GLP 0 and 1.75 mg/kg bw/day The study is reliable with restrictions (only one (low) dose	Glyphosate (Purity: 99.5 %) and Roundup Bioflow (MON 52276, containing 360 g/L of glyphosate acid	The aim of the (pilot) study was to examine whether exposure to glyphosate-based herbicides (GBHs) at a dose of glyphosate considered to be "safe" (the US Acceptable Daily Intake of 1.75 mg/kg bw/day), starting from *in utero* life, affect the development and endocrine system across different life stages in Sprague-Dawley rats. In this (pilot) study, two groups of Sprague-Dawley rats (8/group) were treated from gestation day 6 with either glyphosate (G) (> 99.5% pure) or Roundup Bioflow	*Note: Only the results of glyphosate presented below* <u>1.75 mg/kg bw/day:</u> <u>Dams:</u> There were no statistically significant effects in dams on the following: body weight, water or feed consumption during gestation or lactation, gestational index, mean gestational length, total pups delivered at PND 0, litter size, sex ratio at birth, mean live birth index, dams with reported stillbirths (although the number of dams with stillbirths	CA 5.6.1/016 Manservisi *et al.* (2019). Environmental Health (2019) 18:15

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
tested; small group sizes; blood sampling was done only once (at the end of life) and the timing (9 AM to 3 PM) of sampling could not rule out circadian-dependent modulation of circulating hormones)		(R) (MON 52276) (360 g/L of glyphosate acid) diluted in drinking water to achieve glyphosate dose of 1.75 mg/kg bw/day (the US Acceptable Daily Intake). On post-natal day (PND) 28, the offspring were weaned and randomly distributed into two cohorts: 6-week cohort with 8 pups/sex/group and 13-week cohort with 10 pups/sex/group. After weaning, the pups also received the same dose of glyphosate or Roundup Bioflow as that of dams until their sacrifice (PND 73 ± 2 for the 6-week cohort; and PND 125 ± 2 for the 13-week cohort). Reproductive outcome of dams, and developmental landmarks and sexual characteristics of pups were examined.	was higher (4/8) compared to control (2/8)), number of stillborns, survival index at PND 1 or 21, weight of adrenals, uterus or ovaries and total testosterone levels <u>Offspring:</u> -the anogenital distance (AGD) on PND 4 was statistically significantly increased in males -increased TSH in males of 6-week cohort group	
Perinatal study Oral (drinking water) Swiss mouse GD 0 p.c. PND 20 No guideline No GLP 0, 0.5, 5, 50 mg/kg bw/day	Glyphosate (Purity: ≥99.2 %) and Roundup 3 Plus containing 229 g/l glyphosate isopropylamine salt (170 g/l glyphosate acid equivalent)	In this study, the Swiss mouse were given glyphosate (G) or glyphosate-based herbicide Roundup 3 Plus (R) via drinking water at 0, 0.5, 5 and 50 mg/kg bw/day from embryonic day 0.5 to 20 days post-partum. Male offspring of the mice (at least 5 derived from 3 to 4 different litters in each group) were sacrificed at 5, 20, 35 days or 8 months for the following examinations: epididymis, seminal vesicles and testis weight, testis morphology,	*Note: Only the results of glyphosate presented below* <u>0.5 mg/kg bw:</u> -decreased relative testis weight (at 35 d and 8 months old mice) -increase in vacuoles in seminiferous epithelium (20 d old mice) -decreased serum testosterone (35 d old mice) -the expression *Sall4* were decreased (5 d old mice) <u>5 mg/kg bw/day:</u>	CA 5.6.1/017 Pham *et al*. (2019). Toxicological Sciences, Vol. 169, Issue 1, May 2019, Pages 260–271

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
The study is reliable with restrictions because of the following reasons: small group size, limited description of the study conditions and the results.		spermatozoa in total epididymis, serum testosterone; and the following in only G groups – number of undifferentiated spermatogonia and Sertoli cells (35 days old), expression of several genes in spermatogonia and germ cells number in testis (5 days old).	- decreased no. of undifferentiated spermatogonia (35 d old mice) -increase in vacuoles in seminiferous epithelium (20 d old mice) -empty seminiferous tubulues (20 d old mice) -the expression *Sall4* were decreased (5 d old mice) <u>50 mg/kg bw/day:</u> -decreased serum testosterone (35 d old mice) -increase in vacuoles in seminiferous epithelium (20 d old mice) -the expression of *Kit* and *Sall4* were decreased (5 d old mice)	
Prenatal study Oral (drinking water) ICR Mouse No guideline No GLP GD 0 up to PND 21 Concentration: 0.5% (a drinking water concentration of 0.5% (5g glyphosate/L) will corresponds to 1000 mg/kg bw/day using a	Glyphosate (purity not specified) and Roundup (glyphosate as isopropylamine salt)	10 ICR mice/group were given glyphosate (G) or glyphosate-based herbicide Roundup (R) via drinking water at a single dose level of 0.5% of glyphosate in both groups from gestation day (GD) 1 to GD19. A similar group of animals were given distilled water and served as the control group. Five dams/group were sacrificed on GD19 and fetuses were examined. The liver and serum samples of the fetus/offspring (2/sex/litter preferred; on PND7 and PND21) were collected to examine the	*Note: only the results for glyphosate are reported below:* <u>0.5%</u> <u>Offspring:</u> hepatic steatosis, increased serum and liver concentrations of lipids	CA 5.6.1/018 Ren *et al*. (2019). Environmental Pollution 254 (2019) 112906

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
default value of 0.2 for subacute studies according to EFSA guidance document on default values (EFSA Journal 2012;10(3):2579) Study reliable with restrictions (glyphosate used is not sufficiently characterised, only one dose level was tested, there was large inter animal variability observed and too few animals per dose level were analysed)		following: the serum biochemical indexes, histopathological observations, lipid concentrations and mRNA gene expression levels that are related to lipogenesis and lipid catabolism in the livers.		
in vitro oocyte cultures mice (Kunming mice) No guideline No GLP 50, 100, 200, 500 µM The study is reliable with restrictions (test material identity, in particular purity, not specified; only a single concentration level tested in the experiments (except one); a positive control was missing)	Glyphosate (purity: not reported)	In this *in vitro* study, oocytes from Kunming mice were treated with 50, 100, 200 or 500 µM glyphosate to evaluate the ratio of germinal vesicle breakdown (GVBD) and first polar body extrusion (PBE) and with 500 µM glyphosate to evaluate the reactive oxygen species (ROS) levels, spindle morphology, mitochondrial function, DNA integrity, cell apoptosis and autophagy. At least 50 oocytes from 24, 12 or 6 mice were analysed for each experiment, with at least three biological replicates.	<u>50 and 100 µM:</u> No effects <u>200 µM:</u> - GVBD and PBE decreased <u>500 µM:</u> - GVBD and PBE decreased (indicating effects on oocyte developmental competence) -mRNA expression of sod3, gpx and cat genes (suggesting enhanced ROS production) -misaligned chromosomes, abnormal spindle morphology and reduced p-MAPK protein levels in oocytes -mitochondrial membrane potential was lowered (suggesting interference with the	CA 5.6.1/019 Zhang *et al*. (2019). Chemosphere 237 (2019) 124435

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
			mitochondrial function in oocytes) -expression of Bcl-2 protein decreased, while that of Bax protein increased (suggesting induced early apoptosis in oocytes). -the mRNA expression of autophagy-related genes (Ic3, atg14 and mtor) and expression of autophagy-related proteins (LC3 and Atg12) was increased (suggesting induced autophagy in oocytes)	
2-week Orally (gavage) SD rat No guideline No GLP 0, 2.5, 25 mg/kg bw/day The study is reliable with restrictions as the exposure duration was short (2 weeks only), endpoints were limited (for e.g., testes were not weighed) and there were only 2 dose levels (for glyphosate; and only 1 for Glyfonova).	Glyphosate (purity: ≥96 %) and Glyfonova (herbicide formulation)	Adult Sprague-Dawley male rats (10/group) were treated via gavage with glyphosate at 2.5 and 25 mg/kg bw/day; and with a glyphosate-based herbicide Glyfonova at 25 mg/kg bw/day equivalent glyphosate dose for 2 weeks. Following parameters were investigated: intra-testicular testosterone levels; expression of key marker genes in the testes; testis histopathology, protein expression analysis and apoptotic activity.	2.5 and 25 mg/kg bw/day: No effects observed on either of the various testicular parameters examined	CA 5.6.1/020 Johansson *et al*. (2018). Reproductive Toxicology, Vol. 82, December 2018, Pages 25-31

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
13-week (pilot study) Orally (drinking water) Design of the study derives from the 13-week cohort protocol of the National Toxicology Program's (NTP) Modified One-Generation Reproduction Study 2011 (as stated in article) GLP: Yes SD rat 8 animals/sex for 6-week cohort 10 animals/sex for 13- week cohort Treatment starting from prenatal life, i.e. GD 6 of their mothers to PND 73 or 125 1.75 mg/kg bw/day The study is reliable with restrictions (low dose only, few animals, actual levels of test compounds that reached the foetus during gestation or that were ingested postnatally by the offspring during the period of lactation were not estimated).	Glyphosate (Purity: >99.5 %) and Roundup Bioflow	Sprague-Dawley rats were orally via drinking water exposed to 1.75 mg/kg bw/day starting from prenatal life, i.e. gestational day (GD) 6 of their mothers. One cohort was continuously dosed until sexual maturity (6-week cohort) and another cohort was continuously dosed until adulthood (13-week cohort). The endpoints investigated were mortality, body weight, water and food consumption, and clinical signs in dams and offspring and litter data.	1.74 mg/kg bw/day Survival, body weights, food and water consumption of rats were not affected by the treatment with glyphosate. No clinical changes were observed in the animals of the dosed groups. Furthermore, litter sizes were fully comparable among groups. In the treated rats, the majority of glyphosate was excreted in urine unchanged at levels of about 100-fold higher than that of AMPA and the mean urinary concentration of glyphosate increased with the duration of treatment.	CA 5.6.1/021 Panzacchi *et al*. (2018). Environmental Health 17:52
in vitro bovine granulosa and theca cell	Glyphosate (purity: not specified)	In this study effects of glyphosate on ovarian cell proliferation, steroid production and gene expression were evaluated using	0.01, 0.03, 0.5 and 1.7 µg/mL No effects 5 µg/mL	CA 5.6.1/022 Perego *et al*. (2017). J. Appl. Toxicol, 37: 692-698

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No guideline No GLP 0, 0.01, 0.03, 0.5, 1.7, 5.0 µg/mL The study is reliable with restrictions (glyphosate used is not sufficiently characterised, no positive controls were used and the tests were conducted with few test concentrations)		bovine granulosa cells (GC) and theca cells (TC) as *in vitro* models.	Impaired granulosa cells proliferation and estradiol production	
5 weeks Orally (gavage) Sprague Dawley rat No guideline No GLP 0, 5, 50, 500 mg/kg bw/day Reliable with restrictions. Limited parameters investigated in the study, low number of animals used, and no details about clinical signs.	Isopropylamine salt of glyphosate (purity 90 %)	The potential toxicity of glyphosate to the male reproductive system of the rat has been investigated after oral treatment with glyphosate for 5 weeks at dose levels up to 500 mg/kg bw. The endpoints studied were body weight, food intake, daily weight gain, absolute and relative reproductive organ weight, serum hormone levels, oxidative stress parameters, testicular histopathology and expression of AR in testis.	<u>5 mg/kg bw/day:</u> -a trend towards decreased serum concentrations with dose for testosterone and progesterone <u>50 mg/kg bw/day:</u> -a trend towards decreased serum concentrations with dose for testosterone and progesterone -decreased weight of seminal vesicle gland, coagulating gland (22%) -reduced bw (10%) not statistically significant <u>500 mg/kg bw/day:</u> -reduced food consumption -decreased weight of seminal vesicle gland, coagulating gland (30%) -decreased total sperm count -a trend towards decreased serum concentrations with dose for testosterone and progesterone	CA 5.6.1/023 Dai *et al*. (2016). Acta Histochemica 118, 519-526

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
			-reduced bw (9%) not statistically significant	
in vitro murine Leydig cell culture (Mouse Leydig BLTK1 (BLT-1 cells, clone K1) No guideline No GLP 1, 3, 10, 30, 100, 300, 600 µM Reliable with restrictions because the test substance was not characterised and the results of only one concentration level were reported.	Isopropylamine salt of glyphosate (purity: 90% w/w)	In this study, recombinant human chorionic gonadotropin (rhCG) and forskolin (FSK) were used as positive controls for the induction of steroidogenesis, as measured by increases in progesterone, testosterone and 17β-estradiol levels in culture media. Murine BLTK1 Leydig cells were investigated as a novel model for evaluating the effects of chemicals on steroidogenesis.	When tested in this system glyphosate was not found to induce testosterone production or alter rhCG induction of testosterone.	CA 5.6.1/024 Forgacs *et al*. (2012) Toxicological Sciences 127(2), 391–402

GD gestational day
PND post-natal day

Table 2.6.6.1-38: Summary table of other studies on sexual function and fertility which are not considered further for the endpoint of reproductive toxicity

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
60 days Oral Rat (Wistar)	Glyphosate-based herbicide marked as "Tackle" (purity not reported), manufactured by The Candel FZE F-08-004, along N4-E7 road, Lekki, Lagos, Nigeria	The aim of the study was to explore the impact of glyphosate on the characteristics of spermatozoa and pregnancy success rate in females following oral administration of glyphosate in male rats. The specific objectives include the examination of the effects of glyphosate on sperm count, motility	<u>400 mg/kg bw/day:</u> -decreased sperm count (280.60 compared to 323.60 in control) (not stat. sign) -abnormal morphology 26 (compared to 9 in control) -reduced litter size (1.8 compared to 8.2 in control)	Sakpa C. L. *et al*. (2018). Ann Biomed Sci Vol. 17, No. 2, pp. 156-164

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
5 males/dose (age not reported) As from day 61 of the experiment male rats from each group were allowed to mate with females in their groups Total: 15 males and 15 females used in study No guideline stated No GLP 0, 400, 2000 mg/kg bw/day Supplementary data The test substance used was a glyphosate-based formulation ("Tackle") and effects caused by co-formulants cannot be excluded. The study is reliable with restrictions because of the following reasons: the test substance is not sufficiently characterised, only two doses tested, small group sizes, clinical observations not		and morphology in adult Wistar rats and to determine the pregnancy success rates and litter size in females mated with glyphosate treated male Wistar rats.	-reduced pregnancy outcome (40% compared to 100% in control) <u>2000 mg/kg bw/day:</u> -decreased sperm count (193.60 compared to 323.60 in control) -abnormal morphology 66 (compared to 9 in control -reduced litter size (0.6 compared to 8.2 in control) -reduced pregnancy outcome (20% compared to 100% in control)	

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
presented, body weights not recorded)				
Review article The reference is considered as supplementary data only (review article) Several factors may interfere with the results such as intraspecific variability, age of animals at start of experiments, dose and duration exposure, body weight and potential strain differences. Furthermore, the test substance used in the selected studies is not sufficiently characterized in this review report, and the selected studies are inadequately described.	Not applicable	The aim of this study was to explore the potential adverse effects of glyphosate on the reproductive function of male rats and mice. A systematic and comprehensive literature search was performed using five different databases with different combinations of glyphosate exposure and sperm concentration. As stated by study author, eight studies were identified as qualified and a random effect model was conducted. A comprehensive literature search was performed on the association between glyphosate exposure on rodents and change of sperm concentration. The search was conducted in Pub Med, Web of Sciences, MEDLINE, TOXLINE, Embase, CNKI, and Wanfang databases from January 1990 up to November 2016, with a combination of the following keywords: glyphosate; round up; reproductive toxicity; testicular; testes; sperm reserves; sperm quality sperm concentrations; male; animal; rats; and mice. Further, titles and abstracts were examined of all papers obtained to identify other potential articles. The search and evaluation were conducted in November 2016.	The results suggest that exposure to glyphosate caused a decrease in sperm concentration in both rats and mice.	Cai W. *et al*. (2017). Environmental Toxicology and Pharmacology (2017), Vol. 55, pp. 148-155
12 weeks Oral (gavage) Rat (albino) No guideline	Roundup (360 g/L of glyphosate in the form of 441 g/L potassium salt) from Monsanto Europe S.A./N.V., Antwerp, Belgium, O611, F-1059 3379	The aim of the study was to assess the effect of Roundup on the reproductive capacity of adult male albino rats. Eight male albino rats/dose were administered daily via gavage with 3.6, 50.4 and 248.4 mg/kg bw of glyphosate in 0.25 ml/100 g for 12 weeks. The control group was administered 0.25 ml/100 g distilled water for 12 weeks. At the	The reproductive hormones testosterone, FSH and LH levels in blood appeared to be dose dependently decreased while the prolactin levels appeared to be dose dependently increased. Oxidative stress markers in testes were observed to be dose dependently reduced compared to control rats with exception of MDA which	Owagboriaye F. O. *et al*. (2017). Experimental and Toxicologic Pathology (2017), Vol. 69, No. 7, pp. 461-468.

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No GLP 8 males/dose 0, 3.6, 50.4 and 248.4 mg/kg bw Supplementary data The study is carried out with a formulation of glyphosate, thus effects caused by co-formulants cannot be excluded. The study is reliable with restrictions because of the following reasons: the test substance is not sufficiently characterised (particularly, purity and batch not specified), small group sizes of animals used, strain of animals not specified, no details on food, clinical signs and body weight not specified, no historical control data and positive control missing.		end of the study, blood sample, epididymal sperm cells and testicular tissue were collected from the rats to assess reproductive hormones (testosterone, luteinizing hormone [LH], follicle-stimulating hormone [FSH] and prolactin), oxidative stress indices, epididymal sperm morphology, sperm count and motility and testicular histopathology.	increased with increased doses of Roundup. Sperm motility and count were also decreased in rats exposed to Roundup. Histopathology assessment of the testes from the control group revealed normal cellular architectural structure while progressive degenerative lesions were observed in rat testes exposed to Roundup.	
Perinatal study Oral (feeding)	Glyphosate formulation MAGNUM SUPER II (66.2 % glyphosate potassium salt (equivalent	The aim of the study was to investigate whether perinatal exposure of 2 and 200 mg/kg bw/day of a glyphosate-based herbicide (GBH) alters female reproductive	GBH exposure was not found to alter the body weight gain, vaginal opening onset, pregnancy rates, number of corpora lutea or resorption sites of F1 females. However,	Milesi, M. M. *et al*. (2018). Archives of Toxicology, Vol. 92, No. 8, pp. 2629 2643

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
Rat (Wistar-inbred strain) No guideline No GLP 7 pregnant females/group 0, 2 or 200 mg/kg bw/day Exposure from GD 9 until LD 21 Supplementary data The study is carried out with a formulation of glyphosate, thus effects caused by co-formulants cannot be excluded. The study is not reliable due to uncertainties with regard to statistical analyses. Furthermore, the test substance is not sufficiently characterised (particularly, purity and batch not specified), small group sizes of animals used, inbred strain of animals used, no	to 54 % w/v glyphosate acid) Grupo Agros S.R.L., Argentina	performance, and/or induces second-generation effects related to congenital anomalies or growth alterations. Pregnant rats (F0) were administered GBH through food in a dose of 2 mg or 200 mg of glyphosate/kg bw/day from gestational day 9 until weaning. The serum concentrations of glyphosate and AMPA were determined at the end of the lactation period. Body weight gain and vaginal canal opening of F1 females were recorded. Sexually mature F1 females were mated and their reproductive performance assessed by determination of pregnancy rate and on gestational day 19, the number of corpora lutea, the implantation sites and resorption sites. To evaluate possible second generation effects on F2 offspring, foetal morphology on gestational day19, foetal length and weight, and the placental weight were analysed.	the number of implantation sites were lower in F1 females of both exposed groups with an increased pre-implantation loss. F2 offspring from both GBH exposed groups showed lower foetal weight and length. Also a higher incidence of small for gestational age foetuses, higher placental weight and structural congenital anomalies (conjoined foetuses and abnormally developed limbs) were found in F2 offspring from 200 mg/kg bw/day treated dams.	

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
details on food, clinical observations not presented, no historical control data and positive control missing. It could also be noted that the rats were not exposed during the whole period of organogenesis since exposure started at GD 9 instead of GD 5 as recommended in the OECD TG 414.				

2.6.6.1.1 Short summary and overall relevance of the provided information on adverse effects on sexual function and fertility – generational studies

The potential of glyphosate to cause effects on sexual function and fertility was examined in several generational studies in the rat (Table 57). Furthermore, studies from the open literature were taken into account to evaluate intrinsic properties of glyphosate on reproductive tissues and organs (Table 59).

All studies listed in Table 57 were submitted and evaluated in previous EU evaluations (RAR 2013 and/or DAR 1998). There are no new standard toxicity studies (generational studies) submitted for this report.

Nine multigenerational studies are available of which 5 studies are considered to be of acceptable quality, one is considered supplementary, and 3 studies are considered not acceptable. Furthermore, two one-generation studies are available, one considered supplementary and the other one not acceptable.

The studies considered as not acceptable (CA 5.6.1/014, CA 5.6.1/013, CA 5.6.1/011, CA 5.6.1/012) were included in Table 57 (text in grey colour) but were not considered further in this section. The scientific value of the studies CA 5.6.1/011, CA 5.6.1/012, CA 5.6.1/014 was considered limited mainly because the dose levels tested were much too low for the identification of harmful effects of glyphosate administration on reproduction. For study CA 5.6.1/013 reporting deficiencies and major deviations from OECD TG 416 were reported. For more detailed study summaries, please see Vol. 3-B.6 (AS), section B.6.6.1 (Generational studies).

Generational studies

In the most recent two-generation study by [redacted] (2007) (CA 5.6.1/001-003, Report No. 2060/0013) using Sprague-Dawley rats, treatment-related effects on the parental and offspring generations were observed. Briefly, increased liver weights (13%) were observed in females of both generations at 15000 ppm, and increased kidney weights (11%) were observed in F0 females at the same dose level. No similar findings were detected in males. However, it could be noted that the achieved dose level for males (1063 mg/kg bw/day) was less when compared to females (1634 mg/kg bw/day). In the absence of clinical chemistry investigations in the study, the increased liver weight of 13% noted in females was considered adverse, although no histopathological changes were observed in the liver. Further, at 15000 ppm a significant decrease in homogenisation resistant spermatids (HRS, cauda epididymis) was counted in F0 males (control: 400 million/gram; 15000 ppm: 309 million/gram) (23% reduction compared to control). No sperm changes were seen in testis, and histopathological examinations did not reveal any changes in the testis or epididymis. In F1 male offspring, preputial separation was delayed at 15000 ppm without any additional developmental retardation indicating a delay in male sexual maturation. Although, the later onset of preputial separation in male offspring at 15000 ppm had obviously no impact on reproductive performance in week 29, a treatment related effect on sexual maturation at high dose level cannot be excluded. It could be noted that the findings of decreased HRS in cauda epididymis in F0 males and delayed sexual maturation in F1 males occurred at limit dose (1000 mg/kg bw/day). General toxicity was observed for females only. Based on the mentioned effects above, a NOAEL of 5000 ppm (ca. **351 mg/kg bw/day**) was considered for parental, reproductive and offspring toxicity (the NOAELs set in previous evaluation RAR (2015) remains).

In a two-generation study by [redacted] (2000) (CA 5.6.1//004, Report No.: [redacted]/P/6332) using Wistar rats, fertility and reproductive performance was not adversely affected by treatment. No impact on sexual maturation was observed up to the highest dose level of 10000 ppm (985 mg/kg bw/day). A reduction in body weight of F1A pups (10%) was observed at the highest applied dose of 10000 ppm resulting in a subsequent reduction (5%) in body weight of the selected F1 parent males for the duration of the mating period.

The NOAEL for parental toxicity was set at 10000 ppm (**985 mg/kg bw/d**ay) (highest dose) (in previous RAR (2015), the parental NOAEL was set at 3000 ppm based on “a lower body weight in F1 pups and a subsequent reduction also in body weight of F1 adult males at 10000 ppm”)

The NOAEL for offspring toxicity was set at 3000 ppm (**293 mg/kg bw/day**) based on reduction in the body weight of the F1A pups in the 10000 ppm group.

The NOAEL for reproductive toxicity was set at 10000 ppm (**985 mg/kg bw/day**, mean daily intake of glyphosate during pre-mating phase in F0 males) (highest dose).

In a two-generation study by [redacted] (1997) (CA 5.6.1/005, Report No.: [redacted] 96-0031) using Sprague Dawley rats, treatment was associated with a number of parentally toxic effects at the highest dose of 30000 ppm (>2000

mg/kg bw/day). Following effects were observed: loose stool (males of both generations), reduced body weight (<10%, males of both generations), lower fertility indices (F1 females, 79.2% compared to 95.8% in control, not statistically significant), increased liver weights (F1 males and females), increased kidney weights (males of both generation and F1 females), decreased prostate weights (F1 males) and distension of the caecum (males and females of both generations). Offspring toxicity consisted of significantly decreased body weight and distension of caecum observed in F1 and F2 pups at 30000 ppm. Sexual maturation (preputial separation and vaginal opening) was not examined in this study.

Based on the results, the NOAEL for parental and offspring toxicity was considered to be 6000 ppm (**417 mg/kg bw/day**).

A NOAEL of 6000 ppm (**417 mg/kg bw/day**) was considered for reproductive toxicity based on lower fertility indices observed at 30000 ppm (2150 mg/kg bw/day), although not statistically significant (in previous RAR (2015), the reproductive NOAEL was set at 30000 ppm)

Supplementary data indicating no hazard on reproduction, are obtained by the two-generation study conducted by [redacted] (1993) (CA 5.6.1/006, Report No. TOXI 885-RP-G2) using random bred Wistar rats. Parameters like general health, growth of parents, gestation/lactation period, body weight and food consumption, gross necropsy findings of pups and parents were unaffected by treatment up to the highest tested dose of 10000 ppm. Further, glyphosate did not affect mortality incidence, parturition performance, mean litter size, pup weight and male and female fertility index. It could however be noted that the study was limited (effect dose lacking). No sperm analyses were performed in the study, and sexual offspring development was not investigated. The NOAEL for parental, offspring and reproductive toxicity was set at 10000 ppm (**about 700-800 mg/kg bw/day**) (the NOAELs set in previous evaluation RAR (2015) remains).

In the study by [redacted] (1992) (CA 5.6.1/007-008, Report No. [redacted] 47/911129) oral administration of glyphosate to Sprague-Dawley rats by dietary admixture at a maximum dose level of 10000 ppm for two successive generations resulted in effects consisting of increased food and water consumption of F1 females at 10000 ppm. Histopathological findings in the salivary gland (hypertrophy of acinar cells with prominent granular cytoplasm) were observed in parental animals of both generations at ≥3000 ppm. The severity grade of the findings was minimal. The increased incidence of parotid findings showed a statistically significant trend with NOAEL at 1000 ppm for F0 and F1 generation females, and 3000 ppm for F0 and F1 generation males. The increased incidence of submaxillary findings showed a statistically significant trend with NOAEL at 1000 ppm for F0 females and 3000 ppm for F1 females. No historical control data are available. Since effects on salivary gland weights were not investigated in this study, the LOAEL for effect on salivary gland was set at 3000 ppm as a precautionary approach although the severity grade of findings observed in the study was minimal. No effects were observed in pups. Sperm analysis was not performed in this study.

The NOAEL for parental toxicity was 1000 ppm (**66 mg/kg bw/day**) based on changes observed in salivary glands observed at ≥3000 ppm (≥197 mg/kg bw/day) (in previous RAR (2015), the parental NOAEL was set at 3000 ppm)

The NOAEL for offspring toxicity was set at 10000 ppm (**668 mg/kg bw/day**) (highest dose) (in previous RAR (2015), the offspring NOAEL was set at 3000 ppm)

The NOAEL for reproductive toxicity was 10000 ppm (**668 mg/kg bw/day**) (highest dose) (the NOAEL for reproductive toxicity set in previous evaluation RAR (2015) remains).

In the respective one-generation dose-range finding study performed by [redacted] (1991) (CA 5.6.1/009, Report No. [redacted] 42/90619) 3000, 10000 and 30000 ppm were applied from day 3 of pregnancy through to termination of the study. Maternal toxic effects were observed in F0 females at 10000 ppm (soft faeces) and 30000 ppm (one mortality for which cause of death was not identified, soft faeces and reduced bw and body weight gain). Macroscopical (enlarged/firm/congestion/swollen) and histopathologic changes in salivary glands were recorded in all treatment groups. The microscopical changes in salivary glands consisted of granular basophilic cytoplasm of acinar cells and hypertrophy of acinar cells observed in F0 females at all dose levels and prominent mitoses in acinar cells observed in F0 females at 30000 ppm. The acinar cells hypertrophy was moderate at 10000 ppm and marked at 30000 ppm. Furthermore, macroscopic gastro-intestinal changes were observed in F0 females at all dose levels (content watery and/or dark and stomach distended and/or congested observed at ≥3000 ppm, distended caecum observed at 30000 ppm). Findings in pups consisted of reduced pup weights observed at ≥3000. Furthermore, macroscopic changes in salivary gland (congested) were observed in one pup at 3000 ppm and in four pups at 10000 ppm, but the significance of this finding was not clear since this effect did not occur in the highest dose group (30000 ppm). No adverse effect on reproduction parameters were observed. The study is not suitable for NOAEL setting

(few animals used, limited parameters investigated, no statistical analyses conducted). However, it is noteworthy that effects occurred in this study at lower dose levels than in the main study. The findings of reduced pup weights observed at the low dose level of 3000 ppm (236 mg/kg bw/day) were not confirmed in the main study using sufficient number of animals and test doses up to 10000 ppm, nor in other available generational studies for which much more animals were employed.

In the two-generation study by [redacted] (1990) (CA 5.6.1/010, Report No. [redacted]-10387) maternal toxicity was evident in the high dose group at 30000 ppm (about 2000 mg/kg bw/day) indicated by soft stool and reduced body weights. At the same dose level, pups showed decreased body weights when compared to controls. A slight reduction in the average litter size (13%) was observed in the F0 dams of the 30000 ppm dose group, and to a lesser degree in the F1 dams. The reduction was non-statistically significant and not noted when F1 animals were re-mated, and treatment-relation was considered to be equivocal. A reduction in litter size was not confirmed in the study by [redacted] (1997) (CA 5.6.1/005, Report No.: [redacted] 96-0031), where the same strain of animal and dietary concentrations of glyphosate were tested. The NOAEL for parental, offspring and reproductive toxicity was set at 10000 ppm (**666 mg/kg bw/day**) (the NOAELs set in previous evaluation RAR (2015) remains).

Overall summary (generational studies)
Effects on adult animals consisted of a significant decrease in homogenisation-resistant spermatid count and equivocal effects on litter size observed in rats at limit test dose. Furthermore, lower fertility indices were observed in one study with Sprague Dawley rats at high dose level (above 2000 mg/kg bw/day) but this finding was not statistically significant. It could be noted that this effect occurred at a lower dose level (462.2 mg/kg bw/day) in the [redacted] (1985) study (CA 5.6.1/013) conducted with Wistar rats. However, this latter study was not acceptable due to reporting deficiencies and major deviations from OECD TG 416.

Effects on the offspring consisting of reduced pup weight and delayed preputial separation were observed at limit test dose (1000 mg/kg bw/day), and distended caecum was observed at a very high dose of 30000 ppm (above 2000 mg/kg bw/day).

Studies from the open literature

In studies from the open literature summarised in Table 59, the effects of the active substance glyphosate and Round-up formulations have been investigated. In the following, the focus is on effects after glyphosate treatment. For more detailed study summaries, please see Vol. 3-B.6 (AS), section B.6.6.3 (Reproductive toxicity- Information from public literature).

Male reproductive system:

Effects on the male reproductive system were investigated in two *in vitro* and four *in vivo* studies following different treatment protocols. Briefly, *in vitro* exposure of Sertolli cells to glyphosate was reported to alter Sertolli cell junction barrier permeability and to decrease testosterone-stimulated TER. Further, a redistribution of claudin11 was observed (Gorga *et al*., 2020). No effects on the induction of testosterone production or alteration of recombinant human CG induction of testosterone was reported in Leydig cells up to 600 µM (Forgacs, 2012).

Under *in vivo* conditions, a decrease in absolute (but not relative) weight of the seminal vesicle gland and coagulating gland was observed in Sprague Dawley rats after subacute (5 weeks) exposure to ≥50 mg/kg bw glyphosate/day, and the total sperm count was significantly decreased at 500 mg/kg bw/day. Also, there was a trend towards decreased serum concentrations with dose for testosterone and progesterone (Dai *et al*., 2016). Further, sperm-depleted seminiferous tubuli were reported in 35 days old Swiss mice exposed to 5 mg glyphosate/kg bw/day in drinking water from embryonic day 0.5 to 20 days post-partum (Pham *et al*., 2019). No similar effect was observed in the same study in mice sacrificed at later time points. Further, no dose response was observed since the effect was only seen in the mid-dose group.

No effects on testes have been observed in SD rats following *in vivo* exposure up to 25 mg/kg bw/day over 14 weeks in a NTP study. The parameters investigated in the study were as follows: intra-testicular testosterone levels, expression of key marker genes in the testes, testis histopathology, protein expression analysis and apoptotic activity (Johansson *et al*., 2018). No effects on sperm parameters (number of mature spermatids in the testis, daily sperm production, number and sperm transit time through caput/corpus and cauda epididymis and morphology) were observed in the study by Manservisi *et al*., 2019) where rats were exposed to glyphosate at 1.75 mg/kg bw/day for 13 weeks.

Overall, the published data might suggest that the male reproduction system was adversely affected by glyphosate exposure leading to reduced sperm counts at doses of 500 mg/kg bw/day (Dai *et al*., 2016). Likewise, a significant decrease in homogenisation-resistant spermatid count in F0 males was observed at ca. 1000 mg/kg bw/day in the regulatory study performed by [redacted] (2007). The finding in study by Dai *et al* (2016) was however not given appropriate weight since the study was reliable with restrictions (few animals used and limited parameters investigated).

Female reproductive system:

Effects on maturation of oocytes was investigated in two studies under *in vitro* conditions. Zhang *et al*. (2019) reported that 500 µM glyphosate treatment negatively affected development of mouse (Kunming) oocytes as indicated by reduced rates of germinal vesicle breakdown and first polar body extrusion. Further, molecular analysis indicated increased reactive oxygen species and an influence of glyphosate on DNA stability and intracellular signaling pathways relevant for apoptosis. Only one single concentration level was tested in the experiment, no positive controls were used and the purity of test substance was missing. Further, Perego *et al*. (2017) investigated ovarian function in bovine granulosa and theca cells after glyphosate stimulation. A slight, non-dose-related alteration in bovine granulosa cell proliferation and estradiol production was observed at 5 µg/mL. Due to the isolated occurrence of the observed effects without any dose-response relationship, the biological significance of those findings was requested.

Overall, the effects observed *in vitro* could only be considered to be of limited *in vivo* relevance and are not considered to conclusively indicate a hazard on female fertility.

Further *in vivo* studies investigating reproductive and developmental toxicity:

Manservisi *et al*. (2019) performed a pilot study in Sprague-Dawley rats (8/group) for an extended-one generation study (OECD 443). In this study the F0 female breeders received the test item from gestation day (GD) 6 to the end of lactation, while the offspring (F1) continued to be exposed after weaning for an additional 6 or 13 weeks. The test item, glyphosate (G) (> 99.5% pure), was diluted in drinking water to achieve glyphosate dose of 1.75 mg/kg bw/day (the US Acceptable Daily Intake). The endpoints analysed in the study were body weight, water and food consumption, gestational parameters, litter parameters, landmarks of sexual development, estrous cyclicity, gross and histopathology of reproductive and endocrine tissues, sperm parameters and serum and plasma hormone levels. Reproductive parameters remained to be unaffected by glyphosate exposure at 1.75 mg/kg bw/day. The anogenital distance (AGD) on PND 4 was statistically significantly increased in males. Furthermore, increased TSH level in plasma was reported in male animals at this dose level.

Overall, a delay in male sexual development indicated by increased anogenital distance observed on PND 4, and an increased TSH level in plasma was reported in male rats. Due to relevant methodological limitations, including the low number of test animals and timing of blood sample collection, interpretation of the reported findings should be considered with caution. However, a significant delay in sexual maturation in male offspring (F1) indicated by delayed preputial separation (occurring after 45.9 days in the mean versus 43.0 days in the control group) was also observed in the regulatory study performed by [redacted]. (2007) which became apparent at the top dose level of 15000 ppm (higher than 1000 mg/kg bw/day). Thus, following a weight-of-evidence approach a negative effect on male sexual development cannot be fully excluded. With regard to the effect of increased TSH level in plasma in male animals, RMS is of the view that the increased TSH should be considered as indicative of the thyroid-related activity at a low dose of glyphosate tested in this study and should be considered along with the outcome of thyroid parameters in other repeated dose toxicity studies with glyphosate.

In the study by Panzacchi *et al*. (2018) Sprague-Dawley rats were orally via drinking water exposed to 1.75 mg/kg bw/day starting from prenatal life, i.e. gestational day (GD) 6 of their mothers. One cohort was continuously dosed until sexual maturity (6-week cohort) and another cohort was continuously dosed until adulthood (13-week cohort). The endpoints investigated were mortality, body weight, water and food consumption, and clinical signs in dams and offspring and litter data. Survival, body weights, food and water consumption of rats were not affected by the treatment with glyphosate. No clinical changes were observed in the animals of the dosed groups. Furthermore, litter sizes were fully comparable among groups.

In the study by Ren *et al*. (2019), 10 ICR mice/group were given glyphosate via drinking water at a single dose level of 0.5% of glyphosate from gestation day (GD) 1 to GD19. A similar group of animals were given distilled water and served as the control group. Five dams/group were sacrificed on GD19 and foetuses were examined. The liver and serum samples of the foetus/offspring (2/sex/litter preferred; on PND7 and PND21) were collected to examine the following: the serum biochemical indexes, histopathological observations, lipid concentrations and mRNA gene

expression levels that are related to lipogenesis and lipid catabolism in the livers. A statistically significant decrease in body weight gain of the offspring was observed on PND21. There was no difference though when the values were considered on the basis of sex. Furthermore, liver histopathology showed increased vacuoles with lipid droplets, more red areas representing lipid substances and clusters of monocytes (PND7 females). The study is reliable with restrictions because the glyphosate used is not sufficiently characterised, only one dose level was tested, there was large inter animal variability observed and too few animals per dose level were analysed. Thus, it not possible to clearly attribute any of the observed differences to glyphosate exposure.

There are several epidemiological studies available in the open literature reporting reproductive outcomes such as miscarriage, fecundity, pre-term delivery, gestational diabetes mellitus, birth weights, congenital malformations, neural tube defects, attention-deficit disorder/attention-deficit hyperactive disorder (ADD/ADHD) (e.g. Arbuckle *et al.* (2001)[12], Savitz *et al.* (1997)[13], Garry *et al.* (2002)[14]) Due to the uncertainties regarding type of formulation, exposure levels, simultaneous exposure to more than one pesticide, statistically significant positive association and the of recall bias the reliability of this information is difficult to assess, but this data is not considered to establish a clear link between exposure to the active substance and reproductive toxicity.

Overall summary (open literature): A review of available published literature did not provide conclusive evidence that glyphosate exposure negatively affects reproduction.

Table 2.6.6.1-39: Relevant parental NOAEL

Study	NOAEL for parental toxicity	LOAEL for parental toxicity	Parental effects at LOAEL (adverse effects in bold text)
[redacted] (2000) CA 5.6.1/004 Report No.: [redacted]/P/6332 Alpk:APfSD rat Dose levels: 0, 1000, 3000, 10000 ppm equivalent to mean achieved dose levels of: F0: 0, 99.4, 292.6, 984.7 and 0, 104.4, 322.8, 1054.3 mg/kg bw/day for males and females, respectively during pre-mating period F1: 0, 116.5, 351, 1161 and 0, 123.3, 370.8, 1218.1 mg/kg bw/day for males and females, respectively, during the premating period	985 mg/kg bw/day	-	-
[redacted] (2007) CA 5.6.1/001-003 Report No. 2060/0013 Sprague Dawley rat	351 mg/kg bw/day	1063 mg/kg bw/day	↑ **liver weight** (F0 females: abs weight: 13%, rel weight: 8%; F1 females: absolute weight: 10%, relative weight: 8%) ↑ **kidney weight** (F0 females: abs. weight: 11%, rel. weight: 7%)

12 Arbuckle, T. E. Lin, Z. Mery, L. S. (2001). An exploratory analysis of the effect of pesticide exposure on the risk of spontaneous abortion in an Ontario farm population. Environmental Health Perspectives Volume: 109

13 Savitz, D.A. Arbuckle, T. Kaczor, D. Curtis, K.M. (1997). Male pesticide exposure and pregnancy outcome. American Journal of Epidemiology Volume: 146, Number: 12, Pages: 1025-1036

14 Garry, V. F. Harkins, M. E. Erickson, L. L. Long-Simpson, L. K. Holland, S. E. Burroughs, B. L. (2002). Birth defects, season of conception, and sex of children born to pesticide applicators living in the Red River Valley of Minnesota, USA. Environmental Health Perspectives Volume: 110 Pages: 441-449

Dose levels: 0, 1500, 5000 and 15000 ppm glyphosate technical equivalent to mean achieved dose levels of: 0, 104, 351 and 1063 mg/kg bw/day for males, and 0, 162, 530 and 1634 mg/kg bw/day for females			
[redacted] (1997) CA 5.6.1/005 Report No.: [redacted]96-0031 Sprague-Dawley Dose levels: 0, 1200, 6000, 30000 ppm Equivalent to: F0: 0, 83.6, 417, 2151 and 0, 96.9, 485, 2532 mg/kg bw/day in males and females, respectively F1: 0, 91.7, 458, 2411 and 0, 104.8, 530, 2760 mg/kg bw/day in males and females, respectively	417 mg/kg bw/day	2151 mg/kg bw/day	-**clinical signs** (loose stool) (F0, F1, both sexes) ↓bw (F0 males: 8%; F1 males: 7%) -**organ weight changes** (liver: F1 males: abs weight: ↑13%; F1 females: abs weight: ↑ 22%, rel weight: ↑ 20%; kidney: F0 males: rel weight: ↑11%; F1 males: rel weight: ↑14%; F1 females: abs weight: ↑17%, rel weight: ↑15%; prostate: F1 males abs and rel weight:↓32%) -**lower fertility indices** (F1 females, not statistically significant) (79.2% compared to 95.8% in control) -**distension of caecum** (F0, F1) (both sexes)
[redacted] (1992) CA 5.6.1/007-008 Report No.: [redacted] 47/911129 Sprague-Dawley Dose levels: 0, 1000, 3000, 10000 ppm Equivalent to: F0: 0, 66, 197, 668 and 0, 75, 226, 752 mg/kg bw/day in males and females, respectively F1: 0, 76, 230, 771 and 0, 82, 245, 841 mg/kg bw/day in males and females, respectively)	66 mg/kg bw/day	197 mg/kg bw/day	3000 ppm (197 mg/kg bw/day) -**histopathological changes in salivary gland** (minimal hypertrophy of acinar cells with prominent granular cytoplasm) (Parotid, males: F0: 3/28, F1: 4/23; Parotid females: F0: 5/28, F1: 4/24; Submaxillary, females: F0: 4/28; F1: 0/24) 10000 ppm (668 mg/kg bw/day) ↑water consumption (F1 females, 17%) ↑food intake (F1 females) (3%) ↓ mean bw (F1 males, 1-7%) -**histopathological changes in salivary gland** (increased incidence of minimal hypertrophy of acinar cells with prominent granular cytoplasm) (Parotid, males: F0: 12/26, F1: 11/23; Parotid females: F0: 17/28, F1: 9/23; Submaxillary, females: F0: 14/28; F1: 3/23)
[redacted] (1990) CA 5.6.1/010 Report No. [redacted]-10387 Sprague-Dawley 0, 2000, 10000, 30000 ppm	666 mg/kg bw/day	1983 mg/kg bw/day	-**clinical signs** (soft stool) ↓**bw** (Terminal bw: F0 males: 8%, F1 males: 13%, F1 females: 10%; Maternal bw during gestation: Day 1: F0 females: 7%, F1 females first mating: 12%, F1 females second mating: 13%; Day 21: F0 females: 7%, F1 females first mating: 8%, F1 females second mating: 8%) ↓ litter size (F0 dams: 13%)

corresponding to 132-140, 666-711, 1983-2230 mg/kg bw/day for males and 160-163, 777-804, 2322-2536 mg/kg bw/day for females (calculated for F0 and F1A adults)			
[redacted] (1993) CA 5.6.1/006 Report No.: TOXI 885-RP-G2 Wistar (random bred) Dose levels: 0, 100, 1000, 10000 ppm Dietary level would correspond to a mean daily compound intake of 0, 7.7, 77 and 770 mg/kg bw/day. [The mean daily intake was not reported for all dietary levels, but for the low level of 100 ppm a corresponding average value of 7.7 mg/kg bw/d was given in the original report]. Supplementary data (effect dose lacking, limited parameters investigated)	700 mg/kg bw/day	-	No treatment related effects

Parental toxicity observed in the studies indicated effects on salivary gland, gastrointestinal disturbances, reduced body weight and organ weight changes. The lowest parental NOAEL of 66 mg/kg bw/day was observed in the [redacted] (1992) study (CA 5.6.1/007-008, Report No.:[redacted] 47/911129) where effects on salivary glands were observed at 197 mg/kg bw/day. Based on this effect the parental NOAEL was **66 mg/kg bw/day**.

Table 2.6.6.1-40: Relevant offspring NOAEL

Study	NOAEL for offspring toxicity	LOAEL for offspring toxicity	Effects in offspring at LOAEL
[redacted] (2000) CA 5.6.1/004 Report No.:[redacted]/P/6332 Alpk:APfSD rat Dose levels: 0, 1000, 3000, 10000 ppm equivalent to mean achieved dose levels of: F0: 0, 99.4, 292.6, 984.7 and 0, 104.4, 322.8, 1054.3 mg/kg bw/day for males and females, respectively during pre-mating period F1: 0, 116.5, 351, 1161 and 0, 123.3, 370.8, 1218.1 mg/kg bw/day for males and females, respectively, during the premating period	293 mg/kg bw/day	985 mg/kg bw/day	↓ **pup bw** (10%) (F1A)
[redacted] (2007)	351 mg/kg bw/day	1063 mg/kg bw/day	**-delayed sexual maturation** (delayed

CA 5.6.1/001-003 Report No. 2060/0013 Sprague Dawley rat <u>Dose levels:</u> 0, 1500, 5000 and 15000 ppm glyphosate technical equivalent to mean achieved dose levels of: 0, 104, 351 and 1063 mg/kg bw/day for males, and 0, 162, 530 and 1634 mg/kg bw/day for females			preputial separation, Days at completion: 45.9 compared to 43.0 in control) (F1 generation)
[redacted] (1997) CA 5.6.1/005 Report No.: [redacted] 96-0031 Sprague-Dawley <u>Dose levels:</u> 0, 1200, 6000, 30000 ppm Equivalent to: F0: 0, 83.6, 417, 2151 and 0, 96.9, 485, 2532 mg/kg bw/day in males and females, respectively F1: 0, 91.7, 458, 2411 and 0, 104.8, 530, 2760 mg/kg bw/day in males and females, respectively	417 mg/kg bw/day	2151 mg/kg bw/day	↓ **pup weights** (F1 males:14%, F1 females: 13%; F2 males: 9%, F2 females: 8%) **-distension of caecum** (F1 and F2 litters)
[redacted] (1992) CA 5.6.1/007-008 Report No.: [redacted] 47/911129 Sprague-Dawley <u>Dose levels:</u> 0, 1000, 3000, 10000 ppm Equivalent to: <u>F0:</u> 0, 66, 197, 668 and 0, 75, 226, 752 mg/kg bw/day in males and females, respectively <u>F1:</u> 0, 76, 230, 771 and 0, 82, 245, 841 mg/kg bw/day in males and females, respectively)	668 mg/kg bw/day	-	No treatment-related effects in offsprings
[redacted] (1990) CA 5.6.1/010 Report No. [redacted]-10387 Sprague-Dawley 0, 2000, 10000, 30000 ppm corresponding to 132-140, 666-711, 1983-2230 mg/kg bw/day for males and 160-163, 777-804,	666 mg/kg bw/day	1983 mg/kg bw/day	↓ **pup weight** (Day 21: F0 males: 13%, F0 females: 11%; F1A males and females: 14%; F1B males: 19%, F1B females: 13%)

2322-2536 mg/kg bw/day for females (calculated for F0 and F1A adults)			
[redacted] (1993) CA 5.6.1/006 Report No.: TOXI 885-RP-G2 Wistar (random bred) Dose levels: 0, 100, 1000, 10000 ppm Dietary level would correspond to a mean daily compound intake of 0, 7.7, 77 and 770 mg/kg bw/day. [The mean daily intake was not reported for all dietary levels, but for the low level of 100 ppm a corresponding average value of 7.7 mg/kg bw/d was given in the original report]. Supplementary data (effect dose lacking, limited parameters investigated)	700 mg/kg bw/day	-	No treatment related effects

In offspring, body weight was reduced at limit dose (1000 mg/kg bw/day) (CA 5.6.1/004). Preputial separation was delayed in F1 generation in one study at ca 1000 mg/kg bw/day (CA 5.6.1/001-003). Further, distension of caecum was observed in pups (F1 and F2 litters) in one study at very high dose above 2000 mg/kg bw/day (CA 5.6.1/005). A NOAEL for offspring toxicity was set at **293 mg/kg bw/day**.

Table 2.6.6.1-41: Relevant reproductive NOAEL

Study	NOAEL for reproductive toxicity	LOAEL for reproductive toxicity	Effects on sexual function and fertility
[redacted] (2007) CA 5.6.1/001-003 Report No. 2060/0013 Sprague Dawley rat Dose levels: 0, 1500, 5000 and 15000 ppm glyphosate technical equivalent to mean achieved dose levels of 0, 104, 351 and 1063 mg/kg bw/day for males, and 0, 162, 530 and 1634 mg/kg bw/day for females	351 mg/kg bw/day	1063 mg/kg bw/day	**-changes in sperm parameters** (↓ number of homogenisation resistant spermatid in cauda epididymis) (309 million/gram compared to 400 million/gram in control) (F0 generation)
[redacted] (1997) CA 5.6.1/005 Report No.: [redacted] 96-0031 Sprague-Dawley Dose levels: 0, 1200, 6000, 30000 ppm Equivalent to:	417 mg/kg bw/day	2151 mg/kg bw/day	-lower fertility indices (F1 females, not statistically significant) (79.2% compared to 95.8% in control)

F0: 0, 83.6, 417, 2151 and 0, 96.9, 485, 2532 mg/kg bw/day in males and females, respectively F1: 0, 91.7, 458, 2411 and 0, 104.8, 530, 2760 mg/kg bw/day in males and females, respectively			
[redacted] (1990) CA 5.6.1/010 Report No. [redacted]-10387 Sprague-Dawley 0, 2000, 10000, 30000 ppm corresponding to 132-140, 666-711, 1983-2230 mg/kg bw/day for males and 160-163, 777-804, 2322-2536 mg/kg bw/day for females (calculated for F0 and F1A adults)	666 mg/kg bw/day	1983 mg/kg bw/day	↓ **litter size** (F0 dams: 13%)
[redacted] (1992) CA 5.6.1/007-008 Report No.: [redacted] 47/911129 Sprague-Dawley <u>Dose levels:</u> 0, 1000, 3000, 10000 ppm Equivalent to: <u>F0:</u> 0, 66, 197, 668 and 0, 75, 226, 752 mg/kg bw/day in males and females, respectively <u>F1:</u> 0, 76, 230, 771 and 0, 82, 245, 841 mg/kg bw/day in males and females, respectively)	668 mg/kg bw/day	-	No treatment-related effects on sexual function and fertility
[redacted] (1993) CA 5.6.1/006 Report No.: TOXI 885-RP-G2 Wistar (random bred) Dose levels: 0, 100, 1000, 10000 ppm Dietary level would correspond to a mean daily compound intake of 0, 7.7, 77 and 770 mg/kg bw/day. [The mean daily intake was not reported for all dietary levels, but for the low level of 100 ppm a corresponding average value of 7.7 mg/kg bw/d was given in the original report]. Supplementary data (effect dose lacking, limited parameters investigated)	700 mg/kg bw/day	-	No treatment related effects

[redacted] (2000) CA 5.6.1/004 Report No.: [redacted]/P/6332 Alpk:APfSD rat <u>Dose levels:</u> 0, 1000, 3000, 10000 ppm equivalent to mean achieved dose levels of: F0: 0, 99.4, 292.6, 984.7 and 0, 104.4, 322.8, 1054.3 mg/kg bw/day for males and females, respectively during pre-mating period F1: 0, 116.5, 352, 1161 and 0, 123.3, 370.8, 1218.1 mg/kg bw/day for males and females, respectively, during the premating period	985 mg/kg bw/day	-	No treatment related effects on sexual function and fertility

Regarding reproductive toxicity, equivocal effects on litter size was observed in one study at the very high dose of 2000 mg/kg bw/day (CA 5.6.1/010). Also, at a very high dose (2151 mg/kg bw/day) lower fertility indices (not statistically significant) was observed in F1 generation (CA 5.6.1/005). In another study, a significant decrease in homogenisation resistant spermatid count in F0 males were observed at ca 1000 mg/kg bw/day (CA 5.6.1/001-003). Based on this latter effect, the NOAEL for reproductive toxicity was **351 mg/kg bw/day**.

2.6.6.1.2 Comparison with the CLP criteria regarding adverse effects on sexual function and fertility

According to Regulation 1272/2008 (CLP), substances are classified for reproductive toxicity in Category 1A (known human reproductive toxicant) based largely on evidence from humans or in 1B (presumed human reproductive toxicant) or 2 (suspected human reproductive toxicant) largely based on animal data. The animal data required for 1B classification shall provide *"clear evidence of an adverse effect on sexual function and fertility in the absence of other toxic effects"* or if occurring together with other toxic effects *"the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects".*

Substances are classified in Category 2 when there is "*some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility, and where the evidence is not sufficiently convincing to place the substance in Category 1",* further *"the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects"*

As glyphosate is not a known human reproductive toxicant and there is no human data available for glyphosate providing clear evidence of an adverse effect on sexual function, the criteria for category 1A is not fulfilled.
Effects of glyphosate on sexual function and fertility were investigated in rats in five two-generational studies considered of acceptable quality and a further two-generation study considered as supplementary data. In addition, a range-finding one-generation toxicity study (CA 5.6.1/009) is available but the study is limited and considered as supplementary data only. Also, data from the open literature were taken into account to evaluate intrinsic properties of glyphosate on reproductive tissues and organs. These data are limited and regarded as reliable with restrictions.

The effects noted in the generational studies, that are considered potentially relevant for classification are as follows: changes in sperm parameters, delayed sexual maturation, reduced litter size, lower fertility indices, and reduced pup weights. In addition, distension of caecum was observed in pups.

Effects on sperm parameters
In the study by [redacted] (2007) (CA 5.6.1/001-003, Report No. 2060/0013), a significant decrease in homogenisation resistant spermatids (HRS, cauda epididymis) was counted in F0 males (309.0 million/gram compared to 399.9 million/gram in control) at the highest dose level of 15000 ppm (~1000 mg/kg bw/day). No significant effects were observed in the F1 generation. Sperm changes and histopathological examinations did not reveal any changes in the testis or epididymis. It could be noted that this finding occurred at the limit dose level (1000 mg/kg bw/day) only. Parental toxicity in this study consisted of liver and kidney weight changes observed in females at 15000 ppm. No similar findings were detected in males. However, it could be noted that the achieved

dose level for males (1063 mg/kg bw/day) was less when compared to females (1634 mg/kg bw/day). The finding of decreased HRS in cauda epididymis was not confirmed in the study by [redacted] (1997) (CA 5.6.1/005, Report No.: [redacted] 96-0031) using the same strain of rat and higher dosages (above 2000 mg/kg bw/day) or in the study by [redacted] (2000) (CA 5.6.1/004, Report No.: [redacted]/P/6332) using another strain of rat and dose levels up to 10000 ppm (~1000 mg/kg bw/day).

In the open literature, decreased total sperm count was reported in Sprague Dawley rats after subacute (5 weeks) exposure to 500 mg/kg bw glyphosate/day (Dai *et al*. (2016). However, this study was reliable with restrictions (few animals used and limited parameters investigated). Thus, findings in this study are not given appropriate weight.

Overall, effects on sperm parameters were observed, but the findings were confined to high dose (limit dose) with presence of general toxicity. Thus, data do not provide convincing evidence for a classification of the substance in Cat. 2.

Reduced litter size

In the study by [redacted] (1990) (CA 5.6.1/010, Report No. [redacted]-10387), a slight reduction in the average litter size (13%) was observed in the F0 dams of the 30000 ppm (above 2000 mg/kg bw/day) dose group, and to a lesser degree in the F1 dams. The reduction was non-statistically significant and not noted when F1 animals were re-mated, and treatment-relation was considered to be equivocal. Maternal toxicity consisted of clinical signs (soft stool) and reduced body weight observed at this dose level. A reduction in litter size was not confirmed in the study by [redacted] (1997) (CA 5.6.1/005, Report No.: [redacted] 96-0031) using the same strain of rat and where the same dietary concentrations of glyphosate were tested.

Overall, equivocal reduction in litter size was observed, but this finding was confined to very high dose level (above 2000 mg/kg bw/day) and not confirmed in other studies. Thus, data do not provide convincing evidence for a classification of the substance in Cat. 2.

Delayed sexual maturation

In the study by [redacted] (2007) (CA 5.6.1/001-003, Report No. 2060/0013), delayed preputial separation was observed in F1 male offspring at 15000 ppm (~1000 mg/kg bw/day) (Days at completion: 45.9 compared to 43.0 in control). The delayed onset of sexual maturation had no impact on subsequent reproductive performance. It could be noted that this finding occurred at the limit dose level (1000 mg/kg bw/day). General toxicity in this study consisted of liver and kidney weight changes observed in females at 15000 ppm.

In the open literature, a delay in male sexual development (increased anogenital distance) was observed in male Sprague-Dawley rats on PND 4 following administration to glyphosate diluted in drinking water at 1.75 mg/kg. (Manservisi *et al*., 2019). However, this study was reliable with restrictions (low number of test animals and uncertainties with regard to timing of blood sample collection), thus findings in this study are not given appropriate weight.

Overall, there are some data indicating effects on male sexual development, but the findings were confined to limit dose level. Thus, data do not provide convincing evidence for a classification of the substance in Cat. 2.

Lower fertility indices

In the study by [redacted] (1997) (CA 5.6.1/005, Report No.: [redacted] 96-0031), lower fertility indices were observed in F1 females of high dose group (79.2% compared to 95.8% in control). However, it could be noted that the finding was not statistically significant. Furthermore, the finding was observed at a very high dose level (above 2000 mg/kg bw/day) in the presence of general toxicity. Thus, data do not provide convincing evidence for a classification of substance in Cat. 2.

Overall conclusion, available data did not provide convincing evidence for a classification with regard to sexual function and fertility. Therefore, no classification for sexual function and fertility is considered warranted.

2.6.6.2 Adverse effects on development [equivalent to section 10.10.4 of the CLH report template]

Table 60: Summary table of animal studies on adverse effects on development (for transparency, effects not considered adverse but observed in several studies are included in italics)

Reference Method, Guideline, GLP Deviations Study quality	**Species, strain, sex, no/group route, duration of treatment**	**Test substance Dose levels**	**NOAEL**	**LOAEL**	**Targets/ Main effects**
RAT					
CA 5.6.2/001 Report No. [redacted]/P/4819 [redacted] 1996; OECD 414 (1981) GLP Deviations: Shorter treatment period (days 7-16) Parameters not assessed: Weight and histopathological changes of the thyroid glands of the dams, Anogenital distance (AGD) in foetuses, Indication of incomplete testicular descent/cryptorchidism Thyroid hormones (T4, T3 and TSH). Acceptable	Alpk: APfSD Wistar-derived females 24/group Gavage GD 7-16	Glyphosate acid Technical 95.6% 0, 250, 500, 1000 mg/kg bw/d In deionised water	Maternal & developmental: 1000 mg/kg bw/d	>1000 mg/kg bw/d	None
CA 5.6.2/002 Report No. [redacted] 94-0152 [redacted] 1995 OECD 414 (1981) GLP Deviations: Shorter treatment period (days 6-15) Parameters not assessed: Weight and histopathological changes of the thyroid glands of the dams, Anogenital distance (AGD) in foetuses, Indication of incomplete testicular	CD (SD) females 24/group Gavage GD 6-15	HR-001 95.68% 0, 30, 300, 1000 mg/kg bw/d in purified water with 0.5 % sodium carboxymethylcellulose (CMC)	Maternal: 300 mg/kg bw/d Note to AGG: Develop-mental: 1000 mg/kg bw/d	Maternal: 1000 mg/kg bw/d Develop-mental: >1000 mg/kg bw/d	<u>Maternal:</u> Loose faeces 20/22 <u>Development:</u> *skeletal variations not considered treatment-related*

Reference Method, Guideline, GLP Deviations Study quality	**Species, strain, sex, no/group route, duration of treatment**	**Test substance Dose levels**	**NOAEL**	**LOAEL**	**Targets/ Main effects**
descent/cryptorchidism Thyroid hormones (T4, T3 and TSH). Historical control data referred to and further details on the HCD is not included in the report. Acceptable					
CA 5.6.2/003 Report No. [redacted] 43 & 41/90716 OECD 414 (1981) GLP Deviations: Shorter treatment period (days 6-15) Parameters not assessed: Weight and histopathological changes of the thyroid glands of the dams, Anogenital distance (AGD) in foetuses, Indication of incomplete testicular descent/cryptorchidism Thyroid hormones (T4, T3 and TSH). Acceptable	CD, 24/group Gavage GD 6-15	Glyphosate Technical 98.6% 0, 300, 1000, 3500 mg/kg bw/d In purified water with 1% sodium methylcellulose (MC)	Maternal: 300 mg/kg bw/d Develop-mental: 300 mg/kg bw/d	Maternal: 1000 mg/kg bw/d Develop-mental: 1000 mg/kg bw/d	<u>Maternal 3500 mg/kg bw/d:</u> Mortality 2/24 Bw gain (d 6-16) ↓32% Loose faeces (22/24, compared to 0 in controls) Noisy respiration (15/22, compared to 0 in controls) Salivation 22/24, compared to 0 in controls) Gaseous distension in GI tract (of the 2 mortalities) <u>Maternal 1000 mg/kg bw/d:</u> Noisy respiration (2/25) *Bw gain (d 6-16) ↓3%* <u>Development:</u> <u>3500 mg/kg bw/d:</u> Mean foetal weight: ↓6% Reduced ossification 35.7% compared to 11.7% in control <u>1000 mg/kg bw/d:</u> Reduced ossification 28.4% compared to 11.7% in control, skeletal variations at low incidences 3500 mg/kg bw/d is far above the limit dose in OECD TG 414.
CA 5.6.2/004 and CA 5.6.2/005 Report No. [redacted].883.TER-R Guideline not stated GLP Deviations: Shorter treatment period (days 6-15)	Wistar, Treatment: 25 Control: 30 gavage GD 6-15	Glyphosate Technical 96.8% 0, 1000 mg/kg bw/d in Postman brand refined groundnut (peanut) oil	Maternal: 1000 mg/kg bw/d Develop-mental: not applicable (<1000 mg/kg bw/d)	Maternal 1000 mg/kg bw/d Develop-mental: not applicable (<1000	<u>Maternal:</u> no effects <u>Development:</u> *reduced ossification (toxicological significance unknown)*

Reference Method, Guideline, GLP Deviations Study quality	**Species, strain, sex, no/group route, duration of treatment**	**Test substance Dose levels**	**NOAEL**	**LOAEL**	**Targets/ Main effects**
Single dose level Parameters not assessed: Weight and histopathological changes of the thyroid glands of the dams, Anogenital distance (AGD) in foetuses, Indication of incomplete testicular descent/cryptorchidism Thyroid hormones (T4, T3 and TSH). Supportive				mg/kg bw/d)	
CA 5.6.2/006 Report No. not stated [redacted] 1986 Batch: not provided Not acceptable	Study considered non-acceptable (see Volume 3, section B.6.6.2).				
CA 5.6.2/007 Report No. not stated Anonymous 1981 Not acceptable but included as it was considered in the RAC opinion (2017)	CFY, diet, GD 6-18	Glyphosate Technical 96.8%	Maternal & Develop-mental: 544 mg/kg bw/d	Not applicable	Not applicable
CA 5.6.2/008 Report No. 401-054 [redacted] [redacted] 1980 OECD 414 (1981) Non-GLP Deviations: Parameters not assessed: Weight and histopathological changes of the thyroid glands of the dams, Anogenital distance (AGD) in foetuses, Indication of incomplete testicular	Charles River, gavage, GD 6-19	Glyphosate Technical 98.7% 0, 300, 1000, 3500 mg/kg bw/d in 0.5 % aqueous Methocel®	Maternal & Develop-mental: 1000 mg/kg bw/d	Maternal & Develop-mental: 3500 mg/kg bw/d	<u>Maternal 3500 mg/kg bw/d:</u> Mortality 6/25 Soft stool/diarrhoea: 22/25 Reduced body weight gain (23%, day 0-20) <u>Development 3500 mg/kg bw/d:</u> ↓ implantations ↑ post-implantation loss Reduced mean foetal body weight (↓9%) ↓mean number of viable foetuses (20%) ↑visceral and skeletal malformations/ variations

Reference Method, Guideline, GLP Deviations Study quality	Species, strain, sex, no/group route, duration of treatment	Test substance Dose levels	NOAEL	LOAEL	Targets/ Main effects
descent/cryptorchidism Thyroid hormones (T4, T3 and TSH). Food consumption was not recorded Data on clinical observations are not included uterine weight was not recorded fetal body weight by sex Acceptable					3500 mg/kg bw/d is far above the limit dose in OECD TG 414.
CA 5.6.1/004 Report No.: [redacted]/P/6332 [redacted] (2000) Two generation reproduction study (dietary) OECD TG 416 GLP: Yes Deviations from OECD TG 416 (2001): (i) no individual animal data presented in study report (ii) anogenital distance not examined as no treatment-related differences in sex ratio and sexual maturation were observed (iii) the thyroid was not weighed (iv) preimplantation loss not determined (v) pup development investigations restricted to body weight, vaginal opening and	Rat Alpk:APfSD (Wistar-derived) M, F 26/sex/group Administration: daily by dietary admixture throughout the treatment period. Control animals were treated in an identical manner with untreated laboratory diet. Exposure of F0 began at approximately 5 weeks of age. After 10 weeks of exposure F0 rats were mated. The appropriate experimental diet was fed throughout the study, to F0 and F1	Glyphosate acid Purity: 97.6% (w/w) Lot/Batch#: Y04707/082 0, 1000, 3000, 10000 ppm equivalent to mean achieved dose levels of: F0: 0, 99.4, 292.6, 984.7 mg/kg bw/day for males and 0, 104.4, 322.8, 1054.3 mg/kg bw/day for females during pre-mating period F1: 0, 116.5, 351 and 1161 mg/kg bw/day for males, and 0, 123.3, 370.8 and 1218.1 mg/kg bw/day for females, during the premating period .	NOAEL for parental toxicity: 10000 ppm (985 mg/kg bw/day) NOAEL for offspring toxicity: 3000 ppm (293 mg/kg bw/day) NOAEL for reproductive toxicity: 10000 ppm (985 mg/kg bw/day,)		F0 and F1 adults: <u>1000 ppm:</u> No treatment related effects <u>3000 ppm:</u> No treatment related effects <u>10000 ppm:</u> ↓ bw for selected F1 parent males during the pre-mating period (up to 5% reduction compared to control group) Offsprings: <u>1000 ppm:</u> No treatment related effects <u>3000 ppm:</u> No treatment related effects <u>10000 ppm:</u> ↓ bw (10%) (F1A)

Reference Method, Guideline, GLP Deviations Study quality	Species, strain, sex, no/group route, duration of treatment	Test substance Dose levels	NOAEL	LOAEL	Targets/ Main effects
preputial separation Acceptable	parents and offspring until termination				
Two generation reproduction study (dietary) CA 5.6.1/005 Report No.: [redacted] 96-0031 OECD TG 416 GLP: Yes Deviations from OECD TG 416 (2001): (i) testes were not used for enumeration of homogenisation-resistant spermatids but cauda epididymal sperm was enumerated (the guideline recommends both testes and epididymides to be used for enumeration of homogenisation-resistant spermatids and cauda epididymides sperm reserves, respectively) (ii) thyroid and spleen not weighed (iii) vaginal opening and preputial separation not examined (iv) anogenital distance not determined (v) no organ weighed for pups (the guideline recommends brain, speen and thymus to be weighed)	Rat Sprague Dawley Crj:CD (SD) M, F 24/sex/group Administration: daily by dietary admixture throughout the treatment period. Control animals were treated in an identical manner with untreated laboratory diet. Exposure of F0 began at approximately 5 weeks of age. After 10 weeks of exposure F0 rats were mated. The appropriate experimental diet was fed throughout the study, to F0 and F1 parents and offspring until termination. Duration of administration for both F0 and F1 parental	Glyphosate technical Purity: 94.61 % (w/w) Lot No.: T-950308 0, 1200, 6000, 30000 ppm Equivalent to: F0: 0, 83.6, 417, 2151 and 0, 96.9, 485, 2532 mg/kg bw/day in males and females, respectively F1: 0, 91.7, 458, 2411 and 0, 104.8, 530, 2760 mg/kg bw/day in males and females, respectively)	NOAEL for parental, offspring and reproductive toxicity: 6000 ppm (417 mg/kg bw/day		F0 and F1 adults: <u>1200 ppm:</u> No treatment-related effects <u>6000 ppm:</u> No treatment related effects <u>30000 ppm:</u> -clinical signs (loose stool) (F0, F1, both sexes) ↓bw (F0 males: 8%; F1 males: 7%) -organ weight changes (liver: F1 males: abs weight: ↑13%; F1 females: abs weight: ↑ 22%, rel weight: ↑ 20%; kidney: F0 males: rel weight: ↑11%; F1 males: rel weight: ↑14%; F1 females: abs weight: ↑17%, rel weight: ↑15%; prostate: F1 males abs and rel weight:↓32% of caecum (F0, F1) (both sexes) Offspring (F1 and F2): <u>1200 ppm:</u> No treatment-related effects <u>6000 ppm:</u> No treatment-related effects <u>30000 ppm:</u> ↓ pup weights (F1 males:14%, F1 females: 13%; F2 males: 9%, F2 females: 8%) -distension of caecum (F1 and F2 litters)

Reference Method, Guideline, GLP Deviations Study quality	Species, strain, sex, no/group route, duration of treatment	Test substance Dose levels	NOAEL	LOAEL	Targets/ Main effects
(vi) pre-and post-implantation loss not reported (vii) number of corpora lutea not given. (viii) time to mating not reported (ix) number of animals used are not in line with the recommendation by the guideline Acceptable	animals: a total of approximately 18 weeks (for a part of F1 parental animals duration of administration extended to approximately 22 weeks due to a 4-week extension for reciprocal crosses with untreated animals)				
CA 5.6.1/009 Report No. [redacted] 42/90619 One-generation range finding study (dietary) No guideline GLP: No Deviations from OECD TG 416 (2001): (i) only 10 females/group were used (the guideline recommends 20 pregnant females/group) (ii) the F0 females were time-mated (the guideline recommends a mating procedure) (iii) duration of study was only 10 weeks. F0 exposed from Day 3 of pregnancy through the termination of the study, females	Rat Sprague-Dawley Crl:CD (SD) BR VAF/Plus M, F F0 females: 10 time mated/group F1 generation: 10/sex/group Administration: daily by dietary admixture throughout the treatment period. Control animals were treated in an identical manner with untreated laboratory diet. Time-mated F0 females	Glyphosate technical Purity: 98.6 % (w/w) Lot/Batch No.: 206-Jak-25-1 0, 3000, 10000, 30000 ppm Equivalent to: F0 females: 0, 236-311, 799-1010 and 2515-2789 mg/kg bw/day F1 offspring: 0, 368-390, 1291-1335 and 3918-4453 mg/kg/day for males and 355-402, 1191-1271 and 3961-4397 mg/kg/day for females	The study is not suitable for NOAEL setting. Study acceptable as dose range finding study only (low number of animals, limited parameters investigated, no statistics)		Adults (F0): <u>3000 ppm:</u> ↓bw gain by Day 14 of pregnancy (F0 females: 2% compared to control) -macroscopic salivary gland changes (enlarged/firm/congested/swollen) (F0 females: 2/9) -macroscopic gastro-intestinal changes (content watery and/or dark: F0 females (2/9); stomach distended and/or congested: F0 females (2/9)) -microscopical changes in salivary gland (minimal granular basophilic cytoplasm of acinar cells with minimal hypertrophy of acinar cells) (F0 females: 2/9) <u>10000 ppm:</u> -clinical signs (soft faeces and yellow stained sawdust) (F0 females) ↓ bw gain by Day 14 of pregnancy (F0 females: 3% compared to control) -macroscopic gastrointestinal changes

Reference Method, Guideline, GLP Deviations Study quality	Species, strain, sex, no/group route, duration of treatment	Test substance Dose levels	NOAEL	LOAEL	Targets/ Main effects
were allowed to litter and rear their young to weaning, when 10 males and 10 female offspring per group were selected and reared on their respective diets to six weeks of age (the guideline recommends that F0 animals are dosed at least 10 weeks before mating period, dosing continued in both sexes during the 2 week mating period and continued throughout pregnancy and up to the weaning of the F1 offspring. The same procedure for the F1 offspring to produce the F2 generation) (iv) oestrous cycle not evaluated (v) litter parameters limited (vi) sperm parameters not evaluated (vii) sexual maturation not investigated (viii) no organ weighed (ix) histopathology limited (salivary glands investigated only) (x) statistical analyses not performed **Supplementary only** (low number of animals, limited parameters	were used. Exposure of F0 females began at Day 3 of pregnancy and was continued through pregnancy to termination of the study. The study duration was 10 weeks				(content watery and/or dark: F0 females (7/10); stomach distended and/or congested: F0 females (5/10) -macroscopic salivary gland changes (enlarged/firm/congested/swollen) (F0 females 6/10) -microscopical changes in salivary gland (moderate/marked granular basophilic cytoplasm of acinar cells and minimal/moderate hypertrophy of acinar cells (F0 females: 10/10) <u>30000 ppm:</u> -mortality (one F0 animal died on Day 21 post partum, cause of death not known) -clinical signs (soft faeces and yellow stained sawdust) (F0 females) -increased water consumption towards the end of pregnancy (F0 females: 11%) ↓ bw gain during pregnancy/lactation (F0 females: Gestation: up to 23%; Lactation: up to 47%)F0 females: Gestation Day 6 (23%), 14 (22%), 20 (11%); Lactation Day 7 (19%), 14 (39%), 21 (47%) ↓ bw F0 females: Gestation day 6 (2%), 14 (7%), 20 (5%); Lactation Day 7 (5%), 14 (13%), 21 (14%) -macroscopic gastrointestinal changes (content watery and/or dark: F0 females (8/9); stomach distended and/or congested: F0 females (4/9); distended caecum (4/9) -macroscopic salivary gland changes (enlarged/firm/congested/swollen) F0 females (8/9) -microscopical changes in salivary gland (marked granular basophilic

Reference Method, Guideline, GLP Deviations Study quality	Species, strain, sex, no/group route, duration of treatment	Test substance Dose levels	NOAEL	LOAEL	Targets/ Main effects
investigated, no statistics)					cytoplasm of acinar cells and moderate hypertrophy of acinar cells (9/9) and prominent mitoses in acinar cells (2/9)) <u>Pups, F0 generation:</u> <u>3000 ppm:</u> ↓ mean pup weight (Day 21 post partum: 9%) -macroscopical changes in salivary gland (congested) (one pup) (significance unclear) <u>10000 ppm:</u> ↓ mean pup weight (Day 21 post partum: 13%) -macroscopical changes in salivary gland (congested) (4 pups) (significance unclear) <u>30000 ppm:</u> ↓ mean pup weight (at birth: 4.5%, Day 21 post partum: 38%) Offspring from birth to 6 weeks of age (F1 generation): <u>3000 ppm:</u> -macroscopical changes in parotid salivary gland (enlarged/swollen) (1/10 male) <u>10000 ppm:</u> No treatment-related effects <u>30000 ppm:</u> -clinical signs (soft faeces) ↓ bw gain (Day 42 post partum: males: 25%; females: 15%) ↓ food during Weeks 5 (22%) and 6 (12%) (males only) -macroscopical changes in parotid salivary gland

Reference Method, Guideline, GLP Deviations Study quality	Species, strain, sex, no/group route, duration of treatment	Test substance Dose levels	NOAEL	LOAEL	Targets/ Main effects
					(enlarged/swollen) (males 5/10, females 2/10) -macroscopic gastrointestinal changes (soft content: males (7/10), females (9/10))
CA 5.6.1/010 Report No. [redacted]-10387 Two generation reproduction study (dietary) No guideline GLP: Yes Deviations from OECD TG 416 (2001):: (i) minor deviations in housing conditions: Temperature was 18-26ºC (the guideline recommends that the temperature in the experimental animal room should be 22 ± 3°C) (ii) no data on food efficiency (iii) no details on fertility indices, number of live births and pre- and post-implantation loss (iv) no determination of oestrus cycle length (v) sperm analysis not performed (vi) no determination of physical or sexual development landmarks (vii) parental animals: only	Rat Sprague-Dawley M, F 30/sex/group F0 generation rats (30/sex/group) were administered daily by dietary admixture for approximately 11 weeks and then mated to produce the F1a generation; 30 rats/sex/group from the F1 a generation were similarly exposed (approximately 14 weeks) and mated twice, to produce the F2a and F2b generations	Glyphosate Purity: 97.67 % (w/w) Lot No.: XLI-203 0, 2000, 10000, 30000 ppm Corresponding to 132-140, 666-711, 1983-2230 mg/kg bw/day for males and 160-163, 777-804, 2322-2536 mg/kg bw/day for females) (calculated for F0 and F1A adults)	NOAEL for parental, offspring and reproductive toxicity: 10000 ppm (666-711 mg/kg bw/day for males and 777-804 mg/kg bw/day for females)		<u>2000 ppm:</u> No treatment-related effects <u>10000 ppm:</u> No treatment-related effects <u>30000 ppm:</u> -clinical signs (soft stool) ↓bw (Terminal bw: F0 males: 8%, F1 males: 13%, F1 females: 10%; Maternal bw during gestation: Day 1: F0 females: 7%, F1 females first mating: 12%, F1 females second mating: 13%; Day 21: F0 females: 7%, F1 females first mating: 8%, F1 females second mating: 8%) ↓ litter size (F0 dams: 13%) Pups: <u>2000 ppm:</u> No treatment-related effects <u>10000 ppm:</u> No treatment related effects <u>30000 ppm:</u> ↓ pup weight (Day 21: F0 males: 13%, F0 females: 11%; F1A males and females: 14%; F1B males: 19%, F1B females: 13%)

Reference Method, Guideline, GLP Deviations Study quality	Species, strain, sex, no/group route, duration of treatment	Test substance Dose levels	NOAEL	LOAEL	Targets/ Main effects
ovaries and testes with epididymides weighed (uterus, prostate, seminal vesicles, brain, liver, kidneys, spleen, pituitary, thyroid and adrenal glands not weighed) (viii) brain, spleen and thymus of pups not weighed (ix) coagulating gland and cervix not included in the histopathological examination (x) no details on number of pups with grossly visible abnormalities Acceptable					

Reference Method, Guideline, GLP Deviations Study quality	Species, strain, sex, no/group route, duration of treatment	Test substance Dose levels	NOAEL	LOAEL	Targets/ Main effects
RABBIT					
CA 5.6.2/009 Report No. [redacted]/P/5009 [redacted], 1996 OECD 414 (1981) GLP Deviations: Shorter treatment period (days 8-20) Parameters not assessed: - Individual data not available - Fetal body weight by sex was not reported - weight and histopathological changes of the	NZW rabbit, gavage. 20 rabbits/dose group GD 8-20,	Glyphosate Technical 95.6%. 0, 100, 175, 300 mg/kg bw/d in deionised water	Maternal: 100 mg/kg bw/d; Develop-mental: 175 mg/kg bw/d	Maternal: 175 mg/kg bw/d; Develop-mental:300 mg/kg bw/d	<u>Maternal 300 mg/kg bw/d:</u> *Mortality: 2/20 compared to 1/20* Food intake during treatment (d 8-20) ↓19-43% Bw gain (days 8-20) ↓32% ↑Diarrhoea (19/20 compared to 4 in control) <u>Maternal 175 mg/kg bw/d:</u> *Mortality: 2/20* ↓Food intake (19-43%) ↑Diarrhoea (11/20)

Reference Method, Guideline, GLP Deviations Study quality	**Species, strain, sex, no/group route, duration of treatment**	**Test substance Dose levels**	**NOAEL**	**LOAEL**	**Targets/ Main effects**
thyroid glands of the dams, - anogenital distance (AGD) in foetuses, - indication of incomplete testicular descent/cryptorchidism in male foetuses, thyroid hormones (T4, T3 and TSH) Acceptable					<u>Maternal 100 mg/kg bw/d:</u> *Mortality: 2/20* <u>Developmental 300 mg/kg bw/d:</u> Foetal wt ↓8% *Delayed ossification* *Minor skeletal defects*
CA 5.6.2/010 Report No. 434/020 [redacted] 1996 OECD 414 (1981) GLP Deviations: Shorter treatment period (days 8-20) Parameters not assessed: - Individual foetal data not available - Fetal body weight by sex was not reported - weight and histopathological changes of the thyroid glands of the dams, - anogenital distance (AGD) in foetuses, - indication of incomplete testicular descent/cryptorchidism in male foetuses, - thyroid hormones (T4, T3 and TSH) Acceptable	NZW rabbit, GD 7-19, gavage. 18 rabbits/dose group	Glyphosate Technical 95.3%. 0, 50, 200, 400 mg/kg bw/d in 1 % carboxymethyl cellulose	Maternal 50 mg/kg bw/d & Develop-mental >400 mg/kg bw/day	Maternal: 200 mg/kg bw/d Develop-mental: >400 mg/kg bw/day	<u>Maternal 400 mg/kg bw/d:</u> Mortality: 2/18 Scours 16/16 (compared to 5/14 in controls) Food intake during treatment (d 7-19) ↓26-38% Bw gain (days 7-19) ↓>90% ↑Diarrhoea (10/16compared to 0/14 in control) <u>Maternal 200 mg/kg bw/d:</u> Bw gain (days 7-19) ↓24-29%, n.s.s *<u>Developmental 400 mg/kg bw/d:</u>* *↑ post-implantation loss 12.1 compared to 3.7 in control (n.s.s)* *<u>Developmental 200 mg/kg bw/d:</u>* *↑ post-implantation loss 11.5 compared to 3.7 in control (stat.sign)*
CA 5.6.2/011 Report No. [redacted] 94-0153 [redacted], 1995 OECD 414 (1981) GLP Deviations:	Japanese White rabbits (Kbl:JW), 18 rabbits/dose group	Glyphosate Technical 97.56%. 0, 10, 100, 300 mg/kg bw/d In purified water with 0.5%	Maternal: 100 mg/kg bw/d; Develop-mental: >300 mg/kg bw/d	Maternal: 300 mg/kg bw/d; Develop-mental: >300 mg/kg bw/d	<u>Maternal 300 mg/kg bw/d:</u> *Mortality: 1/18* Loose faeces (4/17) Abortions (2/18 compared to 0/18 in controls).

Reference Method, Guideline, GLP Deviations Study quality	**Species, strain, sex, no/group route, duration of treatment**	**Test substance Dose levels**	**NOAEL**	**LOAEL**	**Targets/ Main effects**
Shorter treatment period (days 6-18) Parameters not assessed: - Individual foetal data not available - Insemination is not considered in OECD TG 414 - weight and histopathological changes of the thyroid glands of the dams, - anogenital distance (AGD) in foetuses, - indication of incomplete testicular descent/cryptorchidism in male foetuses, - thyroid hormones (T4, T3 and TSH) Acceptable	Gavage GD 6-18,	carboxymethylcellulose			<u>*Maternal 10 mg/kg bw/d:*</u> *Abortions 2/18* <u>*Developmental 300 mg/kg bw/d:*</u> *↑ Number of litters with malformations 5//14 compared to 1/18 in controls)* *(parietal bone, hemivertebra)*
CA 5.6.2/012/13 Report No. TOXI: 884-TER-RB Amendment (CA 5.6.2/013) [redacted] 1993 OECD 414 (1981) GLP Deviations: Shorter treatment period (days 6-18) Parameters not assessed: - Individual foetal data not available - Insemination is not considered in OECD TG 414 - weight and histopathological changes of the thyroid glands of the dams,	NZW rabbit,. 26, 17, 16 and 15 rabbits in the 0, 20, 100 and 500 mg/kg bw/d dose groups Gavage GD 6-18,	Glyphosate Technical 96.8% 0, 20, 100, 500 mg/kg bw/d in 0.5% aqueous carboxy methyl cellulose	Maternal: 20 mg/kg bw/d; Develop-mental: not applicable	Maternal: 100 mg/kg bw/d; Develop-mental: not established due to low number of foetuses at top dose	<u>Maternal 500 mg/kg bw/d:</u> Mortality: 8/15 Soft/liquid faeces; 12/15 Food consumption (treatment, d 6-19) ↓31% No bwg during treatment compared to 100 g in controls. <u>Maternal 100 mg/kg bw/d:</u> Mortality: 4/16 <u>Developmental, all doses:</u> *stat. sign. increase in viceral malformations ("dilated hearts")*

Reference Method, Guideline, GLP Deviations Study quality	Species, strain, sex, no/group route, duration of treatment	Test substance Dose levels	NOAEL	LOAEL	Targets/ Main effects
- anogenital distance (AGD) in foetuses, - indication of incomplete testicular descent/cryptorchidism in male foetuses, - thyroid hormones (T4, T3 and TSH) GLP supplementary					
CA 5.6.2/014 Report No. [redacted] 45 & 39 (preliminary study with pregnant does) & 40 (preliminary study with non-pregnant does) /901303 [redacted] 1991 OECD 414 (1981) GLP Deviations: Shorter treatment period (days 7-19) Parameters not assessed: - Individual foetal data not available Fetal body weight by sex was not reported - weight and histopathological changes of the thyroid glands of the dams, - anogenital distance (AGD) in foetuses, - indication of incomplete testicular descent/cryptorchidism in male foetuses, - thyroid hormones (T4, T3 and TSH) Acceptable	NZW rabbit, 19, 19, 16 and 20 rabbits in the 0, 50, 150 and 450 mg/kg Gavage. GD 7-19	Glyphosate Technical 98.6% 0, 50, 150, 450 mg/kg bw/d in 1 % methylcellulose	Maternal: 50 mg/kg bw/d; Develop-mental: 150 mg/kg bw/d	Maternal: 150 mg/kg bw/d; Develop-mental: 450 mg/kg bw/d	<u>Maternal 450 mg/kg bw/d:</u> Mortality: 1/20 Soft/liquid stool; 13/20 Food consumption (treatment, d 7-19) ↓6-17%, n.s.s Body weight gain (day 11-29) ↓10%, n.s.s <u>Maternal 150 mg/kg bw/d:</u> Soft/liquid stool; 5/16 Food consumption (d 11-19) ↓12%, n.s.s Body weight gain (day 11-29) ↓21%, n.s.s <u>Developmental 450 mg/kg bw/d:</u> Late embryonic deaths 1.3 compared to 0.2 in controls (HCD 0.1 – 1.3 (0.7)) Post-implantation loss 21% compared to 5,7 % in controls (HCD 6.5 – 17.5 (12.9)) Cardiac malformations 11//95 compared to 1/163 in controls.

Reference Method, Guideline, GLP Deviations Study quality	**Species, strain, sex, no/group route, duration of treatment**	**Test substance Dose levels**	**NOAEL**	**LOAEL**	**Targets/ Main effects**
					Developmental 150 mg/kg bw/d: *Post-implantation loss 15.3% compared to 5.7 % in controls (HCD 6.5 – 17.5 (12.9))* *Developmental 50 mg/kg bw/d:* *Post-implantation loss 19.5% compared to 5,7 % in controls (HCD 6.5 – 17.5 (12.9))*
CA 5.6.2/015 Report No. [redacted] 45 & 39 & 40/901303 [redacted] 1991;	Historical control data: Control Indiviual Incidence Major Anormalities (Interfauna Rabbit 1989)				
CA 5.6.2/016 Report No. not stated	Study considered non-acceptable (see Volume 3, section B.6.6.2).				
CA 5.6.2/017 Report No. not stated	Same study as 5.6.2/007, the part of the study investigating rabbit was not included by the applicant.				
CA 5.6.2/018 Report No. 401-055 [redacted] 1980 Pilot study Pre Guideline/GLP Not in previous RAR	Dutch Belted rabbit 5/dose Gavage GD 6-27	Technical glyphosate 100% Vehicle: 1% aqueous Methocel® 0, 125, 250, 500, 1250 and 2500 mg/kg bw/d in 1% aqueous Methocel®	Maternal: not applicable	Maternal/ Develop-mental: not applicable	Maternal, 2500 mg/kg bw/d: Mortality: 5/5 soft stool, diarrhoea. No effects on maternal bw and bw gain; Maternal, 1250 mg/kg Mortality: 5/5 Maternal, 500mg/kg Mortality: 4/5 Due to high mortality, no meaningful analysis of data can be made. Doses ≥ 500 mg/kg bw/d clearly exceeds the MTD.

Reference Method, Guideline, GLP Deviations Study quality	**Species, strain, sex, no/group route, duration of treatment**	**Test substance Dose levels**	**NOAEL**	**LOAEL**	**Targets/ Main effects**
5.6.2/019 Report No. 401-056 [redacted] 1980 Pre Guideline/GLP Acceptable as supportive information Deviations: Parameters not assessed: -Individual foetal data not available Fetal body weight by sex was not reported Insemination is not considered in OECD TG 414 - weight and histopathological changes of the thyroid glands of the dams, - anogenital distance (AGD) in foetuses, - indication of incomplete testicular descent/cryptorchidism in male foetuses, - thyroid hormones (T4, T3 and TSH) - The number of surviving dams was lower than recommended thus the number of litters in treated groups and controls was too low for a robust assessment. Supportive information	Dutch Belted rabbit, 16, 16, 16 and 16 rabbits in the 0, 75, 175 and 350 mg/kg bw/d dose group Gavage GD 6-27	Technical glyphosate 98.7%. 0, 75, 175, 350 mg/kg bw/d in 1% aqueous Methocel®	Maternal: 75 mg/kg bw/d; Develop-mental: ≥75 mg/kg bw/d	Maternal: 175 mg/kg bw/d; Develop-mental: not established due to low number of foetuses	<u>Maternal,350 mg/kg bw/d:</u> Mortality: 10/17 (59%) Soft stool/diarrhoea reported but no incidences presented <u>Maternal, 175 mg/kg</u> Mortality: 2/16 (12.5%) Soft stool/diarrhoea reported but no incidences presented No effects on maternal bw and bw gain. <u>Development, 350, 175 mg/kg bw/d:</u> Not possible to assess due to high mortality in dams. Mortality above 10 % in the 175 and 350 mg/kg bw/d groups prevents an assessment of developmental toxicity at these doses.

Table 61: Summary table of human data on adverse effects on development

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data available				

Table 62: Summary table of other studies relevant for developmental toxicity

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
Mechanistic study Supplementary data/reliable with restrictions - purity unknown -solvent not stated -no information of the embryonal stage of the embryos -no positive control included for ROS accumulation or disassembly of microtubule organizing centers.	Glyphosate as powder from Research Products International (Mt. Prospect, IL, USA; catalog G36060, CAS 1071-83-6) Purity and batch not stated	Study performed to investigate the effects of glyphosate (0 – 300 µM) on metaphase II mouse oocyte quality and embryo damage to obtain insight on its mechanisms of cellular action and the tolerance of oocytes and embryos towards glyphosate.	The study shows that glyphosate in a dose dependent manner and in concentrations in the range of those found in human blood following accidental acute exposure causes disruption of the microtubule organizing center and chromosomal disorganization. Also, interference with intracellular zinc bioavailability and ROS accumulation were observed in the mouse oocytes. Further, in embryos zinc depletion and accumulation of ROS was also observed in a dose-related manner.	CA 5.8.2 Yahfoufi Z. A. et al.Toxicology, (2020) Vol. 439, Art. No. 152466
Studies on sexual function and fertility which are not considered further for the endpoint of reproductive toxicity				

Teratological effects induced by three pesticides in pregnant rats Supplementary/reliable with restrictions: -performed with formulation thus effects caused by co-formulants cannot be excluded -test substance not sufficiently characterised (particularly, purity and batch not specified) -only one dose used -low number of animals -acclimatisation period not reported -temperature exceeded limit -food consumption not measured -individual data missing, -necropsy of dams not preformed -assessment of AGD, T4, T3 and TSH in dams not performed -sex ratio missing -no historical control data -no positive control	Roundup containing 35% glyphosate E.C. provided by Pesticide Center Institute, Dokki, Cairo, Egypt	Roundup administered by oral gavage to 10 pregnant females from Day 6 to Day 20 of gestation. A similarly constituted group of females received the vehicle and served as control. body weight, clinical signs, gravid uterus weight, the number of resorption sites, implantation sites and live or dead fetuses was recorded. Live fetuses were weighed and examined for any skeletal malformation.	Treatment did not cause mortality, induce clinical signs of toxicity but reduced body weight gain during pregnancy compared to control animals. Further, the mean number of implantation sites, the mean uterus weight and the mean number of total and live fetuses were reduced when compared to control animals. Also the number of resorption was increased. Fetal examination revealed a loss in fetal size, weight and skeletal malformations in the glyphosate treated animals when compared to controls. Glyphosate treatment also caused less ossification of most parts of skull and legs, as well as complete loss of ossification in the digits and caudal vertebrae in comparison with those of control.	CA 5.6 Abou-Amer W. L. et al. (2010) Alexandria Journal of Pharmaceutical Sciences (2010), Vol. 24, No. 1, pp. 21-26

Effects of melatonin in rats in the initial third stage of pregnancy exposed to sub lethal doses of herbicides Supplementary/ reliable with restrictions: -performed with a formulation containing polyethylene tallow amine and effects caused by this or other co-formulants cannot be excluded -the test substance is not sufficiently characterised (particularly, purity and batch not specified) -only day up to day 7 of gestation studied -small group sizes -only one dose used -unclear if controls were administered vehicle -no details on food or food consumption -individual data missing -histopathological examination of implantation sites only provided for animals treated with Glyphosate-Roundup, Paraquat and melatonin simultaneously -clinical observations and historical control data not presented	Roundup made of 360 g/L glyphosate (N phosphonomethyl glycine) and 16% (w/v) polyoxoethylene amine, Sigma Aldrich, St. Louis, Missouri, USA	The aim of the study was to investigate reproductive effects and the induction of oxidative stress in the liver by simultaneous application of 10 mg/kg bw/day melatonin to pregnant female rats exposed to 500 mg/kg bw Glyphosate-Roundup and 50 mg/kg bw/day Paraquat during day 1 to 7 of gestation.	Exposure of Glyphosate-Roundup formulation alone and in association with Paraquat significantly impaired the dams' body weight development and caused changes in reproductive parameters. These changes were not observed when the animals were simultaneously treated with melatonin.	CA 5.6 de Almeida L. L. et al. (2017) Acta Histochemica (2017), Vol. 119, No. 3, pp. 220-227

Low-dose Roundup induces developmental toxicity in bovine preimplantation embryos in vitro. Supplementary/ reliable with restrictions: -performed with a formulation and insufficient information is provided to determine whether it is the EU representative formulation -non guideline, non-GLP -no positive control.	Roundup from Monsanto Co., St. Louis, MO, USA purchased from a commercial source containing 360 g/L of glyphosate	Study investigating the effects of Roundup on in vitro development of bovine preimplantation embryos at different concentrations (0.45, 0.9, and 1.8 ppm).	Roundup impairs the development and quality of bovine preimplantation embryos in a dose-dependent manner even at 0.9 ppm concentration. Roundup increases intracellular calcium levels and induces oxidative stress and apoptosis in bovine embryos.	CA 5.6 Cai W. et al. (2020) Environmental science and pollution research international, (2020) Vol. 27, No. 14, pp. 16451-16459
Glyphosate and pendimethalin in breast milk samples from Egyptian rural areas: a pilot study for infant's risk assessment Not reliable	High purity standards of glyphosate were obtained from Sigma Aldrich Chemie GmbH (Steinheim, Germany) and standard of glyphosate solution were prepared in acetonitrile for chromatography and mass spectrometric analysis	Study to investigate thirty-one samples of breast milk from rural mothers in Egypt and conducted for the herbicides, glyphosate and pendimethalin analysis followed by their impact on infants.	Results state that glyphosate was detected in breast milk. However, the solubility of glyphosate in toluene is 36 mg/L. The highest value in this paper is just below 30 ppm. If correct, 5 ml of breast milk, extracted, evaporated and dissolved in 1 ml of toluene, the solubility of glyphosate must be approximately 150 ppm and 5 times higher than the reported solubility for glyphosate in toluene. Moreover, the HPLC method lists an excitation wavelength that is higher than the emission wavelength. According to Stokes law, the emitted light is always of longer wavelength than the excitation light.	CA 5.9 Abdel-Halim K. Y. et al. (2019) International Journal of Advanced Research, (2019) Vol. 7, No. 9, pp. 991-1002

2.6.6.2.1 Short summary and overall relevance of the provided information on adverse effects on development

RATS

The dossier includes seven developmental toxicity studies performed in different rat strains. All studies except study CA 5.6.2/006 (Report No. not stated) was included in the previous evaluation from 2015. Studies CA 5.6.2/006 (Report No. not stated) and CA 5.6.2/007 (Report No. not stated) are considered non-acceptable since limited investigations and deficiencies in reporting hamper a proper assessment of data. For transparency, study CA

5.6.2/007 is yet presented in volume 3 since this study was taken into consideration by RAC in the opinion from 2017[15].

The five remaining studies were all but one (i.e., CA 5.6.2/008 (Report No.401-054)) performed in accordance with the principles of GLP and in line with OECD TG 414. However, since studies were performed between 1980 and 1996, they do not meet the current recommendations in the updated version of OECD TG 414 from 2018. Therefore, parameters relevant particularly for the assessment of endocrine disruption (i.e., anogenital distance (AGD) in foetuses, indication of incomplete testicular descent/cryptorchidism in male foetuses, weight, and histopathological changes of the thyroid glands in dams, blood samples from dams to assess thyroid hormones (T4, T3 and TSH)) were not included.

Only a few adverse maternal effects were noted among the five acceptable studies performed at doses up to 1000 mg/kg bw/day (see table 60). These included loose faeces in study **CA 5.6.2/002 (Report No. [redacted] 94-0152)** and gastrointestinal disturbances and noisy respiration in study **CA 5.6.2/003 (Report No. [redacted] 43 & 41/90716)**. Mortality was only observed above the limit dose, at a very high dose of 3500 mg/kg bw/day where almost all dams suffered from loose faeces (study **CA 5.6.2/003 (Report No. [redacted] 43 & 41/90716)**).

In study **CA 5.6.2/008 (Report No. 401-054)**, developmental effects such as post-implantation loss (1.2 compared to 0.6 in controls (not statistically significant)) and malformations (10 in 3 litters compared to 3 in 3 litters in control) were observed at the very high dose of 3500 mg/kg bw/day. The malformations included six foetuses from one litter with bent tail, open eyelids, missing kidneys and ureters as well as various skeletal effects. Three foetuses in another litter were reported to have dwarfism. All of the malformations were reported to be within the historical control data range. Nevertheless, since the maternal toxicity was considerable at this dose level (~25% mortality, the results from the 3500 mg/kg bw/day group cannot be properly evaluated and should thus be considered with much caution. There were no maternal effects and no foetuses with malformations in low and mid doses. The mean number of viable foetuses, late or early resorptions, post-implantation loss, corpora lutea, the foetal sex distribution and mean foetal body weight were comparable between low and mid-dose animals and controls. A statistically significant decrease in the mean number of total implantations and viable foetuses was noted in the low dose group but in the absence of similar effects at higher doses it was considered a random finding.

Developmental toxicity was indicated in study **CA 5.6.2/003 (Report No. [redacted] 43 & 41/90716)** as an increased incidence of pre-implantation loss (93%) and reduced foetal weight (6%) in foetuses from high-dose dams. The increase in pre-implantation loss was also observed in the preliminary study but considered unrelated to treatment by the study author since treatment started after implantation. A higher incidence of malformations was observed in high dose animals compared to controls (3 from 2 litters and 1 in 1 litter in high dose and control foetuses, respectively, foetal incidence: 1.1% compared to 0.3%). The types of malformations varied between groups, but interventricular septal defect was observed both in a mid-dose and in a high-dose foetus. Findings were not statistically significant and showed no dose-response thus in line with the conclusions by RAC in 2017, the results are not considered as evidence for developmental toxicity. The mean percentage of foetuses with skeletal anomalies (reduced degree of ossification, distortion of ribs) was 35.7% compared to 11.7% in controls. Besides mortality in 2/24 dams administered 3500 ppm, maternal toxicity was evident as a marked reduction in body weight gain (32 % lower than controls during gestation days 6-16) as well as a reduced food intake during the dosing period. It is therefore likely that reduced ossification in foetuses were secondary to maternal toxicity.

However, some of these skeletal anomalies were seen at lower incidences also in mid-dose foetuses from dams in which maternal toxicity was limited to noisy respiration (2/25 dam) and a slight reduction in bw gain (3% lower than controls during gestation days 6-16). An increased incidence of reduced ossification of sacro-caudal vertebral arches was also seen in the low dose-group and increased incidences of lumbar ribs were similar in all treated groups however the overall incidence of variations was still less in the low dose group. There were no significant changes in implantation rate, post-implantation loss, litter size or sex ratio.

Table 2.6.6.2-1: Foetal effects in rats (Study CA 5.6.2/003), table (modified) from RAC opinion

Dose level (mg/kg bw/d)	0	300	1000	3500
Mean foetal wt (g)	3.96	3.90	3.89	3.71** (↓7%)
Foetuses with wavy ribs (thoracic ribs) / number of foetuses examined	1/155	-/143	3/166	28/144

15 Committee for Risk Assessment, RAC Opinion proposing harmonised classification and labelling at EU level of glyphosate (ISO); N-(phosphonomethyl)glycine, CLH-O-0000001412-86-149/F, Adopted 15 March 2017.

Reduced ossification of 1 or more cranial centres	3/155 in 3 litters	2/143 in 2 litters	12/166 in 8 litters	10/144 In 5 litters
Reduced ossification of sacrocaudal vertebral arches	3/155 (2%) in 2 litters	8/143 (6%) in 6 litters	17/166 (10%) in 11 litters	15/144 (10%) in 10 litters
Foetuses with unossified sternebrae (%)	13.7 23/155 foetuses	28.5 39/143 foetuses	17.6 29/166	33.8** 50/142
Foetuses showing skeletal variation (%)1	11.7	22.6	28.4	35.7**

* statistically significant, $p < 0.05$; ** $p < 0.01$
[1]Historical control range for skeletal variations: 21.9 – 27.2%

Ventricular septal defect noted in study CA 5.6.2/003 was also observed in one foetus each of the 300 and 1000 mg/kg bw/d groups in study **CA 5.6.2.002 (Report No. [redacted] 94-0152)** and a different foetus (from a different litter) in the 300 mg/kg bw/d group displayed a right aortic arch. The concomitant maternal toxicity was mild and limited to slightly loose stool in 20 of 22 dams. There were no significant differences in the mean gravid uterine weights, mean numbers of corpora lutea and implants between the control group and any of the treated groups. Furthermore, no significant differences were observed with respect to the mean number of live foetuses, the mean percent incidence of resorptions, foetal deaths, sex ratio, mean foetal body weights and the mean placental weights.

Neither mortality nor clinical signs were observed in studies **CA 5.6.2.001 (Report No. [redacted]/P/4819)** or **CA 5.6.2.004 ([redacted].883.TER-R))** with amendment (establishment of No Observed Adverse Effect Level (NOAEL)) in **5.6.2/005**, both performed with Wistar rats. The results from study 5.6.2.001 showed no differences in litter data between treated and control and the proportion of foetuses with external/visceral variants and the proportion of foetuses with skeletal variants was actually lower in treated groups compared to controls.

In study **CA 5.6.2.004/005 ([redacted].883.TER-R)**, a significantly increased incidence of delayed ossification (normal variations) including caudal vertebral arch, forelimb proximal phalange and hindlimb distal phalanges was observed in animals administered 1000 mg/kg bw/d, the only treatment group. Otherwise, there was no increased incidences of external, visceral or skeletal malformations. The number of corpora lutea, implantations, embryonic and foetal resorptions, pre-implantation and post-implantation loss was similar between treated animals and controls and there were no significant differences in litter size, the incidence of dead or abnormal foetuses, foetal body weights or foetal sex ratios.

Overall, treatment-related mortality was only observed at a very high dose level of 3500 mg/kg bw/day, i.e., above the limit dose. The clinical signs observed among the rat studies such as mortality, loose faeces, reduced bodyweight gain and noisy respiration were mainly limited to this high dose level although loose faeces and minor effects on bodyweight gain and noisy respiration were also noted at 1000 mg/kg bw/day.

Developmental effects were observed in study **CA 5.6.2/008 (Report No. 401-054)** (post-implantation loss and malformations) at the very high dose (3500 mg/kg/bw/d) also causing excessive maternal toxicity (~25% mortality). According to the CLP legislation (Annex I: 3.7.2.4.4) data from a dose level with such an excessive toxicity shall not normally be considered for further evaluation.

Cardiovascular malformations were reported in two of the six rat studies. In both studies these were single incidences but occurred at two dose levels (i.e. 300 and 1000 mg/kg bw/d in study CA 5.6.2.002 (Report No. [redacted] 94-0152) and at 1000 and 3500 mg/kg bw/d in study CA 5.6.2/003 (Report No. [redacted] 43 & 41/90716). However, considering that effects are single incidences, do not show a clear dose-response or statistical significance and that these effects were not seen in the other three acceptable rat studies at similar doses, these findings are not considered evidence for teratogenicity.

Maternal and developmental NOAEL/LOAEL in rats

The overall maternal and developmental NOAELs set at 300 mg/kg bw/d in the previous evaluation based on the findings in study CA 5.6.2/003 (Report No. [redacted] 43 & 41/90716) (clinical signs, reduced bodyweight gain in dams, reduced ossification, skeletal variations in foetuses) is not fully agreed. The effect on bodyweight gain observed at 1000 mg/kg bw/day is considered mild (3%) and not an appropriate basis for the overall maternal NOAEL.

Loose faeces was observed in almost all animals administered 1000 mg/kg bw/day in study CA 5.6.2/002 (Report

No. [redacted] 94-0152) but was not observed at this dose level in study CA 5.6.2/003 (Report No. [redacted] 43 & 41/90716), also performed in CD rats, and not at this dose level in the other acceptable rat studies. Despite this lack of consistency between studies and the mild nature of the effect, the RMS proposes to maintain the maternal NOAEL at 300 mg/kg bw/day as established in the previous assessment.

Skeletal variations were seen in study CA 5.6.2/003 (Report No. [redacted] 43 & 41/90716) at 1000 mg/kg bw/d, delayed ossification of unclear significance were noted at 1000 mg/kg bw/d in a different study considered acceptable (CA 5.6.2/002 (Report No. [redacted] 94-0152)) and in one supportive study (CA 5.6.2/004 ([redacted].883.TER-R)) whereas no developmental toxicity was observed in two other studies considered acceptable (CA 5.6.2/001 and CA 5.6.2/008 (Report No. 401-054)) at 1000 mg/kg bw d. The developmental NOAEL is proposed to be set at 300 mg/kg bw/day based on the skeletal variations observed at 1000 mg/kg bw/d in study CA 5.6.2/003 (Report No. [redacted] 43 & 41/90716).

RABBITS

The dossier includes eight developmental toxicity studies performed in rabbits. All studies except the pilot study in CA 5.6.2/018 (Report No. 401-055) were included also in the previous evaluation from 2015. Due to the high mortality, the information from study CA 5.6.2/018 (Report No. 401-055) is only of limited use for a proper assessment of possible adverse effects on embryofoetal development.

Study CA 5.6.2/012 (Report No. TOXI: 884-TER-RB) with 5.6.2/013 (amendment to final report - Teratogenicity study in rabbits – Test compound: Glyphosate technical (FSG 03090 H/05 March 1990)) was considered supportive only due to several deficiencies with respect to both methodology and reporting and due to the high mortality hampering a proper assessment of data. Likewise, all deficiencies in study CA 5.6.2/016/Report No. 1086 are considered to prevent a proper assessment and since it is not even possible to set a developmental NOAEL, the study is considered non-acceptable.

The remaining studies were all but CA 5.6.2/019 (Report No. 401-056) performed in accordance with the principles of GLP and in line with OECD TG 414. However, since studies were performed between 1980 and 1996, they do not meet the current recommendations in the updated version of OECD TG 414 from 2018. Therefore, parameters relevant particularly for the assessment of endocrine disruption (i.e., anogenital distance (AGD) in foetuses, indication of incomplete testicular descent/cryptorchidism in male foetuses, weight and histopathological changes of the thyroid glands in dams, blood samples from dams to assess thyroid hormones (T4, T3 and TSH)) were not included.

Based on the effect levels noted among the five studies, pregnant rabbits seemed to be more sensitive to effects of glyphosate compared to pregnant rats. Mortality was observed in two of the rat studies but only at a dose level of 3500 mg/kg bw/day which is far above the limit dose of 1000 mg/kg bw/day. In rabbits, mortality was observed in all except one study at or above doses of 175 mg/kg bw/day. The most prominent clinical sign among rabbits, diarrhoea, was noted at doses of 150 mg/kg bw/day while it was observed in three of the rat studies only at doses at or above the limit dose of 1000 mg/kg bw/day. Similarly, reduced food consumption and reduced body weight gain were observed at 150 mg/kg bw/day and higher in rabbits but only at a dose level of 3500 mg/kg bw/day in rats. In addition to diarrhoea in rats and rabbits, necropsy findings including ulceration in rabbits, indicate an irritative effect of glyphosate on the gastrointestinal tract. It has been argued that this greater sensitivity to glyphosate in pregnant rabbits compared to pregnant rats may be due to rabbits ingesting their caecotrophes (a specialized digestive strategy for the recycling of caecal contents and the extraction of nutrients). From a theoretical point of view, this may either lead to an increased exposure to glyphosate as it is excreted unchanged in faeces or it may lead to undernourishment since loose faeces and diarrhoea prevent the rabbits from ingesting their caecotrophs. It may also be a combination of both with an initially high exposure due to recirculation of the substance followed by undernourishment when caecotrophy is prevented. All high dose animals that died prior to termination were considered treatment-related and gastrointestinal effects were noted in almost all of these.

Analyses of litter parameters showed an increase in post-implantation loss in study **CA 5.6.2/010/Report No. 434/020** in animals administered 400 mg/kg bw/d (12.1 compared to 3.7 in control) or 200 mg/kg bw/d (11.5 compared to 3.7 in control). Maternal toxicity in high-dose dams (400 mg/kg bw/d) included a statistically significant decrease in body weight gain from GD 10-29, diarrhoea, scours and two treatment-related deaths (indicated by necropsy findings of fluid filled large intestines, haemorrhage, ulceration and sloughing of the stomach, congested duodenum and gas distended colon, rectum and appendix) on GD 19 and 20 (the latter killed in extremis). In mid-dose (200 mg/kg bw/d) dams, maternal toxicity was limited to a non-statistically significant decrease in bw gain since the single death observed in this group was due to mis-dosing.

The non-statistically significant increase in late embryo/foetal deaths and post-implantation loss in the 400 mg/kg bw/day was considered unrelated to treatment since it appeared mainly due to one animal with nine late embryonic/foetal deaths (resulting in a post-implantation loss of 69.2% in that specific animal). However, the effect was also noted in the 200 mg/kg bw/d group and statistically significant. There was no apparent dose-response (the mean percentage post-implantation losses: 3.7 ± 6.5, 3.6 ± 8.5, 11.5 ± 11.4 and 12.1 ± 18.6 in control, 50, 200 and 400 mg/kg bw/d dose groups respectively). Since the mean viable litter size at termination was similar between all groups (9.1 ± 2.5, 8.7 ± 2.4, 7.9 ± 2.5 and 8.9 ± 2.6 in the control, low, intermediate and high dose group, respectively) the slight, but statistically significant, increase in post-implantation loss (mainly caused by a non-statistically significant increase in early embryonic/foetal death) in the mid-dose group is considered to have limited toxicological significance.

Effects on foetal viability was also observed in study **CA 5.6.2/014/Report No. [redacted] 45 & 39 (preliminary study with pregnant does) & 40 (preliminary study with non-pregnant does) /901303** in which the incidence of late embryonic deaths in animals administered 450 mg/kg bw/d was 1.3 compared to 0.2 in controls (HCD 0.1 – 1.3 (0.7)) and the post-implantation loss was 19.5 ± 19.8, 15.3 ± 17.2 and 21 ±11.8 at 50, 150 and 450 mg/kg bw/d, respectively compared to 5,7 % in controls (HCD 6.5 – 17.5 (12.9)). Maternal toxicity included treatment-related death of one high-dose dam on GD 20 following abortion, soft/liquid faeces and non-statistically significant reduced food consumption and body weight gain in the mid and high dose groups. The effect was also assessed by RAC in the context of classification and labelling. The RAC opinion concludes "*Overall RAC concludes that following in utero exposure to glyphosate in rabbits no clear relationship between exposure and effects on foetal viability could be determined. Effects on foetal viability were not reported consistently in the four acceptable developmental toxicity studies in rabbits. Actually, only one study [redacted] 1991) reported effects on foetal viability, however, without a clear dose-response relationship and within the historical control range for late- and total embryonic deaths."*

There were no effects on post-implantation loss in the other studies at comparable doses except for study **CA 5.6.2/016/Report No. 1086** in which a slightly higher mean number of embryo/foetal death (1.4 ± 2.20 compared to 0.07 ± 0.26 in controls) and a slightly lower mean number of viable implants/litter (5.2 ± 3.03 compared to 7.3 ± 3.1 in controls) was reported. However, the results may reflect that two high dose dams aborted and consequently had no live foetuses. The study also suffers from several deficiencies (e.g., lack of statistical analyses, lack of individual data and lack of necropsy results) thus results must be interpreted with caution.

A statistically significant reduced foetal weight (8%) was observed in study 6.6.2/009 but no significant effects on foetal weights were observed in the other studies.

Malformations (skeletal and visceral) were reported in several studies. Skeletal malformations mainly affecting the parietal bone was observed in study **CA 5.6.2/011/Report No. [redacted] 94-0153** and cardiac malformations were observed in studies **CA 5.6.2/012/013/ TOXI: 884-TER-RB** and **CA 5.6.2/014/Report No. [redacted] 45 & 39** (preliminary study with pregnant does) & 40 (preliminary study with non-pregnant does) /901303. Other malformations were considered single incidences and unrelated to treatment. The incidences were low and claimed to be within historical control data or in presence of maternal toxicity. Although historical control data included for study **CA 5.6.2/014/Report No. [redacted] 45 & 39 (preliminary study with pregnant does) & 40 (preliminary study with non-pregnant does) /901303** indicated that the total number of malformations (foetuses and affected litters) was within the historical control range reported and values were not statistically different from concurrent control values, the incidence of each type of malformations noted (i.e. interventricular septal defect, enlarged left, reduced right ventricles, retro-oesophageal right subclavian artery and narrow/dilated aortic arch/pulmonary trunk/arterial trunk) was outside the historical control range for the effect. It was also argued that the increase in malformations in the high dose group in this study occurred in the presence of maternal toxicity. However, maternal toxicity was not excessive (mortality of one dam following gastrointestinal disturbances and a 10% non-statistically significant reduction of body weight gain) thus such association is not apparent. The study was assessed by RAC concluding that the reported increase in cardiovascular malformations were to some extent clustered together in the same foetuses and was shown in the presence of maternal toxicity, however, it was not considered marked.

Table 2.6.6.2-2: Results from foetal examination in study CA 5.6.2/014/Report No. [redacted] 45 & 39 (preliminary

study with pregnant does) & 40 (preliminary study with non-pregnant does) /901303

Dose Group (mg/kg bw/day)	0 (control)	50	150	450	Historical control range or x/y □ (mean)
Number of does with live young or litters at Day 29	18	12	15	13	--
Mean foetal weight (g)	43.9	43.3	44.0	44.5	41.4 – 47.6 (44.1)
Thoracic region malformations			--		
No. of foetuses with interventricular septal defect	1	1	1	4	10/1511
%	0.6	1.0	0.9	4.2	0.66
Litter incidence	1	1	1	4	10/188
%	5.56	8.3	6.67	30.8	5.32
Foetuses with enlarged left, reduced right ventricles	0	0	0	2	2/1511
%	0.0	0.0	0.0	2.1	0.13
Litter incidence	0	0	0	2	2/188
%	0	0	0	15.4	1.10
Foetuses with retro-oesophageal right subclavian artery	0	0	3	2	7/1511
%	0.0	0.0	2.7	2.1	0.46
Litter incidence	0	0	1	1	7/188
%	0	0	6.6	7.6	3.72
Foetuses with narrow/dilated aortic arch/pulmonary trunk/arterial trunk	1	1	1	3	8/1511
%	0.6	1.0	0.9	3.2	0.52
Litter incidence	1	1	1	3	8/188
%	5.56	8.3	6.67	23.1	4.25

□ number affected / total number examined
Malformed foetuses are excluded
-- no data

The cardiac malformations cardiomegaly and "seal shaped" heart in study **CA 5.6.2/012/013/ TOXI: 884-TER-RB** and **CA 5.6.2/014/Report No. [redacted] 45 & 39 (preliminary study with pregnant does) & 40 (preliminary study with non-pregnant does) /901303** was observed in a single incidence and without dose-response. The finding "dilated heart" was reported in four foetuses from 3 litters in the 20 mg/kg bw/day dose group, 4 foetuses (3 + 1) from 2 litters in the 100 mg/kg bw/day dose group and all foetuses (4) in one litter and one foetus of another litter at the 500 mg/kg bw/day. There were no significant maternal effects in the doe with 3 cases of dilated heart at 100 mg/kg bw/d. In the doe with 4 cases of dilated heart at 500 mg/kg bw/d, soft stool and diarrhoea was recorded at GD 10. However, due to the high maternal mortality in high and mid dose dams (8/15 and 4/16 respectively) there were only few foetuses and litters available for the assessment (i.e., 28/5, 77/12, 78/13 and 133/20 in high, mid, low dose and controls, respectively. Moreover, there were no historical control data included and the term "dilated heart" was not clearly defined.

Table 2.6.6.2-3: Cardiac findings in study CA 5.6.2/012/013/ TOXI: 884-TER-RB and **CA 5.6.2/014/Report No. [redacted] 45 & 39 (preliminary study with pregnant does) & 40 (preliminary study with non-pregnant does) /901303**

Dose group (mg/kg bw/d)	**0**	**20**	**100**	**500**
No. of foetuses/no. of litters exmined	133/20	78/13	77/12	28/5
Major visceral malformations:				
No. of foetuses/litters with dilated heart	-	4*/3	4*/2	5*/2*
No. of foetuses/litters with cardiomegaly	0	0	1**	0
No. of foetuses/litters with "seal shaped" hearts	1/1	0	1**	0
No. of fooetuses/litters with dilated ventricle	1/1	0	1/1	1/1
No. affected/total no. of foetuses	2/133	4/78	4/77	5/28
Litters affected/total no. of litters	2/133	3/13	2/12	2/5

* statistically significant, $p \leq 0.05$
** same foetus

Ventricular septal defects were observed in (0(0), 1(1), 1(1) and 2(2) foetuses (litters) from the 0, 125, 250 and 500 mg/kg bw/d dose groups in study **CA 5.6.2/016/Report No. 1086**. The results from this study indicated a higher total number of foetuses and litters with malformations in the mid and high dose groups (3 foetuses (3 litters), 6(6), 10(10) and 20(14) from the 0, 125, 250 and 500 mg/kg bw/d dose groups, respectively) but as stated in the RAC opinion, *"it is not clear form the reporting of the study whether the different malformations were found in different foetuses or if some foetuses had multiple malformations. The total number of litters in the high dose with malformations is reported to be 14. However, the number of animals on the study was 15 and out of these 3 were reported as being nonpregnant and 2 as having aborted. However, the number of litters examined is reported to be 12 in the high dose group which implies that aborted foetuses where examined and that data from these 2 litters were included in the analysis."*

The study is considered non-acceptable in this assessment due to several deficiencies in methodology and reporting and the observations are thus not given much weight.

One foetus in the mid dose and one in the high dose group in study CA 5.6.2/009/Report No. [redacted]/P/5009 was reported to have a single heart ventricle, thickened ventricle walls, enlarged aorta and reduced pulmonary artery. However, cardiac malformations (enlarged aorta, a persistent truncus arteriosus) was also observed in a control foetus thus the findings were not considered to indicate a treatment-related effect.

Skeletal malformations were observed among studies but did not appear related to treatment since effects did not show any clear dose-responses.

One high dose foetus in study **CA 5.6.2/009/Report No. [redacted]/P/5009** showed gross malformations of the skull. In addition to this, a statistically significant increase in foetuses (litter) with minor skeletal defects was observed in the low- and high dose group (58 (16), 82 (18), 59 (16) and 79 (17) at 0, 100, 175 and 300 mg/kg bw/d). The individual minor skeletal effects showed a statistically significant increase only in the high dose group for partially ossified transverse process on the 7th cervical vertebrae (8 foetuses in 2 litters as compared to 1 foetus in the controls), unossified transverse process on the 7th lumbar vertebrae (14 foetuses in 4 litters as compared to 4 foetuses in 3 litters in the controls) or partially ossified 6th sternebrae (16 foetuses from 7 litters as compared to 4 foetuses in 2 litters in the controls). A statistically significant increase in foetuses (litter) with skeletal variations was also reported

in the high dose group (119 (17), 129 (18), 116 (17) and 132 (17) at 0, 100, 175 and 300 mg/kg bw/d). These variations included a non-statistically significant increased incidence of foetuses with partially ossified odontoids (62 foetuses in 15 litters compared to 50 foetuses in 15 litters in the controls) or 27 pre-sacral vertebrae (37 foetuses in 12 litters compared to 23 fetuses in 10 litters in the controls). The foetal bw was statistically significantly reduced by 8% in the high-dose group. With respect to maternal toxicity, all animals that aborted (i.e., 1, 2, 1 and 2 rabbits in the 0, 100, 175 and 300 mg/kg bw/d dose groups) died or were sacrificed in extremis. In the high dose group, maternal body weight gain was statistically significantly reduced during treatment by 32% (days 8-20) and accompanied by a by 19-43% reduction in food consumption. Considering the lack of dose-response, these minor effects are not considered related to treatment.

Statistically significant increases in the numbers of litters with skeletal malformations were also reported in study **CA 5.6.2/011/Report No. [redacted] 94-0153** performed with Japanese white rabbits. The litter/foetus incidences were 1/1 (5.6/0.7%), 3/4 (20/3.1%), 2/6 (12.5/4%) and 5/5 (35.7/4.5 %) in the 0, 10, 100 and 300 mg/kg bw/d dose groups, respectively. The most frequent malformations were fissure (0, 1, 3 and 0 foetuses in the low-, mid- and high-dose group, respectively) or splitting (0, 0, 3 and 1 foetuses in the low-, mid- and high-dose group, respectively) of the parietal bones. In the low- and high-dose groups, 1 foetus and 2 foetuses had fusion of parietal bones. The impact of the increase in skeletal malformations was difficult to interpret since a litter is counted whether only one or all foetuses are affected, and for most of the skeletal malformations 1-2 foetuses/litter were affected. Since the types of skeletal malformations were inconsistent and there was no clear dose-response in the number of foetuses showing skeletal malformations, the study author considered this a sporadic alteration rather than treatment-related. The maternal toxicity included one maternal death in the high dose group, abortions (2 in low and 2 in high dose group) and loose stool. Considering that there was no similar craniofacial skeletal effect among the other acceptable rabbit studies at doses up to and including 500 mg/kg bw/d, the skeletal effects observed are not expected to be related to treatment. Also RAC concludes that the skeletal craniofacial malformations reported at low incidences in one study but not found in the other six rabbit developmental toxicity studies were considered to be anomalous and were given less weight in the overall weight of evidence.

In study **CA 5.6.2/019/Report No. 401-056** skeletal malformations were only reported in the low- and mid-dose groups (encephaly, absent rib, malformed rib and fused cervical vertebral centre). However, maternal toxicity was high in the top dose with a mortality rate of 10 in the 350 mg/kg bw/d group compared to 0, 1 and 2 in the 0, 75, 175 mg/kg bw/d dose groups leading to an insufficient number of litters being available for assessing possible adverse effects on foetal development at the high dose level (350).

Maternal and developmental NOAEL/LOAEL in rabbits

The critical effect for the lowest maternal LOAEL in the studies considered acceptable is reduced body weight gain during treatment. In study **CA 5.6.2/010/Report No. 434/020**, the bodyweight gain (days 7-19) was reduced 24-29% in dams administered 200 mg/kg bw/day. Although not statistically significant at this dose level, the same effect and more severe was observed at the next higher dose along with reduced food consumption and mortality. This is further supported by similar findings in study 6.6.2/014 at a LOAEL of 150 mg/kg bw/d. The NOAEL in both studies is 50 mg/kg bw/day.

The critical effect for the developmental LOAEL is a reduced foetal weight observed in study 6.6.2/009 at 300 mg/kg bw/day and the increased incidence of cardiac malformations and statistically significant increase of post-implantation loss in study **CA 5.6.2/014/Report No. [redacted] 45 & 39 (preliminary study with pregnant does) & 40 (preliminary study with non-pregnant does) /901303** at 450 mg/kg bw/day. Due to the lack of a clear dose-response and similar effects at the same dose levels in other studies[16], the lowest adverse effect level for post-implantation loss is considered to be 450 mg/kg bw/d rather than 150 mg/kg bw/d since the latter was yet within the range for historical control data. The NOAEL for reduced foetal weight is 175 mg/kg bw/day and 150 mg/kg bw/day for increased incidences of cardiac malformations and post-implantation loss, respectively. The RMS proposes 150 mg/kg bw/day to represent an overall developmental NOAEL.

Human relevance of effects observed in rabbits

The data on developmental toxicity clearly indicate a higher sensitivity of rabbits compared to rats. Whilst the LOAEL/NOAELs for maternal toxicity in both rabbits and rats are based on reduced body weight gain the

[16] The statistically significant increase of post-implantation loss observed in dams administered 200 mg/kg bw/day in study 6.6.2/010 are not considered treatment-related in the absence of dose-response and no effect on litter size (6.6.2/010).

LOAEL/NOAELS for developmental toxicity in rats and rabbits are set for different effects, skeletal variations in rats and increased incidences of post-implantation loss and cardiac malformations. The differences in sensitivity between pregnant rabbits and pregnant rats may be due to rabbits ingesting their caecotrophes which may either lead to an increased exposure to glyphosate as it is excreted unchanged in faeces or it may lead to an undernourishment due to soft stools and diarrhoea, observed in the studies, prevent the rabbits from ingesting their caecotrophs. It may thus be argued that effects in rabbits are due to this special behaviour leading to a repeated exposure to the substance or a malnutrition that would not exist in other species. Consequently, the rabbit would be a non-representative animal model for effects of glyphosate and the NOAEL for use in risk assessment should not be taken from this type of study.

According to the applicant, *"It is likely that the bolus administration of low pH glyphosate acid stresses the does as well as leads to the irritation of mucosal membrane of the rabbit gastro-intestinal tract. Consequently the associated stress leads to gastro-intestinal stasis. The gross necropsy signs observed in maternal animals in the studies 5.6.2/011, CA 5.6.2/010 and 5.6.2/009, such as hair like boluses in the stomach, fluid filled large intestines and gas distension in the lower gastrointestinal tract are indicative of gastro-intestinal stasis. These findings appear to be relevant to only hindgut fermenters as it is not seen in rats or dogs following administration of an oral bolus dose."*

Furthermore, the applicant states that published literature shows coprophagy to be vital to the rabbit for accessing the necessary nutrition to thrive and survive. Due to the gastrointestinal disturbances in the studies the essential practice of coprophagy was not possible (soft pellets could not form due to diarrhoea), leading to nutritional compromise of the rabbits. Therefore, the applicant considers the rabbit maternal toxicity findings *"clearly not relevant to humans for three simple reasons. Firstly, humans are not exposed to bolus doses of glyphosate acid in their diet, and therefore are not subjected to the irritating effects seen in rabbit gastrointestinal tracts. Secondly, the maternal toxicity in rabbit developmental toxicty studies is not due to subchronic or chronic exposures. Thirdly, humans are not coprophagic; we obtain our nutrients through a balanced diet rather than nutrient recycling via the consumption of faeces."*

However, caecotrophes which are much higher in moisture than the regular hard faeces are often referred to as soft faeces[17] complicating the assessment of the clinical sign diarrhoea/soft faeces noted in the study reports. Moreover, it is clear from studies both in rabbits and rats, that the substance causes gastrointestinal irritation in both species although only at high doses in rats. In rabbits this was seen both as diarrhoea and histopathological changes (studies **CA 5.6.2/010/Report No. 434/020**, **CA 5.6.2/019/Report NO. 401-056)** whereas only diarrhoea/loose faeces were observed in rats. According to Guidance for the setting and application of acceptable operator exposure levels (AOELS), SANCO 7531 - rev.10) the AOEL is based on "…the highest level at which no adverse effect is observed in tests in the most sensitive relevant animal species or, if appropriate data are available, in humans". Since the original study reports do not inform if rabbits were able to eat their caecotrophes or not, it is not considered safe to anticipate that the higher sensitivity in rabbits only results from a species-specific mechanism and thus to dismiss these effect levels. Consequently, it is proposed to take the NOAELs for rabbits into consideration for the derivation of an AOEL for human risk assessment.

In addition to the studies presented above, the data available for the assessment includes published literature identified and categorized by the applicant as "relevant but supplementary" after detailed assessment of full-text articles (Category B studies, summarized in section B.6.6.2 of Vol. 3). While the investigations by Yahfoufi Z. A. *et al* in mouse oocytes (CA 5.8.2) may provide some information that may be useful for a mechanistic understanding, the clinical *in vivo* significance of these observations (i.e. disruption of the microtubule organizing center and chromosomal disorganization, interference with intracellular zinc bioavailability and ROS accumulation in embryos) is unclear in the absence of finding in the OECD 414 studies performed with a larger number of animals and with the purpose of investigating adverse effects in the developing foetus. Except for the study by Yahfoufi Z. A. *et al*., all other studies were performed with glyphosate-based formulations rather than the neat substance and it is thus not possible to conclude if effects observed are caused by glyphosate or by any other co-formulant. Therefore, the categorisation as "supplementary information" is agreed and taking also into account that the quality of studies was regarded "reliable with restrictions", this data is not considered further in the assessment of this endpoint.

There are several epidemiological studies available in the open literature reporting effects such as miscarriage, fecundity, pre-term delivery, gestational diabetes mellitus, birth weights, congenital malformations, neural tube defects, attention-deficit disorder/attention-deficit hyperactive disorder (e.g. Arbuckle, T. E. *et al* 2001[18], Savitz,

[17] Caecotrophy in Rabbits, Amy E. Halls, M.Sc. – Monogastric Nutritionist Shur-Gain, Nutreco Canada Inc., January 2008 (http://www nutrecocanada.com/docs/shur-gain---specialty/caecotrophy-in-rabbits.pdf).

18 Arbuckle, T. E. Lin, Z. Mery, L. S. (2001). An exploratory analysis of the effect of pesticide exposure on the risk of spontaneous abortion in an Ontario farm population. Environmental Health Perspectives Volume: 109

D.A. *et al.* (1997)[19], Garry, V. F *et al.* (2002)[20], Bell *et al.* (2001)[21], Aris (2011)[22], Benítez-Leite *et al* (2009)[23]). Due to uncertainties regarding type of formulation, exposure levels, simultaneous exposure to more than one pesticide, statistically significant positive associations and the influence of recall bias, the reliability of this information is difficult to assess. However, the data is not considered to establish a clear link between exposure to the active substance and developmental toxicity in a way that would reduce the weight of the animal data.

2.6.6.2.2 Comparison with the CLP criteria regarding adverse effects on development

Substances with properties meeting criteria for classification are subcategorised into category 1A (known human reproductive toxicant), 1B (presumed human reproductive toxicant) or 2 (suspected human reproductive toxicant) depending on the strength of evidence.

Classification of a substance in category 1A is largely based on evidence from humans and since no such data is available, this criterion is not fulfilled.

Classification of a substance in category 1B is largely based on data from animal studies. According to CLP guidance, "such data shall provide clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects. However, when there is mechanistic information that raises doubt about the relevance of the effect for humans, classification in Category 2 may be more appropriate."

Substances are classified in Category 2 if there is "some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility, or on development, and where the evidence is not sufficiently convincing to place the substance in Category 1. If deficiencies in the study make the quality of evidence less convincing, Category 2 could be the more appropriate classification."

There were only few effects noted in the seven developmental toxicity studies in rats. These were observed at high doses and there was no consistent pattern of adverse effects. Cardiovascular malformations were reported in two of the rat studies. In both studies these were single incidences occurring at two dose levels. However, considering that effects are single incidences, no clear dose-response was seen, effects were not statistically significant and not seen in the other three rat studies at similar doses, the findings are not considered to fulfil criteria for classification.

Developmental effects were also observed in the reproductive toxicity studies in the rat and consisted of reduced pup weight and distension of caecum (see section 2.6.6.1). Reduced pup weight was observed in several studies. In the study by [redacted] (2000) (CA 5.6.1/004, Report No.: [redacted]/P/6332) using Wistar rats, reduced pup weight was observed at the limit dose (1000 mg/kg bw/day). The finding was observed in F1A pups but not in F2A pups. Further, in the study by [redacted] (1997) (CA 5.6.1/005, Report No.: [redacted] 96-0031) using Sprague-Dawley rats, reduced pup weights were observed at the very high dose of 30000 ppm (above 2000 mg/kg bw/day) but not at 6000 ppm (417 mg/kg bw/day). Maternal toxicity in this study consisted of clinical signs (loose stool) and increased kidney and liver weights observed at 30000 ppm. Also, in the study by [redacted] (1990) (CA 5.6.1/010, Report No. [redacted]-10387) using Sprague Dawley rats, reduced pup weights were observed at the very high dose of 30000 ppm (above 2000 mg/kg bw/day) but not at 10000 ppm (666 mg/kg bw/day). Maternal toxicity in this study consisted of clinical signs (loose stool) and reduced body weight observed at 30000 ppm. It could also be noted that reduced pup weights were observed at low doses (≥236 mg/kg bw/day) in the one-generation range finding study by [redacted] (1991) (CA 5.6.1/009, Report No.: [redacted] 42/90619). However, this study was only considered as supplementary data and not suitable for NOAEL setting (few animals used, limited parameters investigated, no statistical analyses conducted). The finding of reduced pup weight was not confirmed in the main study using sufficient number of animals and test doses up to 10000 ppm (668 and 752 mg/kg bw/day in males and females respectively). Overall, reduced pup weight was observed in several reproductive toxicity studies, but this finding was confined to limit dose. Thus, data do not provide convincing evidence for a classification of the substance in Cat. 2.

Distension of caecum was observed in pups (F1 and F2 litters) in one two-generation toxicity study ([redacted], 1990, CA 5.6.1/010, Report No. [redacted]-10387), but only at the very high dose of 30000 ppm (above 2000 mg/kg bw/day).

19 Savitz, D.A. Arbuckle, T. Kaczor, D. Curtis, K.M. (1997). Male pesticide exposure and pregnancy outcome. American Journal of Epidemiology Volume: 146, Number: 12, Pages: 1025-1036

20 Garry, V. F. Harkins, M. E. Erickson, L. L. Long-Simpson, L. K. Holland, S. E. Burroughs, B. L. (2002). Birth defects, season of conception, and sex of children born to pesticide applicators living in the Red River Valley of Minnesota, USA. Environmental Health Perspectives Volume: 110 Pages: 441-449

21 A Case-Control Study of Pesticides and Fetal Death Due to Congenital Anomalies, Epidemiology, Volume: 12, Number: 2, Pages: 148-156 ASB2012-11559

22 Maternal and fetal exposure to pesticides associated to genetically modified foods in Eastern Townships of Quebec, Canada. Reproductive toxicology Volume: 31, Pages: 528-533 ASB2012-11547

23 Arch Pediatr Urug, Volume: 80, Number: 3, Pages: 237-247ASB2012-11563

Maternal toxicity indicating effects on gastro-intestinal tract (soft faeces) was observed at this dose level. In the one-generation range finding study by [redacted] (1991) (CA 5.6.1/009, Report No.: [redacted] 42/90619) using the same strain of rat, macroscopic gastrointestinal changes (soft faeces) were observed in offspring retained to six weeks of age at 30000 ppm (ca 4000 mg/kg bw/day) but no distended caecum was reported. Thus, the severity of the effect is not considered sufficiently adverse for a classification of the substance in Cat. 2.

Several effects were observed among the studies performed in rabbits and at doses much lower than in rats. The higher sensitivity may be due to rabbits ingesting their caecotrophes either leading to an increased exposure to glyphosate or to undernourishment as soft stools/diarrhoea prevent the rabbits from ingesting their caecotrophes. The adverse effects on development observed included statistically significant increases in late embryo-foetal death, post-implantation loss as well as skeletal and visceral malformations.

Post-implantation loss and late/early embryo-foetal death was reported in only two studies of acceptable quality thus there is no clear evidence for an association between treatment and foetal viability. Effects observed on foetal viability in study CA 5.2/014 [redacted] 45 & 39 (preliminary study with pregnant does) & 40 (preliminary study with non-pregnant does) /901303) did not show a clear dose-response and values were within the historical control range for late- and total embryonic deaths.

Table 2.6.6.2-4: Summary of litter data from the available rabbit developmental toxicity studies (Reproduced from Table A in Annex 1, Background document to the RAC Opinion on Glyphosate, CLH-O- 0000001412-86-149/F. Data is taken from the original study reports)

Study (dose)	Parameter	Control	Low dose	Intermediate dose	High dose
[redacted] 1996 CA 5.6.2/010 Report No. 434/020 (0, 50, 200 & 400 mg/kg bw/d) *p<0.05	Number of dams with viable foetuses at scheduled C-section	14	18	15	15
	Mean foetal weight/litter (g)				
	Number of dams with total litter loss at scheduled C-section	0	0	0	0
	Mean number of implantations/dam	9.5±2.5	9.1±2.3	8.9±2.5	10.3±2.3
	Mean number of early embryonic/foetal deaths/dam	0.21±0.43	0.22±0.55	0.87±1.06	0.47±0.92
	Mean number of late embryonic/foetal deaths/dam	0.14±0.53	0.11±0.32	0.13±0.35	0.93±2.28
	Mean number of total embryonic/foetal deaths/dam	0.36±0.63	0.33±0.77	1.00*±1.00	1.40±2.35
	Mean percentage of post-implantation loss/dam	3.7±6.5	3.6±8.5	11.5±11.4*	12.1±18.6
	Mean number of live foetuses/litter	9.1±2.5	8.7±2.4	7.9±2.5	8.9±2.6
	Mean foetal weight/litter (g)	41.5±5.5	39.4±5.6	41.7±4.5	38.2±5.2
[redacted] 1996	Number of dams with viable foetuses at	17	18	17	17

CA 5.6.2/009 Report No. [redacted]/P/5009 (0, 100, 175 & 300 mg/kg bw/d) *p<0.05	scheduled C-section				
	Number of dams with total litter loss at scheduled C-section	0	0	0	0
	Mean number of implantations/dam	9.65±2.06	9.00±1.78	9.12±2.5	9.82±1.88
	Mean percentage of early intrauterine death/dam	6.2±9.7	7.5±17.0	8.1±8.1	11±16
	Mean percentage of late intrauterine deaths/dam	5.5±10.4	1.9±4.5	4.0±4.9	2.5±8.3
	Mean percentage of post-implantation loss/dam	11.7±12.0	9.5±16.7	12.1±9.7	13.6±16.6
	Mean number of live foetuses/litter	8.41±1.80	8.17±2.20	7.94±2.19	8.47±2.32
	Mean foetal weight/litter	44.4±4.3	43.3±3.9	43.2±5.7	40.7±7.8*
[redacted] 1991 CA 5.6.2/014 [redacted] 45 & 39 (preliminary study with pregnant does) & 40 (preliminary study with non-pregnant does) /901303 (0, 50, 150 & 450 mg/kg bw/d) *p<0.05 **p<0.01 No information available on standard deviations (SD) for the calculations.	Number of dams with viable foetuses at scheduled C-section	18	12	15	13
	Number of dams with total litter loss at scheduled C-section	0	0	0	0
	Mean number of implantations/dam	9.7	10.5	9.0	9.2
	Mean number of early embryonic deaths/dam	0.4	0.9	0.9	0.5
	Mean number of late embryonic deaths/dam	0.2	0.9	0.5	1.3**
	Mean number of total embryonic death/dam	0.6	1.8*	1.5*	1.8**
	Mean percentage of post-implantation loss/litter	5.7	19.5*	15.3*	21.0**
	Mean number of live foetuses	9.1	8.7	7.5	7.3
	Mean foetal weight	43.9	43.3	44.0	44.5
[redacted] 1993 CA5.6.2/012/013 Report No. TOXI: 884-TER-RB	Number of dams with viable foetuses at scheduled C-section	20	13	12	5
	Number of dams with total litter loss at scheduled C-	0	0	0	1

(0, 20, 100 & 500 mg/kg bw/d) # no info on SD * p≤0.05.	section (data included in calculations)				
	Mean number of implantations/dam	8±2.0	8±1.5	9±1.8	6±2.4
	Total number of embryonic resorptions/group (%)	10 (7)	11 (11)	11 (11)	9 (24)
	Total number of foetal resorptions/group (%)	8 (5)	7 (7)	13 (13)	1 (3)
	Total number of post-implantation loss/group (%))	18 (12)	18 (18	24 (24)	10 (26)
	Mean number of viable foetuses/litter#	7	6	7	6
	Mean foetal body weight	32±5.3	35±3.7*	35±2.4*	33±4.9
[redacted],1989[1] CA 5.6.2/016 Report No. 1086 0, 125, 250, 500 mg/kg bw/d)	Number in the study	15	15	15	15
	Number aborted	0	0	0	2
	Number non-pregnant at termination[2]	2	1	1	3
	Number pregnant at termination	13	14	14	12
	Number with no live fetuses[3]	0	0	0	2
	Number of litters examined	13	14	14	12
	Mean number of implantations/dam[4]	9.0±1.2	9.3±1.3	9.4±1.12	8.5±1.05
	Mean number of early resorption/dam[4]	1.7±3.22	1.1±2.53	1.0±2.56	1.9±2.43
	Mean number of non-viable implants/dam[4]	0.07±0.26	0.13±0.35	0.27±0.59	1.4±2.2
	Mean number of viable implants/dam[4]	7.3±3.1	8.0±2.59	8.0±2.48	5.2±3.03
	Mean foetal body weight[4]	40.6±16.6	47.1±0.95	47.5±1.38	48.7± 1.87
[redacted]1995 CA 5.6.2/011 Report No. [redacted] 94-0153 (0,10,100, 300 mg/kg bw/d)	Number of dams with total litter loss at scheduled C-section (not included in calculations)	0	1	2	1
	Number of dams with viable litters at scheduled C-section	18	15	16	14

	Mean number of implantations/dam	8.5±2.8	9.8±2.9	10.4±2.9	8.6±3.3
	Mean number of live foetuses/dam	7.8±2.4	8.7±3.2	9.4±2.7	8.0±3.2
	Percentage fetal resorptions and deaths	7.1	13.8	8.7	6.5
	Mean foetal body weight (M)	35.8±8.1	37.3±5.4	36.7±3.3	36.2±5.4
	Mean foetal weight (F)	35.7±6.7	36.1±5.1	36.0±3.9	34.9±4.4
[redacted] 1980 CA 5.6.2/019 Report No. 401-056 (0, 75, 175, 350 mg/kg bw/d) *p<0.05	Number of dams with viable litters at scheduled C-section	12	15	11	6
	Number of dams with total litter loss at scheduled C-section	0	0	0	0
	Mean number of implantations/dam	5.9±2.39	8.0±1.81	6.1±2.84	7.2±2.93
	Mean number of post-implantation loss/dam	0.7±0.89	0.4±0.63	0.2±0.4	0.8±1.33
	Mean number of early resorptions/dam	0.4 ± 0.9	0.3±0.59	0.1±0.3	0.5±0.84
	Mean number of late resorptions/dam	0.3±0.45	0.1±0.35	0.1±0.3	0.3±0.52
	Mean number of viable foetuses/dam	5.3±2.73	7.6*±1.84	5.9±2.77	6.3±2.25
	Foetal body weight	33.4±7.27	30.9±4.43	29.9±7.21	29.3±4.82

[1] Study with serious deficiencies in conduct and reporting, thus the data is presented exactly as reported in the summary table I of the study report.
[2] Normally the term "non-pregnant" is used to define animals that have no implantations at C-section. As revealed from the individual litter data in the study report, all animals in the study had implantations and it appears that the "non-pregnant animals" in fact were animals that had total litter loss.
[3] This data is not in line with the data presented in the individual litter data.
[4] Data from "non-pregnant" as well as female rabbits that aborted during the study have been included in the calculations.

Visceral and skeletal malformations were reported in five out of the seven rabbit studies but only three of these five studies were considered acceptable. Increases in visceral malformations including interventricular septal defects were observed in study CA 5.6.2/014 ([redacted] 45 & 39 (preliminary study with pregnant does) & 40 (preliminary study with non-pregnant does) /901303), ventricular septal defects in study CA 5.6.2/016 (Report No. 1086) (non-acceptable) and the increase in dilated heart in study CA 5.6.2/012/013 (Report No. TOXI: 884-TER-RB) (study considered supportive only) raise concern that cardiovascular malformations in the heart can be induced following *in utero* exposure to glyphosate in rabbits. The maternal mortality rate in studies CA 5.6.2/012/013 (Report No. TOXI: 884-TER-RB) and CA 5.6.2/019 was excessive thus the number of foetuses was too low for a robust assessment. Since cardiovascular malformations following treatment with glyphosate was not reported consistently among the four studies of acceptable quality and the study considered supportive and, when reported, the incidences were low, without a clear dose-response relationship and also reported in the control groups, RAC concluded in 2017 that criteria for classification were not fulfilled. The RMS agrees with this conclusion. Likewise, since a statistically significant increase in skeletal craniofacial malformations were not seen in the other rabbit developmental toxicity studies considered acceptable, the skeletal malformations reported in study CA 5.6.2/011 (Report No. [redacted] 94-0153) are not considered to fulfil criteria for classification.

Table 2.6.6.2-4: Summary of malformations in the rabbit developmental toxicity studies (Reproduced from Table A in Annex 1, Background document to the RAC Opinion on Glyphosate, CLH-O- 0000001412-86-149/F. Data is taken from the original study reports)

Study (dose)	Parameter	Control	Low dose	Intermediate dose	High dose
[redacted] 1980 CA 5.6.2/019 Report No. 401-056 (0, 75, 175, 350 mg/kg bw/d)	Number of fetuses (litters) examined	63(12)	114(15)	65(11)	38(6)
	Total number of fetuses (litters) with malformations	0	3(3)	2(2)	2(1)
	- External/Visceral	0	0	0	2(1)
	- Skeletal	0	3(3)	2(2)	0
	- Cardiovascular	0	0	0	0
[redacted] 1993 CA5.6.2/012/013 Report No. TOXI: 884-TER-RB (0, 20, 100 & 500 mg/kg bw/d) #significantly different from control by Contingency testing	Number of fetuses (litters) examined	133(20)	79(13)	77(12)	28(5)
	Total number of fetuses (litters) with malformations	Not reported	Not reported	Not reported	Not reported
	- External	2(2)	2(1)	1(1)	0
	-Visceral	4(3)	6(3)	6(4)	8#(2)
	- Skeletal	11(4)	5(3)	0#	1(1)
	- Cardiovascular	2(2)	4(3)	6(4)	6(2)
[redacted] 1996 CA 5.6.2/009 Report No. [redacted]/P/5009 (0, 100, 175 & 300 mg/kg bw/d)	Number of fetuses (litters) examined	143(17)	147(18)	135(17)	144(17)
	Total number of fetuses (litters) with malformations	3(2)	1(1)	0	2(2)
	- External/Visceral	2(2)	1(1)	0	2(2)
	- Skeletal	3(2)	0	0	1(1)
	- Cardiovascular	1(1)	1(1)	0	1(1)
[redacted] 1995 CA 5.6.2/011 Report No. [redacted] 94-0153 0,10,100, 300 mg/kg bw/d) *p≤0.05	Number of fetuses (litters) examined	140(18)	130(15)	150(16)	112(14)
	Total number of fetuses (litters) with malformations	1(1)	Not reported (3)	Not reported (3)	5(5*)
	- External	0	0	2(1)	
	-Visceral	0	1(1)	3(2)	
	- Skeletal	1(1)	4(3)	6(2)	
	- Cardiovascular	0	0	1	
[redacted] [redacted] 1996 CA 5.6.2/010 Report No. 434/020 0, 50, 200 & 400 mg/kg bw/d)	Number of fetuses (litters) examined	128(14)	157(18)	119(15)	134(15)
	Total number of fetuses (litters) with malformations	2(2)	3(2)	2(2)	1(1)
	- External/Visceral	1(1)	2(1)	2(2)	1
	- Skeletal	1(1)	1(1)	1(1)	0

	- Cardiovascular	0	0	1(1)	0
[redacted] 1991. CA 5.6.2/014 [redacted] 45 & 39 (preliminary study with pregnant does) & 40 (preliminary study with non-pregnant does) /901303 (0, 50, 150 & 450 mg/kg bw/d)	Number of fetuses (litters) examined	163(18)	104(12)	112(15)	95(13)
	Total number of fetuses (litters) with malformations	3(3)	3(3)	5(3)	6(5)
	- Cardiovascular	1(1)	1(1)	4(2)	5(4)
[redacted] [redacted][2], 1989 CA 5.6.2/016 Report No. 1086 (0, 125, 250, 500 mg/kg bw/d)*	Number of fetuses (litters) examined	109(13)	113(14)	120(14)	78(122)
	Total number of fetuses (litters) with malformations	3(3)	6(6)	10(10)	20(142)
	- External	1(1)	2(2)	3(3)	3(3)[2]
	-Visceral	1(1)	4(4)	5(5)	12(9)[2]
	- Skeletal	1(1)	0	2(2)	5(2)[2]
	- Cardiovascular	0	1(1)	1(1)	2(2)[2]

[1]The study report only presented summary information regarding number of foetuses (litters) with malformations.
[2] Study with serious deficiencies in conduct and reporting The reporting of the data is unclear. The total number of litters in the high dose with malformations is reported to be 14. However, the number of animal on the study was 15 and out of these 3 were reported as being nonpregnant and 2 as having aborted. However, the number of litters examined is reported to be 12 in the high dose group which implies that aborted foetuses where examined and that data from these 2 litters were included in the analysis. Consequently, it is unclear to what extent the data for the high dose group represents finding in aborted foetuses. Foetal effects noted in the generational studies in section 2.6.6.1 (reduced foetal bodyweight (≤ 10%) and a decrease in litter size (considered an equivocal finding) occurred at doses at or above the limit dose of 1000 mg/kg bw/day and are not considered to indicate that criteria for classification are fulfilled.

In conclusion, there were no effects observed in the developmental toxicity studies in rats and rabbits that are considered to fulfil criteria for classification for developmental toxicity.

2.6.6.3 Adverse effects on or via lactation [equivalent to section 10.10.7 of the CLH report template]

Table 63: Summary table of animal studies on effects on or via lactation

Method, guideline, deviations[1] if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results** **- NOAEL/LOAEL** **- target tissue/organ** **- critical effects at the LOAEL**	**Reference** **New data for renewal: Yes/No**
Two generation reproduction study (dietary) OECD TG 416 (2001) Rat	Glyphosate technical Purity: 95.7% (w/w) Lot/Batch#: H05H016A 0, 1500, 5000, 15000 ppm (equivalent to mean achieved dose levels of 0,	<u>F0 and F1 adults:</u> <u>1500 ppm:</u> No treatment-related effects <u>5000 ppm:</u> -one F0 female in extremis due to suspected prolonged parturition (not	[redacted] (2007) CA 5.6.1/001 CA 5.6.1/002 CA 5.6.1/003

Method, guideline, deviations[1] if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results** **- NOAEL/LOAEL** **- target tissue/organ** **- critical effects at the LOAEL**	**Reference** **New data for renewal: Yes/No**
Sprague-Dawley Crl:CD (SD) IGS BR M, F 28/sex/group GLP: Yes Acceptable No deviations from OECD TG 416 (2001)	104, 351 and 1063 mg/kg bw/day for males, and 0, 162, 530 and 1634 mg/kg bw/day for females) Administration: daily by dietary admixture throughout the treatment period. Control animals were treated in an identical manner with untreated laboratory diet. Exposure of F0 began at approximately 8 weeks of age. After 10 weeks of exposure F0 rats were mated. The appropriate experimental diet was fed throughout the study, to F0 and F1 parents and offspring until termination.	considered attributable to the administration of glyphosate according to study author) <u>15000 ppm:</u> -one F0 female found dead on day 97 possibly due to complications during parturition (not considered attributable to the administration of glyphosate according to study author) ↑ liver weight (F0 females: abs weight: 13%, rel weight: 8%; F1 females: absolute weight: 10%, relative weight: 8%) ↑ kidney weight (F0 females: abs. weight: 11%, rel. weight: 7%) -changes in sperm parameters (↓ number of homogenisation resistant spermatid in cauda epididymis) (309 million/gram compared to 400 million/gram in control) (F0 generation) <u>Offsprings (F0-F1 and F1-F2 generations):</u> <u>1500 ppm:</u> No treatment-related effects <u>5000 ppm:</u> No treatment-related effects <u>15000 ppm:</u> **-delayed sexual maturation** (delayed preputial separation, Days at completion: 45.9 compared to 43.0 in control) (F1 generation) NOAEL for parental, offspring and reproductive toxicity: 5000 ppm (351 mg/kg bw/day).	Report No. 2060/0013 New data for renewal: No
Two generation reproduction study (dietary) OECD TG 416 Rat Alpk:APfSD (Wistar-derived) M, F	Glyphosate acid Purity: 97.6% (w/w) Lot/Batch#: Y04707/082 0, 1000, 3000, 10000 ppm equivalent to mean achieved dose levels of: F0: 0, 99.4, 292.6, 984.7 mg/kg bw/day for males	<u>F0 and F1 adults:</u> <u>1000 ppm:</u> No treatment related effects <u>3000 ppm:</u> No treatment related effects <u>10000 ppm:</u> ↓ bw for selected F1 parent males during the pre-mating period (up to 5% reduction compared to control group)	[redacted] (2000) CA 5.6.1/004 Report No.: [redacted]/P/6332 New data for renewal: No

Method, guideline, deviations[1] if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results** **- NOAEL/LOAEL** **- target tissue/organ** **- critical effects at the LOAEL**	**Reference** **New data for renewal: Yes/No**
26/sex/group GLP: Yes Acceptable The study was checked for compliance with current OECD TG 416 (2001) and following deviations were observed: (i) no individual animal data presented in study report (ii) anogenital distance not examined as no treatment-related differences in sex ratio and sexual maturation were observed (iii) the thyroid was not weighed (iv) preimplantation loss not determined (v) pup development investigations restricted to body weight, vaginal opening and preputial separation	and 0, 104.4, 322.8, 1054.3 mg/kg bw/day for females during pre-mating period F1: 0, 116.5, 351 and 1161 mg/kg bw/day for males, and 0, 123.3, 370.8 and 1218.1 mg/kg bw/day for females, during the premating period Administration: daily by dietary admixture throughout the treatment period. Control animals were treated in an identical manner with untreated laboratory diet. Exposure of F0 began at approximately 5 weeks of age. After 10 weeks of exposure F0 rats were mated. The appropriate experimental diet was fed throughout the study, to F0 and F1 parents and offspring until termination.	<u>Offsprings:</u> <u>1000 ppm:</u> No treatment related effects <u>3000 ppm:</u> No treatment related effects <u>10000 ppm:</u> ↓ **bw** (10%) (F1A) NOAEL for parental toxicity: 10000 ppm (985 mg/kg bw/day) NOAEL for offspring toxicity: 3000 ppm (293 mg/kg bw/day) NOAEL for reproductive toxicity: 10000 ppm (985 mg/kg bw/day)	
Two generation reproduction study (dietary) OECD TG 416 Rat	Glyphosate technical Purity: 94.61 % (w/w) Lot No.: T-950308 0, 1200, 6000, 30000 ppm Equivalent to:	<u>F0 and F1 adults:</u> <u>1200 ppm:</u> No treatment-related effects <u>6000 ppm:</u> No treatment-related effects <u>30000 ppm:</u>	[redacted] (1997) CA 5.6.1/005 Report No.: [redacted] 96-0031 New data for renewal: No

Method, guideline, deviations[1] if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results** **- NOAEL/LOAEL** **- target tissue/organ** **- critical effects at the LOAEL**	**Reference** **New data for renewal: Yes/No**
Sprague Dawley Crj:CD (SD) M, F 24/sex/group GLP: Yes Acceptable The study was checked for compliance with current OECD TG 416 (2001) and following deviations were observed: (i) testes were not used for enumeration of homogenisation-resistant spermatids but cauda epididymal sperm was enumerated (the guideline recommends both testes and epididymides to be used for enumeration of homogenisation-resistant spermatids and cauda epididymides sperm reserves, respectively) (ii) thyroid and spleen not weighed (iii) vaginal opening and preputial separation not examined (iv) anogenital distance not determined	F0: 0, 83.6, 417, 2151 and 0, 96.9, 485, 2532 mg/kg bw/day in males and females, respectively F1: 0, 91.7, 458, 2411 and 0, 104.8, 530, 2760 mg/kg bw/day in males and females, respectively) Administration: daily by dietary admixture throughout the treatment period. Control animals were treated in an identical manner with untreated laboratory diet. Exposure of F0 began at approximately 5 weeks of age. After 10 weeks of exposure F0 rats were mated. The appropriate experimental diet was fed throughout the study, to F0 and F1 parents and offspring until termination. Duration of administration for both F0 and F1 parental animals: a total of approximately 18 weeks (for a part of F1 parental animals duration of administration extended to approximately 22 weeks due to a 4-week extension for reciprocal crosses with untreated animals)	-clinical signs (loose stool) (F0, F1, both sexes) ↓bw (F0 males: 8%; F1 males: 7%) -organ weight changes (liver: F1 males: abs weight: ↑13%; F1 females: abs weight: ↑ 22%, rel weight: ↑ 20%; kidney: F0 males: rel weight: ↑11%; F1 males: rel weight: ↑14%; F1 females: abs weight: ↑17%, rel weight: ↑15%; prostate: F1 males abs and rel weight:↓32% -lower fertility indices of F1 females (not statistically significant) (79.2% compared to 95.8% in control) -distension of caecum (F0, F1) (both sexes) Offsprings (F1 and F2): 1200 ppm: No treatment-related effects 6000 ppm: No treatment-related effects 30000 ppm: ↓ **pup weights** (F1 males:14%, F1 females: 13%; F2 males: 9%, F2 females: 8%) **-distension of caecum** (F1 and F2 litters) NOAEL for parental, offspring and reproductive toxicity: 6000 ppm (417 mg/kg bw/day)	

Method, guideline, deviations[1] if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL	Reference New data for renewal: Yes/No
(v) no organ weighed for pups (the guideline recommends brain, speen and thymus to be weighed) (vi) pre-and post-implantation loss not reported (vii) number of corpora lutea not given. (viii) time to mating not reported (ix) number of animals used are not in line with the recommendation by the guideline			
Two generation reproduction study (dietary) OECD TG 416 (1983) Rat Wistar rats (Random bred) M, F 30/sex/group GLP: Yes Supplementary only (effect dose lacking, limited parameters investigated in study) The study was checked for compliance with current OECD TG 416 (2001)	Glyphosate technical Purity: 96.8 % (w/w) Batch No.: 60 0, 100, 1000, 10000 ppm Dietary level would correspond to a mean daily compound intake of 0, 7.7, 77 and 770 mg/kg bw/day. [The mean daily intake was not reported for all dietary levels, but for the low level of 100 ppm a corresponding average value of 7.7 mg/kg bw/d was given in the original report]. Administration by dietary admixture. Control animals were treated in an identical manner with untreated laboratory diet. P0 generation: Treatment commenced at 8th week age of parental generation	<u>F0 and F1 adults:</u> <u>100 ppm:</u> No treatment-related effects <u>1000 ppm:</u> No treatment-related effects <u>10000 ppm:</u> No treatment-related effects <u>Offsprings (F1 and F2):</u> <u>100 ppm:</u> No treatment-related effects <u>1000 ppm:</u> No treatment-related effects <u>10000 ppm:</u> No treatment-related effects NOAEL for parental, offspring and reproductive toxicity: 10000 ppm (would correspond to a mean daily compound intake of 700-800 mg/kg bw/d)	[redacted] (1993) CA 5.6.1/006 Report No.: TOXI 885-RP-G2 New data for renewal: No

Method, guideline, deviations[1] if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL	Reference New data for renewal: Yes/No
and following deviations were observed: (i) the highest dose level was too low (the guideline recommends that the highest dose level should be chosen with the aim to induce toxicity but not death or severe suffering (ii) details of achieved concentrations not presented (iii) Parental animals were dosed at least 8 weeks before the mating period (the guideline recommend that dosing shall be continued for at least 10 weeks before the mating period) (iv) estrous cycle monitoring not performed (v) pre-coital interval not recorded (vi) data for gestation length not presented (vii) sperm analysis not performed (viii) monitoring of physical and sexual offspring development not performed (ix) organ weights not determined (x) a quantitative evaluation of	and continued throughout the experimental period until P1 generation was weaned P1 generation: Treatment commenced from weanling and continued throughout the experimental period until F2 litters were weaned and parents and pups were sacrificed		

Method, guideline, deviations[1] if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL	Reference New data for renewal: Yes/No
primordial follicles not conducted (xi) no histopathology (only for organs found abnormal in the macroscopical investigation)			
Two generation reproduction study (dietary) OECD TG 416 (1983) Rat Sprague-Dawley Crl:CD (SD) BR VAF/Plus M, F 28/sex/group GLP: Yes Acceptable The study was checked for compliance with current OECD TG 416 (2001) and following deviations were observed: (i) relative humidity in experimental animal room was 46%±24% (the guideline recommends the relative humidity to be at least 30%) (ii) pre-mating oestrous cycles not determined	Glyphosate technical Purity: 99.2 % (w/w) Batch No.: 206-JaK-119-1 0, 1000, 3000, 10000 ppm Equivalent to: F0: 0, 66, 197, 668 and 0, 75, 226, 752 mg/kg bw/day in males and females, respectively F1: 0, 76, 230, 771and 0, 82, 245, 841 mg/kg bw/day in males and females, respectively) Administration: daily by dietary admixture throughout the treatment period. Control animals were treated in an identical manner with untreated laboratory diet. Exposure of F0 began at approximately 6 weeks of age. After 10 weeks of exposure F0 rats were mated. The appropriate experimental diet was fed throughout the study, to F0 and F1 parents and offspring until termination. F0 animals were treated from 6 weeks of age for up to 29 weeks, F1 animals were treated up to 37 weeks of age	F0 and F1 adults: 1000 ppm: No treatment-related effects 3000 ppm: -histopathological changes in salivary gland (minimal hypertrophy of acinar cells with prominent granular cytoplasm) (Parotid, males: F0: 3/28, F1: 4/23; Parotid females: F0: 5/28, F1: 4/24; Submaxillary, females: F0: 4/28; F1: 0/24) 10000 ppm: ↑water consumption (F1 females, 17%) ↑food intake (F1 females) (3%) ↓ mean bw (F1 males, 1-7%) -histopathological changes in salivary gland (increased incidence of minimal hypertrophy of acinar cells with prominent granular cytoplasm) (Parotid, males: F0: 12/26, F1: 11/23; Parotid females: F0: 17/28, F1: 9/23; Submaxillary, females: F0: 14/28; F1: 3/23) Offsprings (F1 and F2): 1000 ppm: No treatment-related effects 3000 ppm: No treatment-related effects 10000 ppm: No treatment-related effects The NOAEL for parental toxicity was set at 1000 ppm (66 mg/kg bw/day)	[redacted] (1992) CA 5.6.1/007 CA 5.6.1/008 Report No.: [redacted] 47/911129 New data for renewal: No

Method, guideline, deviations[1] if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL	Reference New data for renewal: Yes/No
(iii) pre- and post-implantation loss were not reported (iv) sperm analysis not performed (v) uterus, spleen and thyroid of parental animals not weighed (vi) brain, spleen and thymus of pups not weighed		The NOAEL for offspring and reproductive toxicity was set at 10000 ppm (668 mg/kg bw/day).	
One-generation range finding study (dietary) No guideline Rat Sprague-Dawley Crl:CD (SD) BR VAF/Plus M, F F0 females: 10 time mated/group F1 generation: 10/sex/group GLP: No Supplementary only (low number of animals, limited parameters investigated, no statistics) The study was checked for compliance with OECD TG 416 (2001) and following	Glyphosate technical Purity: 98.6 % (w/w) Lot/Batch No.: 206-Jak-25-1 0, 3000, 10000, 30000 ppm Equivalent to: <u>F0 females:</u> 0, 236-311, 799-1010 and 2515-2789 mg/kg bw/day <u>F1 offspring:</u> 0, 368-390, 1291-1335 and 3918-4453 mg/kg/day for males and 355-402, 1191-1271 and 3961-4397 mg/kg/day for females Administration: daily by dietary admixture throughout the treatment period. Control animals were treated in an identical manner with untreated laboratory diet. Time-mated F0 females were used. Exposure of F0 females began at Day 3 of pregnancy and was continued through pregnancy to termination of the study. The study duration was 10 weeks	*Note: No statistically analyses conducted in this study* <u>Adults (F0):</u> <u>3000 ppm:</u> ↓bw gain by Day 14 of pregnancy (F0 females: 2% compared to control) -macroscopic salivary gland changes (enlarged/firm/congested/swollen) (F0 females: 2/9) -macroscopic gastro-intestinal changes (content watery and/or dark: F0 females (2/9); stomach distended and/or congested: F0 females (2/9)) -microscopical changes in salivary gland (minimal granular basophilic cytoplasm of acinar cells with minimal hypertrophy of acinar cells) (F0 females: 2/9) <u>10000 ppm:</u> -clinical signs (soft faeces and yellow stained sawdust) (F0 females) ↓ bw gain by Day 14 of pregnancy (F0 females: 3% compared to control) -macroscopic gastro-intestinal changes (content watery and/or dark: F0 females (7/10); stomach distended and/or congested: F0 females (5/10) -macroscopic salivary gland changes (enlarged/firm/congested/swollen) (F0 females 6/10) -microscopical changes in salivary gland (moderate/marked granular basophilic cytoplasm of acinar cells and	[redacted] (1991) CA 5.6.1/009 Report No. [redacted] 42/90619 New data for renewal: No

Method, guideline, deviations[1] if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results** **- NOAEL/LOAEL** **- target tissue/organ** **- critical effects at the LOAEL**	**Reference** **New data for renewal: Yes/No**
deviations were observed: (i) only 10 females/group were used (the guideline recommends 20 pregnant females/group) (ii) the F0 females were time-mated (the guideline recommends a mating procedure) (iii) duration of study was only 10 weeks. F0 exposed from Day 3 of pregnancy through the termination of the study, females were allowed to litter and rear their young to weaning, when 10 males and 10 female offspring per group were selected and reared on their respective diets to six weeks of age (the guideline recommends that F0 animals are dosed at least 10 weeks before mating period, dosing continued in both sexes during the 2 week mating period and continued throughout pregnancy and up to the		minimal/moderate hypertrophy of acinar cells (F0 females: 10/10) <u>30000 ppm:</u> -mortality (one F0 animal died on Day 21 *post partum*, cause of death not known) -clinical signs (soft faeces and yellow stained sawdust) (F0 females) -increased water consumption towards the end of pregnancy (F0 females: 11%) ↓ bw gain F0 females: Gestation Day 6 (23%), 14 (22%), 20 (11%); Lactation Day 7 (19%), 14 (39%), 21 (47%) ↓ bw F0 females: Gestation day 6 (2%), 14 (7%), 20 (5%); Lactation Day 7 (5%), 14 (13%), 21 (14%) -macroscopic gastro-intestinal changes (content watery and/or dark: F0 females (8/9); stomach distended and/or congested: F0 females (4/9); distended caecum (4/9) -macroscopic salivary gland changes (enlarged/firm/congested/swollen): F0 females (8/9) -microscopical changes in salivary gland (marked granular basophilic cytoplasm of acinar cells and moderate hypertrophy of acinar cells (9/9) and prominent mitoses in acinar cells (2/9)) <u>Pups to F0 generation:</u> <u>3000 ppm:</u> ↓ **mean pup weight** (Day 21 *post partum*: 9%) -macroscopical changes in salivary gland (congested) (one pup) (significance unclear) <u>10000 ppm:</u> ↓ **mean pup weight** (Day 21 *post partum*: 13%) -macroscopical changes in salivary gland (congested) (4 pups) (significance unclear) <u>30000 ppm:</u> ↓ **mean pup weight** (at birth: 4.5%, Day 21 *post partum*: 38%)	

Method, guideline, deviations[1] if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL	Reference New data for renewal: Yes/No
weaning of the F1 offspring. The same procedure for the F1 offspring to produce the F2 generation) (iv) oestrous cycle not evaluated (v) litter parameters limited (vi) sperm parameters not evaluated (vii) sexual maturation not investigated (viii) no organ weighed (ix) histopathology limited (salivary glands investigated only) (x) statistical analyses not performed		Offspring from birth to 6 weeks of age (F1 generation): 3000 ppm: -macroscopical changes in parotid salivary gland (enlarged/swollen) (1/10 male) 10000 ppm: No treatment-related effects 30000 ppm: -clinical signs (soft faeces) ↓ bw gain (Day 42 *post partum*: males: 25%; females: 15%) ↓ food during Weeks 5 (22%) and 6 (12%) (males only) -macroscopical changes in parotid salivary gland (enlarged/swollen) (males 5/10, females 2/10) -macroscopic gastro-intestinal changes (soft content: males (7/10), females (9/10)) The study is not suitable for NOAEL setting. Study acceptable as dose range finding study only (low number of animals, limited parameters investigated, no statistics)	
Two generation reproduction study (dietary) No guideline Rat Sprague-Dawley M, F 30/sex/group GLP: Yes Acceptable The study was checked for compliance with	Glyphosate Purity: 97.67 % (w/w) Lot No.: XLI-203 0, 2000, 10000, 30000 ppm Corresponding to 132-140, 666-711, 1983-2230 mg/kg bw/day for males and 160-163, 777-804, 2322-2536 mg/kg bw/day for females) (calculated for F0 and F1A adults) F0 generation rats (30/sex/group) were administered daily by dietary admixture for	Adults: 2000 ppm: No treatment-related effects 10000 ppm: No treatment-related effects 30000 ppm: -clinical signs (soft stool) ↓bw (Terminal bw: F0 males: 8%, F1 males: 13%, F1 females: 10%; Maternal bw during gestation: Day 1: F0 females: 7%, F1 females first mating: 12%, F1 females second mating: 13%; Day 21: F0 females: 7%, F1 females first mating: 8%, F1 females second mating: 8%) ↓ litter size (F0 dams: 13%) Pups:	[redacted] (1990) CA 5.6.1/010 Report No. [redacted]-10387 New data for renewal: No

Method, guideline, deviations[1] if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results** **- NOAEL/LOAEL** **- target tissue/organ** **- critical effects at the LOAEL**	**Reference** **New data for renewal: Yes/No**
current OECD TG 416 (2001) and following deviations were observed: (i) minor deviations in housing conditions: Temperature was 18-26ºC (the guideline recommends that the temperature in the experimental animal room should be 22 ± 3°C) (ii) no data on food efficiency (iii) no details on fertility indices, number of live births and pre- and post-implantation loss (iv) no determination of oestrus cycle length (v) sperm analysis not performed (vi) no determination of physical or sexual development landmarks (vii) parental animals: only ovaries and testes with epididymides weighed (uterus, prostate, seminal vesicles, brain, liver, kidneys, spleen, pituitary, thyroid and adrenal glands not weighed)	approximately 11 weeks and then mated to produce the F1a generation; 30 rats/sex/group from the F1 a generation were similarly exposed (approximately 14 weeks) and mated twice, to produce the F2a and F2b generations	<u>2000 ppm:</u> No treatment-related effects <u>10000 ppm:</u> No treatment-related effects <u>30000 ppm:</u> ↓ **pup weight** (Day 21: F0 males: 13%, F0 females: 11%; F1A males and females: 14%; F1B males: 19%, F1B females: 13%) NOAEL for parental, offspring and reproductive toxicity: 10000 ppm (666-711 mg/kg bw/day for males and 777-804 mg/kg bw/day for females)	

Method, guideline, deviations[1] if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL	Reference New data for renewal: Yes/No
(viii) brain, spleen and thymus of pups not weighed (ix) coagulating gland and cervix not included in the histopathological examination (x) no details on number of pups with grossly visible abnormalities			

Table 64: Summary table of human data on effects on or via lactation

Type o data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data.				

Table 65: Summary table of other studies relevant for effects on or via lactation

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
13-week (dose range finding study) Oral (drinking water) Rat (Sprague-Dawley) 24 female animals (17 weeks old) No guideline No GLP 0 and 1.75 mg/kg bw/day The study is reliable with restrictions (only one (low) dose tested; small group sizes; blood sampling was done only once (at the end of life) and the timing (9 AM to 3 PM) of sampling could not rule out circadian-dependent modulation of circulating hormones)	Glyphosate (Purity: 99.5 %) and Roundup Bioflow (MON 52276, containing 360 g/L of glyphosate acid	The aim of the (pilot) study was to examine whether exposure to glyphosate-based herbicides (GBHs) at a dose of glyphosate considered to be "safe" (the US Acceptable Daily Intake of 1.75 mg/kg bw/day), starting from *in utero* life, affect the development and endocrine system across different life stages in Sprague-Dawley rats. In this (pilot) study, two groups of Sprague-Dawley rats (8/group) were treated from gestation day 6 with either glyphosate (G) (> 99.5% pure) or Roundup Bioflow (R) (MON 52276) (360 g/L of glyphosate acid) diluted in drinking water to achieve glyphosate dose of 1.75 mg/kg bw/day (the US Acceptable Daily Intake). On post-natal day (PND) 28, the offspring were weaned and randomly distributed into two cohorts: 6-week cohort with 8 pups/sex/group and 13-week cohort with 10 pups/sex/group. After weaning, the pups also received the same dose of glyphosate or Roundup Bioflow as that of dams until their sacrifice (PND 73 ± 2 for the 6-week cohort; and PND 125 ± 2 for the 13-week cohort).	*Note: Results of glyphosate only are presented below* <u>1.75 mg/kg bw/day:</u> <u>Dams:</u> There were no statistically significant effects in dams on the following: body weight, water or feed consumption during gestation or lactation, gestational index, mean gestational length, total pups delivered at PND 0, litter size, sex ratio at birth, mean live birth index, dams with reported stillbirths (although the number of dams with stillbirths was higher (4/8) compared to control (2/8)), number of stillborns, survival index at PND 1 or 21, weight of adrenals, uterus or ovaries and total testosterone levels <u>Offspring:</u> -the anogenital distance (AGD) on PND 4 was statistically significantly increased in males -increased TSH in males of 6-week cohort group	Manservisi *et al*. (2019). Environmental Health 18:15

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
		Reproductive outcome of dams, and developmental landmarks and sexual characteristics of pups were examined.		
Perinatal study Oral (drinking water) Swiss mouse GD 0 p.c. PND 20 No guideline No GLP 0, 0.5, 5, 50 mg/kg bw/day The study is reliable with restrictions because of the following reasons: small group size, limited description of the study conditions and the results.	Glyphosate (Purity: ≥99.2 %) and Roundup 3 Plus containing 229 g/l glyphosate isopropylamine salt (170 g/l glyphosate acid equivalent)	In this study, the Swiss mouse were given glyphosate (G) or glyphosate-based herbicide Roundup 3 Plus (R) via drinking water at 0, 0.5, 5 and 50 mg/kg bw/day from embryonic day 0.5 to 20 days post-partum. Male offspring of the mice (at least 5 derived from 3 to 4 different litters in each group) were sacrificed at 5, 20, 35 days or 8 months for the following examinations: epididymis, seminal vesicles and testis weight, testis morphology, spermatozoa in total epididymis, serum testosterone; and the following in only G groups – number of undifferentiated spermatogonia and Sertoli cells (35 days old), expression of several genes in spermatogonia and germ cells number in testis (5 days old).	*Note: Results of glyphosate only are presented below* <u>0.5 mg/kg bw:</u> -decreased relative testis weight (at 35 d and 8 months old mice) -increase in vacuoles in seminiferous epithelium (20 d old mice) -decreased serum testosterone (35 d old mice) -the expression *Sall4* were decreased (5 d old mice) <u>5 mg/kg bw/day:</u> - decreased no. of undifferentiated spermatogonia (35 d old mice) -increase in vacuoles in seminiferous epithelium (20 d old mice) -empty seminiferous tubules (20 d old mice) -the expression *Sall4* were decreased (5 d old mice) <u>50 mg/kg bw/day:</u> -decreased serum testosterone (35 d old mice) -increase in vacuoles in seminiferous epithelium (20 d old mice)	Pham *et al.* (2019). Toxicological Sciences, Vol. 169, Issue 1, May 2019, Pages 260–271

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
			-the expression of *Kit* and *Sall4* were decreased (5 d old mice)	
13-week (pilot study) Orally (drinking water) Design of the study derives from the 13-week cohort protocol of the National Toxicology Program's (NTP) Modified One-Generation Reproduction Study 2011 (as stated in article) GLP: Yes SD rat 8 animals/sex for 6-week cohort 10 animals/sex for 13- week cohort Treatment starting from prenatal life, i.e. GD 6 of their mothers to PND 73 or 125 1.75 mg/kg bw/day The study is reliable with restrictions (low dose only, few animals, actual levels of test compounds that reached the foetus during gestation or that were ingested postnatally by the offspring during the period of lactation were not estimated).	Glyphosate (Purity: >99.5 %) and Roundup Bioflow	Sprague-Dawley rats were orally via drinking water exposed to 1.75 mg/kg bw/day starting from prenatal life, i.e. gestational day (GD) 6 of their mothers. One cohort was continuously dosed until sexual maturity (6-week cohort) and another cohort was continuously dosed until adulthood (13-week cohort). The endpoints investigated were mortality, body weight, water and food consumption, and clinical signs in dams and offspring and litter data.	<u>1.74 mg/kg bw/day</u> Survival, body weights, food and water consumption of rats were not affected by the treatment with glyphosate. No clinical changes were observed in the animals of the dosed groups. Furthermore, litter sizes were fully comparable among groups. In the treated rats, the majority of glyphosate was excreted in urine unchanged at levels of about 100-fold higher than that of AMPA and the mean urinary concentration of glyphosate increased with the duration of treatment.	Panzacchi *et al.* (2018). Environmental Health 17:52

2.6.6.3.1 Short summary and overall relevance of the provided information on effects on or via lactation

Note: The studies listed in Tables 63 and 65 are also presented in section 2.6.6.1. There are no specific studies submitted for this endpoint.

Generational studies

In the most recent two-generation study by [redacted] (2007) (CA 5.6.1/001-003, Report No. 2060/0013) using Sprague-Dawley rats, delayed preputial separation was observed in F1 male offspring at 15000 ppm without any additional developmental retardation indicating a delay in male sexual maturation. Although, the later onset of preputial separation in male offspring at 15000 ppm had obviously no impact on reproductive performance in week 29, a treatment related effect on sexual maturation at high dose level cannot be excluded. It could be noted that the finding of delayed sexual maturation in F1 males occurred at limit dose (1000 mg/kg bw/day). General toxicity was observed for females only and consisted of increased liver weights (F0 females: 13%, F1 females: 10%) and increased kidney weights (F0 females: 11%). A NOAEL of 5000 ppm (ca. 351 mg/kg bw/day) was considered for parental, reproductive and offspring toxicity (the NOAELs set in previous evaluation RAR (2015) remains).

In the two-generation study by [redacted] (2000) (CA 5.6.1/004, Report No.: [redacted]/P/6332) using Wistar rats, no impact on sexual maturation was observed up to the highest dose level of 10000 ppm (985 mg/kg bw/day). A reduction in body weight of F1A pups (10%) was observed at the highest applied dose of 10000 ppm. The NOAEL for parental toxicity was set at 10000 ppm (985 mg/kg bw/day) (highest dose) (in previous RAR (2015), the parental NOAEL was set at 3000 ppm based on "a lower body weight in F1 pups and a subsequent reduction also in body weight of F1 adult males at 10000 ppm"). The NOAEL for offspring toxicity was set at 3000 ppm (293 mg/kg bw/day) based on reduction in the body weight of the F1A pups in the 10000 ppm group.

In a two-generation study performed by [redacted] (1997) (CA 5.6.1/005, Report No.: [redacted] 96-0031) using Sprague-Dawley rats, treatment was associated with a number of parentally toxic effects at the highest dose of 30000 ppm (>2000 mg/kg bw/day). Following effects were observed: loose stool (males of both generations), reduced body weight (<10%, males of both generations), lower fertility indices (F1 females, 79.2% compared to 95.8% in control, not statistically significant), increased liver weights (F1 males and females), increased kidney weights (males of both generation and F1 females), decreased prostate weights (F1 males) and distension of the caecum (males and females of both generations). Offspring toxicity consisted of significantly **decreased body weight** and **distension of caecum** observed in F1 and F2 pups at 30000 ppm. Based on the results, the NOAEL for parental and offspring toxicity was considered to be 6000 ppm (417 mg/kg bw/day).

No effects were observed in pups in the study by [redacted] (1992) (CA 5.6.1/007-008, Report No. [redacted] 47/911129) where Sprague-Dawley rats were orally administered glyphosate by dietary admixture at a maximum dose level of 10000 ppm for two successive generations.

The NOAEL for parental toxicity was 1000 ppm (66 mg/kg bw/day) based on changes observed in salivary glands observed at ≥3000 ppm (≥197 mg/kg bw/day) (in previous RAR (2015), the parental NOAEL was set at 3000 ppm). The NOAEL for offspring toxicity was set at 10000 ppm (668 mg/kg bw/day) (highest dose) (in previous RAR (2015), the offspring NOAEL was set at 3000 ppm).

In the respective one-generation dose-range finding study performed by [redacted] (1991) (CA 5.6.1/009, Report No.: [redacted] 42/90619) 3000, 10000 and 30000 ppm were applied from day 3 of pregnancy through to termination of the study. Maternal toxic effects were observed in F0 females at 10000 ppm (soft faeces) and 30000 ppm (one mortality for which cause of death was not identified, soft faeces and reduced body weight gain). Macroscopical (enlarged/firm/congestion/swollen) and histopathologic changes in salivary glands were recorded in all treatment groups. Findings in pups consisted of **reduced pup weights** observed at ≥3000. Furthermore, macroscopic changes in salivary gland (congested) were observed in one pup at 3000 ppm and in four pups at 10000 ppm, but the significance of this finding was not clear since this effect did not occur in the highest dose group (30000 ppm). The study is not suitable for NOAEL setting (few animals used, limited parameters investigated, no statistical analyses conducted). However, it is noteworthy that effects occurred in this study at lower dose levels than in the main study. The findings of reduced pup weights observed at the low dose level of 3000 ppm (236 mg/kg bw/day) were not confirmed in the main study using sufficient number of animals and test doses up to 10000 ppm, nor in other available generational studies in which much more animals were employed.

In the two-generation study (Sprague-Dawley rat) by [redacted] (1990) (CA 5.6.1/010, [redacted]-10387) maternal toxicity

was evident in the high dose group at 30000 ppm (about 2000 mg/kg bw/day) indicated by soft stool and reduced body weights. At the same dose level, pups showed **decreased body weights** when compared to controls. The NOAEL for parental, offspring and reproductive toxicity was set at 10000 ppm (666 mg/kg bw/day) (the NOAELs set in previous evaluation RAR (2015) remains).

Overall summary (generational studies): Effects on the offspring consisting of reduced pup weight was observed in individual studies at limit dose level (1000 mg/kg bw/day) and dose levels above limit dose. Delayed sexual maturation (preputial separation) was observed at limit test dose (1000 mg/kg bw/day), and distended caecum was observed at the very high dose of 2000 mg/kg bw/day.

Studies from the open literature

In relevant studies from the open literature, summarised in Table 65, the effects of the active substance glyphosate and Round-up formulations have been investigated. In the following, the focus is on effects after glyphosate treatment. For more detailed study summaries, please see Vol. 3-B.6 (AS), section B.6.6.3 (Reproductive toxicity-Information from public literature).

Manservisi *et al*. (2019) performed a pilot study in Sprague-Dawley rats (8/group) for an extended-one generation study (OECD 443). In this study the F0 female breeders received the test item from gestation day (GD) 6 to the end of lactation, while the offspring (F1) continued to be exposed after weaning for an additional 6 or 13 weeks. The test item, glyphosate (G) (> 99.5% pure), was diluted in drinking water to achieve glyphosate dose of 1.75 mg/kg bw/day (the US Acceptable Daily Intake). The endpoints analysed in the study were body weight, water and food consumption, gestational parameters, litter parameters, landmarks of sexual development, estrous cyclicity, gross and histopathology of reproductive and endocrine tissues, sperm parameters and serum and plasma hormone levels. Reproductive parameters remained to be unaffected by glyphosate exposure at 1.75 mg/kg bw/day. The **anogenital distance (AGD) on PND 4 was statistically significantly increased** in males. Furthermore, increased TSH level in plasma was reported in male animals at this dose level.

In the study by Panzacchi *et al*. (2018) Sprague-Dawley rats were orally via drinking water exposed to 1.75 mg/kg bw/day starting from prenatal life, i.e. gestational day (GD) 6 of their mothers. One cohort was continuously dosed until sexual maturity (6-week cohort) and another cohort was continuously dosed until adulthood (13-week cohort). The endpoints investigated were mortality, body weight, water and food consumption, and clinical signs in dams and offspring and litter data. Survival, body weights, food and water consumption of rats were not affected by the treatment with glyphosate. No clinical changes were observed in the animals of the dosed groups. Furthermore, litter sizes were fully comparable among groups.

In the study by Pham *et al.* (2019), **sperm-depleted seminiferous tubuli** were reported in 35 days old Swiss mice exposed to 5 mg glyphosate/kg bw/day in drinking water from embryonic day 0.5 to 20 days post-partum. No similar effect was observed in the same study in mice sacrificed at later time points. Further, no dose response was observed since the effect was only seen in the mid-dose group. The study is reliable with restrictions because of the following reasons: small group size, limited description of the study conditions and the results. Thus, findings in this study are not given appropriate weight.

Overall summary (open literature): A review of available published literature did not provide conclusive evidence that glyphosate exposure negatively affects reproduction. The studies from the open literature were considered reliable with restrictions.

2.6.6.3.2 Comparison with the CLP criteria regarding effects on or via lactation

According to the CLP Guidance Table 3.7.1(b) a substance should be classified for lactation effects when the following applies:

"(a) human evidence indicating a hazard to babies during the lactation period; and /or
(b) results of one or two generation studies in animals which provide clear evidence of adverse effect in the offspring due to transfer in the milk or adverse effect on the quality of the milk; and/or
(c) absorption, metabolism, distribution and excretion studies that indicate the likehood that the substance is present in potentially toxic levels in breast milk."

No data is available to address criteria (a) and (c).

As **a conclusion**, available data did not provide clear evidence of adverse effect in the offspring due to transfer in the milk.

2.6.6.4 Conclusion on classification and labelling for reproductive toxicity

No classification and labelling of glyphosate for reproductive or developmental effects is proposed.

2.6.7 Summary of neurotoxicity

Table 66: Summary table of animal studies on neurotoxicity

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results: - NOAEL/LOAEL - target tissue/organ -critical effect at LOAEL**	**Reference**
OECD 424 (acute neurotoxicity), GLP Deviations: none Study acceptable Rat, Alpk:APfSD, 10/sex/dose	Glyphosate, batch P24, purity 95.6% Single oral dose by gavage at 0, 500, 1000, 2000 mg/kg bw	<u>2000 mg/kg bw:</u> Mortality in 1 female. Clinical signs in this animal consisting of decreased activity, subdued behaviour, hunched posture, sides pinched in, tip-toe gait and/or hypothermia. 3/10 females also showed subdued behaviour, decreased activity, hunched posture and/or hypothermia on the day of treatment, but recovered at day 2. No effect on neurotoxic parameters and histopathological evaluation. NOAEL for systemic effects: 1000 mg/kg bw/day No evidence for neurotoxicity up to the highest dose	[redacted]/P/4866, 1996
OECD424 (sub-chronic neurotoxicity), GLP Deviations: None Study acceptable Rat, Sprague-Dawley, 10/sex/dose	Glyphosate, batch H05H016A, purity 95.7% 90-day dietary dose at 0, 1000, 5000 or 20000 ppm (equal to 0, 77, 395, or 1499 mg/kg bw/day in males and 0, 78, 404, or 1555 mg/kg bw/day in females	<u>20000 ppm:</u> Reduced body weight in males (-12%) Reduced body weight gain in males (-15%) Reduced food consumption in males during week 1-3 (up to -17%) NOAEL for systemic effects: 5000 ppm No evidence for neurotoxicity up to the highest dose	2060-0010, 2006
OECD424 (sub-chronic neurotoxicity), GLP Deviations[1] Study acceptable but with restrictions	Glyphosate, batch P24, purity 95.6% 13-week dietary dose at 0, 2000, 8000 or 20000 ppm (equal to 0, 155.5, 617.1 and 1546.5	<u>20000 ppm</u>: Reduced body weight gain in males (-12%) NOAEL for systemic effects: 8000 ppm No evidence for neurotoxicity up to the highest dose	[redacted]/P/4867, 1996

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results: - NOAEL/LOAEL - target tissue/organ -critical effect at LOAEL**	**Reference**
Rat, Alpk:APfSD, 12/sex/dose	mg/kg bw/day for males, and 0, 166.3, 672.1 and 1630.6 mg/kg bw/day for females)		
OECD418 (acute delayed neurotoxicity), GLP Deviations[2] Study acceptable but with restrictions Chicken, Lohmann Brown, 20/females/ Glyphosate/group 12/females/control groups	Glyphosate, batch P24, purity 95.6% Single oral dose by gavage at 0 or 2000 mg/kg bw. Positive control: tri-ortho-cresyl phosphate at 1000 mg/kg bw	No adverse effect observed. NOAEL for acute delayed neurotoxicity and systemic toxicity is 2000 mg/kg bw, the highest dose tested.	[redacted]/C/3122, 1996
OECD 419 (28-day delayed neurotoxicity), GLP Deviations[3] Study unacceptable Chicken, gallus domesticus, 3/females/dose	Glyphosate, batch and purity not reported. Oral daily doses at 0, 250, 500 and 1000 mg/kg bw/day	1000 mg/kg bw/day Reduced body weight (-18%) Minor haematological changes of unclear relevance due to lack of statistical analysis and low number of animals. No indication of neurotoxicity No NOAEL derived as study is considered unacceptable.	CA 5.7.2/002 (no report number provided), 1987

[1] Functional tests were conducted at -1, 5, 9 and 14 instead of prior to exposure, during the first and second week and monthly thereafter

[2] Neuropathy target esterase (NTE) activity was measured in 3 animals instead of 6.

[3] Number of animals too low (3 instead of 12 per group), only 21 day exposure, no post treatment observations conducted, no NTE activity measured.

In an acute neurotoxicity study (report number [redacted]/P/4866) groups of 10 male and female Alpk:APfSD rats per sex were administered single oral doses of 0, 500, 1000 or 2000 mg/kg bw glyphosate acid by gavage. The study was conducted in accordance with OECD 424.

Clinical signs of toxicity (including decreased activity, subdued behaviour, hunched posture, sides pinched in, tip-toe gait and/or hypothermia) occurred during Day 1 but were limited to 3 females at approximately 6 hours post treatment, in the highest dose group (2000 mg/kg/day). One of these females was subsequently found dead on Day 2. These clinical signs were considered to reflect general toxicity associated with the administration of high dose levels of glyphosate acid. Slight reductions in food consumption, without any associated effects on body weight, were also observed during Week 1 for both sexes in the highest dose group. Quantitative assessment of

neurotoxic parameters and histopathological evaluation of the central and peripheral nervous system confirmed no neurotoxic potential for glyphosate.

The NOAEL for neurotoxicity was concluded to be 2000 mg/kg bw/day, the highest dose tested. The NOAEL for systemic toxicity was 1000 mg/kg bw/day based on the observed clinical signs and mortality in females.

In a subchronic neurotoxicity study (report # 2060-0010), groups of 10 male and 10 female Sprague-Dawley (Crl:CD® (SD) IGS BR) rats were fed diets containing 0, 1000, 5000 or 20000 ppm glyphosate (equal to a mean achieved dosage of 0, 77, 395, or 1499 mg/kg bw/day in males and 0, 78, 404, or 1555 mg/kg bw/day in females for 90 consecutive days. The study was conducted in accordance with OECD 424.

The only adverse effect observed was a decrease in body weight (-12%), body weight gain (-15%) and food consumption in high dose males (up to -17%). There were no treatment-related changes in neurological parameters.

The NOAEL for neurotoxicity was concluded to be 20000 ppm, the highest dose tested. The NOAEL for systemic toxicity was 5000 ppm (equal to 395 mg/kg bw/day) based on the decrease in body weight (gain) and food consumption in males.

In a second subchronic neurotoxicity study (report # [redacted]/P/4867), groups of 12 male and 12 female Alpk:APfSD (Wistar-derived) rats were fed diets containing 0, 2000, 8000 or 20000 ppm glyphosate acid for 13 weeks. The study was conducted in accordance with OECD 424 with the exception that functional tests were conducted at -1, 5, 9 and 14 instead of prior to exposure, during the first and second week and monthly thereafter. Therefore, the study was concluded to be acceptable but with restrictions.

The only adverse effect observed was decreased body weight gain and food efficiency in high dose males. There were no treatment related changes in neurological parameters.

The NOAEL for neurotoxicity was concluded to be 20000 ppm, the highest dose tested. The NOAEL for systemic toxicity was 8000 ppm (equal to 1546.5 mg/kg bw/day) based on the decrease in body weight gain in males.

In an acute delayed neurotoxicity study (report # [redacted]/C/3122), 20 female hens were given a single dose of glyphosate acid at a level of 2000 mg/kg bw and observed for the following 21/22 days. Twelve negative control (vehicle, distilled water) and twelve positive control (tri-ortho-cresyl phosphate, TOCP, 1000 mg/kg bw) hens were also dosed. The study was conducted in accordance with OECD 418 with the exception that NTE activity was measured in 3 animals per group instead of 6. The study was therefore concluded to be acceptable with restriction.

No adverse effects were noted in the study and the NOAEL was concluded to be 2000 mg/kg bw, the highest dose tested.

A repeated dose delayed neurotoxicity study (reported as CA 5.7.2/002) in hens was also provided which did not give an indication of a neurotoxic potential for glyphosate. However, the number of animals in the study was too low (n=3), NTE activity was not measured, the exposure period too short (21 days) and no post treatment observation was conducted. Therefore, the study was concluded to be unreliable.

Because there were no indications for a neurotoxic potential of glyphosate in acute and subchronic neurotoxicity studies and no evidence of neurological disturbances in pups in the multi-generation studies in rats, a developmental neurotoxicity study (DNT) is not needed.

Three public literature studies were submitted (refer to Vol 3 CA Section B.6.7 and B.6.9). One study (Martinez *et al.*, 2019; B.6.7.3.1) evaluated that effect of glyphosate and its metabolite AMPA on the blood-brain barrier *in vitro* which both did not indicate a clear neurotoxic potential. The authors concluded that while some minimal effects were observed they occurred at concentrations significantly higher than baseline exposure levels. The study by Chorfa *et al*., 2013 (B.6.7.3.3) evaluated the effect of glyphosate and other pesticides on α-syn levels in human neuroblastoma (SH-SY5Y) and melanoma (SK-MEL-2) cell lines. Glyphosate did not have any impact on the endpoints measured in this study. The publication by Martinez *et al.* 2018 (B.6.7.3.2) did observe an effect of glyphosate on neurotransmitter levels in rat brain regions after oral dosing by gavage at 35, 75, 150, 800 mg/kg bw/day for 6 days. However, the study was a non-guideline *in vivo* study with no concurrent positive control and no positive and negative historical controls included and it is therefore difficult to interpret the biological relevance of the observed changes. A fourth study (Ait-Bali, 2020; B.6.7.3.4) investigated behavioural, neurochemical and molecular changes after pre- and post-natal exposure of mice to a Roundup formulation (glyphosate concentration: 360 g/l as isopropylamine salt 486 g/l). In this study, groups of 10 female Swiss mice received Roundup by gavage at concentrations of 250 or 500 mg/kg bw/day from gestational day 0 (G0) to postnatal day 21 (PND21). At postnatal day 60 (PND60) the downstream effects at the behavioural, neurochemical and molecular levels were examined. The results show that pre- and neonatal exposure to the Roundup formulation impairs fertility and reproduction parameters as well as maternal behaviour of exposed mothers. In offspring, exposed animals show a delay in innate

reflexes and a deficit in motor development. At the adult age, exposed animals showed a decrease of locomotor activity, sociability, learning and short- and long-term memory associated with alterations of cholinergic and dopaminergic systems. The formulation also activated microglia and astrocytes, sign of neuroinflammation event in the medial prefrontal cortex and hippocampus. At the molecular level, a downregulation of BDNF expression and an up-regulation of TrkB, NR1 subunit of NMDA receptor as well as TNFα were found. As in the study only a formulation - and not the active substance glyphosate alone - was investigated, any effect of the co-formulant(s) cannot be excluded. Therefore, the study is considered as supplementary data. The study is reliable with restrictions because of the following reasons: formulation used, only two doses tested, no OECD guideline followed, no GLP status stated, no positive controls used and no HCD provided.

There are three isolated case report of Parkinson's disease developing in individuals with a history of glyphosate product exposure. In one case, Parkinson's disease of relatively acute onset was diagnosed 6 months following incidental dermal exposure to a glyphosate-surfactant product (Barbosa *et al.*, 2001 (B.6.9.8.15)). The second case (Wang *et al.*, 2011 (B.6.9.8.28)) reports the development of Parkinson's of a 44-year old woman who had been employed in a glyphosate manufacturing facility. The third case described a woman who developed transient Parkinsonism that was reportedly reversed by the administration of atropine and pralidoxime (Zheng *et al*., 2018 (B.6.9.8.29)). In all instances, there is no evidence for causation other than a history of prior exposure. In the last case, it is notable that the patient recovered with the treatment for organophosphate exposure, which suggests a completely different aetiology as glyphosate does not require treatment with anticholinergic agents. No other human or animal data support the contention that Parkinson's disease results from exposure to glyphosate, even following massive ingestion or prolonged exposure.

During the previous assessment, several additional public literature studies were evaluated. These were not included in the evaluation of the applicant for the AIR-5 renewal. The applicant is requested to submit these publications together with an evaluation (including a relevance and reliability assessment) and an overall assessment.

The following section is copied from the previous assessment (RAR, 2015) and not re-evaluated yet:

" The main focus of the available studies was on a possible link between an exposure to glyphosate and the development of Parkinson`s disease. This hypothesis but also a link with other neurological diseases was examined in mechanistic studies in different systems such as *Caenorhabditis elegans* worms, in rats or cell cultures (Astiz *et al.*, 2009, ASB2012-11549; Negga *et al.*, 2011, ASB2012-11923; Gui *et al.*, 2012, ASB2012-11835). Sometimes, positive evidence was reported but these findings are not considered relevant when the extremely huge database in laboratory animals with no evidence of neurotoxicity and the absence of suggestive epidemiological data in humans is taken into consideration. Chorfa *et al.* (2013, ASB2014-9328) studied the effects of four pesticides (paraquat, rotenone, maneb and glyphosate) on different molecular events in cell lines which are considered to be related to Parkinson`s disease. Three of the four pesticides triggered molecular events involved in Parkinson`s disease but glyphosate was the only one that did not exhibit such an effect.

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

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

Cattani *et al.* (2014, ASB2014-3919) studied neurotoxic effects of the formulation Roundup in the hippocampus of immature rats following acute (30 min) and chronic (during pregnancy and lactation) exposure. Results showed that acute exposure to Roundup increased the Ca^{2+} influx leading to oxidative stress and neuronal cell death. It was hypothesised that Roundup might lead to excessive extracellular glutamate levels and to glutamate excitotoxicity

and oxidative stress in rat hippocampus. For re-evaluation of glyphosate, these findings obtained with a formulation are without relevance. Furthermore, they are not supported by the huge database of toxicological studies in rats and other species.

Epidemiology

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

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

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

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

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

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

- end of previous assessment (RAR, 2015) -

Overall, the available information does not indicate a neurotoxic potential for glyphosate.

2.6.8 Summary of other toxicological studies

2.6.8.1 Toxicity studies of metabolites and impurities

2.6.8.1.1 Studies with AMPA

Table 2.6.8.1.1-1 Summary table of toxicokinetic studies of the metabolite AMPA

Method	Results	Remarks	Reference
No guideline stated Not GLP Deviations: reporting deficiencies (batch, purity) one dose tested Study unacceptable Wistar rats, male, number unknown AMPA, batch not reported, purity not reported Single oral dose at 6.7 mg/kg bw	Limited absorption and fast elimination (20% in urine, 74% in faeces) AMPA is excreted unmetabolized.	Termination at 120 hours post dosing.	CA 5.8.1/001; Report no. 303

Table 2.6.8.1.1-2 Summary table of animal studies on acute oral toxicity of the metabolite AMPA

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Value LD_{50}	Reference
OECD 401, GLP Deviations: first observation 1 hour after exposure instead of 30 min Study acceptable	Mice, Crj:CD-1, Males and females, 5/sex/dose	AMPA, Batch: A-960719, Purity: 99.33%	5000 mg/kg bw/d, Single oral dose	> 5000 mg/kg bw/d (males and females)	CA 5.8.1/002; Report no. [redacted] 96-0075
In accordance with OECD 401, GLP No deviations Study acceptable	Rat, Sprague-Dawley, Males and females, 5/sex/dose	AMPA, Batch: 286-JRJ-73-4 Purity: 99.2%	5000 mg/kg bw/d, Single oral dose	> 5000 mg/kg bw/d (males and females) Some clinical signs observed	CA 5.8.1/003; Report no. 8763
In accordance with OECD 401, GLP Deviations: control group included; acclimatization period of 2 days Study acceptable	Rat, Wistar, Males and females, 5/sex/dose	(N-methyl-N-phosphonomethyl)glycine, Batch: 244-KMA-9.1, Purity: 97.3%	5000 mg/kg bw/d, Single oral dose	> 5000 mg/kg bw/d (males and females) Some clinical signs observed	CA 5.8.1/004; Report no. 12837
In accordance with OECD 401, GLP Deviations: rats were fasted for up to 24 hours	Rat, Wistar-derived albino, Alpk:APfSD, Males and females, 5/sex/dose	Aminomethyl phosphonic acid, Batch: Y06384/001/001, Purity: 100 % (assumed)	5000 mg/kg bw/d, Single oral dose	> 5000 mg/kg bw/d (males and females) Some clinical signs observed	CA 5.8.1/005; Report no. [redacted]/P/2266

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Value LD_{50}	Reference
Study acceptable					
No guideline followed, non-GLP Deviations[1] Study not acceptable	Rat, no further specifications on the strain, Males and females, 5/dose (mixed gender groups)	CP 50435, Batch: XHD – 16, Purity: not specified	5010, 6310, 7940, 10000 mg/kg bw, Single oral dose	Not determined due to unacceptability of the study	CA 5.8.1/006; Report no. Y-73-19

[1] Doses were not in line with OECD 401, animals were observed for 7 days only, weight not recorded. Purity and stability of the test substance not provided, lack of details on test species and housing conditions.

Table 2.6.8.1.1-3 Summary table of animal studies on acute dermal toxicity of the metabolite AMPA

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Value LD_{50}	Reference
OECD 402, GLP Deviations: animals too young at exposure, application under occlusive conditions Study acceptable	Rat, CD/Crl: CD, Males and females, 5/sex/dose	AMPA, Batch: FA005563, Purity: 98.0%	2000 mg/kg bw/d, Single dermal dose	> 2000 mg/kg bw/d (males and females)	CA 5.8.1/007; Report no. 16168/02
OECD 402, GLP Deviations: temperature and rel. humidity, timepoint of clinical observations on the day of treatment not specified, specific endpoints measured not reported, application under occlusive conditions Study acceptable	Rat, Sprague-Dawley, Males and females, 5/sex/dose	AMPA, Batch: 286-JRJ-73-4, Purity: 99.2%	2000 mg/kg bw/d, Single dermal dose	> 2000 mg/kg bw/d (males and females)	CA 5.8.1/008; Report no. 8764

Table 2.6.8.1.1-4 Summary table of animal studies on skin and eye irritation of the metabolite AMPA

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Results - Observations and time point of onset[2] - Mean scores/animal - Reversibility	Reference
No guideline followed, non-GLP[1] Skin irritation study Deviations[2] Study not acceptable	Rat, no further specifications on the strain, Males and females, Number of animals not reported	CP 50435, Batch: XHD – 16, Purity: not specified	0.5 g test material, 24 hours	No skin reactions were observed after 24 hours of exposure during the 7-day observation period. No further observations were reported	CA 5.8.1/009; Report no. Y-73-19
No guideline followed, non-GLP[1] Eye irritation study Deviations[3] Study not acceptable	Rat, no further specifications on the strain, Males and females, Number of animals not reported	CP 50435, Batch: XHD – 16, Purity: not specified	100 mg test material, Duration of exposure not reported	Signs of oedema, erythema and discharge in all animals; resolved until 120 hours after treatment.	CA 5.8.1/010; Report no. Y-73-19

[1] Study conducted prior to implementation of GLP principles.
[2] Exposure lasted 24 hours instead of 4, experimental procedures not well described, purity and stability not reported, details on test species and housing conditions lacking.
[3] Individual scores for eye irritation not reported, purity and stability of the test item missing, details on test species and housing conditions are lacking.

Table 2.6.8.1.1-5 Summary table of animal studies on skin sensitisation of the metabolite AMPA

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels duration of exposure	Results	Reference
OECD 406, GLP Deviations: animals too young at exposure Study acceptable	Guinea pig, Dunkin-Hartley, Males, 10/test group, 5/negative control, 20/positive control	AMPA, Batch: FA005563, Purity: 98%	1st induction: 5%, intracutaneous 2nd induction: 50%, topical, SLS-treated skin, Challenge: 50%, topical	No skin reactions were observed 24 or 48 h after the challenge treatment with AMPA in the control or test group	CA 5.8.1/011, Report no. 16169/02
OECD 406 No deviations Study acceptable	Guinea pig, Dunkin-Hartley, Females, 20/test group, 20/negative control, 20/positive control	AMPA, Batch: 286-JRJ-73-4, Purity: 99.2%	1st induction: 10%, intracutaneous 2nd induction: 25%, topical, SLS-treated skin, Challenge: 25%, topical	No skin reactions were observed 24 or 48 h after the challenge treatment with AMPA in the control or test group	CA 5.8.1/012, Report no. 8765

Table 2.6.8.1.1-6 Summary table of animal studies on repeated dose toxicity (short-term and long-term toxicity) STOT RE (specific target organ toxicity - repeated exposure) of the metabolite AMPA

Method, guideline, deviations[1] if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results - NOAEL/LOAEL - adverse effects	Reference
No guideline followed Not GLP Deviations: Compared to OECD 407 absence of housing conditions, certificate of analysis, haematology, clinical chemistry, gross necropsies and histopathology Study unacceptable Rat, Wistar, male and female, 5/sex/dose	AMPA, batch not reported, purity 99.9% 14-day, dietary dose Doses of 1000, 2000, 4000 mg/kg bw/day	1000 and 2000 mg/kg bw/day No adverse effects observed 4000 mg/kg bw/day Decreased body weight gain in males; Decreased food consumption in males and females; Red coloured material in urine in one male.	CA 5.8.1/013, Report No. 401-026
No guideline followed GLP Deviations: Preliminary study; absence of haematology, clinical chemistry, gross pathology, limited histopathology and organ weights Acceptable as preliminary study only Rat, Sprague-Dawley, male and female, 5/sex/dose	AMPA, batch 286-JRJ-73-4, purity 99.2% Vehicle: 0.5% carboxymethylcellulose in distilled water 28-day, cannula dosing (10 mL/kg bw) Doses of 10, 100, 350 and 1000 mg/kg bw/day	10 and 100 mg/kg bw/day No adverse effects observed 350 mg/kg bw/day Increased kidney weight in males 1000 mg/kg bw/day Decreased body weight gain in females; Increased kidney weight in males NOAEL: 100 mg/kg bw/day LOAEL: 350 mg/kg bw/day	CA 5.8.1/014, Report No. 148-GLY
OECD 409 (1981) GLP Deviations: absence of ophthalmology, urinalysis and limited organ weights and histopathology Study unacceptable Dog, Beagle, male and female, 2/sex/dose	AMPA, batch HET-9001-1463T, purity 94,38% 28-day, gelatine capsule administration Doses of 10, 30, 100, 300 and 1000 mg/kg bw/day	10, 30 and 100 mg/kg bw/day No adverse effects observed 300 mg/kg bw/day Decreased reticulocyte count, haematocrit and haemoglobin in females 1000 mg/kg bw/day Increased diarrhoea in males and females Decreased erythrocyte count, haematocrit in males and females Increased reticulocyte count in males and females	CA 5.8.1/015, Report No. [redacted]-11127

OECD 408 (1981) GLP Deviations: absence of FOB, platelet count, cholesterol, HDL, LDL, urea, only 2 liver enzymes measured, T4, T3, TSH and vaginal smears not investigated. Study acceptable but with restrictions Rat, Sprague-Dawley, male and female, 10/sex/dose	AMPA, batch 286-JRJ-73-4, purity 99.2% Vehicle: 0.5% carboxymethylcellulose in distilled water 90-day, cannula dosing (10 mL/kg bw) Doses of 10, 100 and 1000 mg/kg bw/day	10, 100 and 1000 mg/kg bw/day No adverse effects observed NOAEL: ≥ 1000 mg/kg bw/day	CA 5.8.1/016, Report No. 7866
Performed according to OECD 408 Not GLP Deviations: absence of ophthalmoscopy, FOB, blood clotting time/potential, sodium, potassium, HDL, LDL, urea, creatinine, T4, T3, TSH, vaginal smears, limited organ weights, macroscopy and histopathology. Study acceptable but with restrictions Rat, Charles River CD, males and females, 20/sex/dose	AMPA, batch XHI 45 and XHI-136, purity of XHI-136 99.96% 90-day, dietary dose Doses of 400, 1200 and 4800 mg/kg bw/day	400 mg/kg bw/day No adverse effects observed 1200 mg/kg bw/day Increased urothelial hyperplasia of urinary bladder in males and females 4800 mg/kg bw/day Decreased food consumption and body weight in males; Increased LDH in males and females; Increased urothelial hyperplasia of urinary bladder in males and females; Increased hyperplasia of kidney epithelium in males and females. NOAEL: 400 mg/kg bw/day LOAEL: 1200 mg/kg bw/day	CA 5.8.1/017, Report No. 401-050
OECD 409 (1981) GLP Deviations: gall bladder, uterus, thymus, spleen and heart not weighed Study acceptable Dog, Beagle, males and females, 5/sex/dose	AMPA, batch PIT-9008-2407-T, purity 87.8% 90-day, gelatine capsule administration Doses of 10, 30, 100 and 300 mg/kg bw/day (corrected for purity: 8.8, 26.3, 87.8 and 263 mg/kg bw/day)	8.8, 26.3, 87.8 and 263 mg/kg bw/day No adverse effects observed NOAEL: 263 mg/kg bw/day	CA 5.8.1/018, Report No. [redacted]-50173

Table 2.6.8.1.1-7 Summary table of genotoxicity/germ cell mutagenicity tests *in vitro* of the metabolite AMPA

Method, guideline, deviations if any	**Test substance**	**Relevant information about the study including rationale for dose selection (as applicable)**	**Observations /Results**	**Reference**
OECD 471/472, GLP Deviations:	AMPA Batch: A-960719,	Ames test, ±S9 mix for metabolic activation, 313-5000 µg/plate, pre-incubation assay,	Number of revertants of the positive and negative control was in the expected range (although no HCD were included in the study report). No	CA 5.8.1/019, Report no. IET 96-0076

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations /Results	Reference
No HCD included, 2-AA as sole positive control in the presence of metabolic activation, parameters in both experiments identical Study acceptable but with restrictions	Purity: 99.33%	Strains: *S. typhimurium* TA 100, TA 98, TA 1535 and TA 1537 and *E. coli* WP2 uvrA	relevant increase observed in any experiment with the tested concentrations neither in the presence nor absence of metabolic activation. AMPA is considered non-mutagenic under the conditions of this assay.	
OECD 471, GLP Deviations: No HCD included, 2-AA as sole positive control in the presence of metabolic activation, only four strains tested Study acceptable but with restrictions	AMPA Batch: 286-JRJ-73-4, Purity: 99.2%	Ames test, ±S9 mix for metabolic activation, 303-5000 µg/plate, standard plate and pre-incubation assay, Strains: *S. typhimurium* TA 100, TA 98, TA 1535 and TA 1537	Number of revertants of the positive and negative control was in the expected range (although no HCD were included in the study report). No relevant increase observed in any experiment with the tested concentrations neither in the presence nor absence of metabolic activation. Only exception for TA 1535 at 630 µg/plate in the absence of S9 mix, which is considered incidental only. AMPA is considered non-mutagenic under the conditions of this assay.	CA 5.8.1/020, Report no. 13269
OECD 471, GLP Deviations: No HCD included, 2-AA as sole positive control in the presence of metabolic activation, evaluation of cytotoxicity and precipitation not reported, bacterial cell density at treatment not reported, acceptance and evaluation criteria not fully	Aminomethyl phosphonic acid, Batch: 48F-3893, Purity: > 99%	Ames test, ±S9 mix for metabolic activation, 1.6-5000 µg/plate, standard plate assay, Strains: *S. typhimurium* TA 100, TA 98, TA 1535, TA 1537 and TA 1538 and *E. coli* WP2 uvrA	Number of revertants of the positive and negative control was in the expected range (although no HCD were included in the study report). Statistically significant increase in the mean number of revertant colonies for all strains at single test item concentrations in the presence or absence of metabolic activation. Some statistically significant increases were observed in both experiments. Although no clear dose-response relationship is observed, the biological relevance of the observations cannot be assessed, amongst others because the HCD are missing. In conclusion, the results of the study are considered equivocal.	CA 5.8.1/021, Report no. CTL/P/2206

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations /Results	Reference
according to OECD 471 Study acceptable but with restrictions				
No guideline followed, non-GLP Deviations: Only single experiment, no HCD included, WP2 hcr used, 2-AA as sole positive control, cytotoxicity and precipitation not reported, acceptance and evaluation criteria not specified. Study not acceptable	Aminomethyl phosphonic acid, Batch: not reported, Purity: 99%	Ames test, ±S9 mix for metabolic activation, 10-5000 µg/plate, standard plate assay, Strains: *S. typhimurium* TA 100, TA 98, TA 1535, TA 1537 and TA 1538 and *E. coli* WP2 hcr	The test item did not induce a statistically significant increase in the number of revertants in any of the tester strains at any concentration when compared to solvent controls, neither in the presence nor in the absence of metabolic activation. Due to multiple deviation, however, the study is not accepted for evaluation.	CA 5.8.1/022, Report no. ET-80-402
OECD 471, GLP Deviations regarding the selection of positive control, however, no impact on the study outcome expected. Study acceptable	AMPA (batch: 107785, purity: 99.2%)	Reverse Mutation Assay (Ames Test) using *S. typhimurium* (TA 98, TA 100, TA 1535 TA 1537) and *E. coli* WP2 uvrA; 1.5-5000 µg/plate, ±S9 standard plate and pre-incubation test	AMPA did not induce a biologically relevant increase in the number of revertants under any of the test conditions. Under the conditions of this test, AMPA is not considered to be mutagenic with and without metabolic activation.	CA 5.8.1/045, Report no. 8442150
OECD 476, GLP Multiple deviations compared to OECD 490; a.o. cytotoxicity based on cloning efficiencies, not on RTG/SG/RSG,	AMPA, Batch: 286-JRJ-73-4, Purity: 99.2%	Mouse lymphoma assay, ±S9 mix for metabolic activation, 310-5000 µg/mL (1st experiment), 630-5000 µg/mL (2nd experiment)	Mutation frequencies of the positive and negative control were within the expected range (although no HCD were included in the study report). No statistically significant increase in mutation frequency observed upon treatment with AMPA in both experiments at any of the tested concentrations, neither in the presence, nor in the absence of metabolic activation. AMPA is considered non-mutagenic under the conditions of this assay.	CA 5.8.1/023, Report no. 13270

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations /Results	Reference
number of treated cells too low, no HCD included. Study acceptable but with restrictions				
OECD 476 (2016) GLP Study acceptable	AMPA (batch: 107785, purity: 99.2%)	Chinese hamster V79 cells, HPRT locus, ±S9, 34.69-1110 µg/mL (eq. to 10 mM)	Under the conditions of this *in vitro* mammalian gene mutation assay, AMPA is considered negative for mutagenicity.	CA 5.8.1/046, Report no. 8441963
OECD 487 (2016) GLP Study acceptable (minor deviation in extended treatment schedule; no impact on study outcome)	AMPA (batch: 107785, purity: 99.2%)	Micronucleus assay in human peripheral lymphocytes, ±S9, 34.69-1110 µg/mL (eq. to 10 mM)	Under the conditions of this *in vitro* micronucleus assay, AMPA did not induce chromosome breaks and/or gain or loss (negative for clastogenicity and aneugenicity).	CA 5.8.1/047, Report no. 8442149
OECD 482, GLP No deviations, however, OECD 482 was deleted in 2014 and the UDS assay is no longer a standard method. Study **not** acceptable	AMPA, Batch: A015478701, Purity: 99.9%	*In vitro* unscheduled DNA synthesis (UDS) in primary rat hepatocytes, 0.625-10 mM (1st and 2nd experiment)	Mean number of net nuclear grain counts of the positive and negative control were within the expected range of the HCD. Under the conditions of this test, AMPA did not induce unscheduled DNA synthesis in primary rat hepatocytes *in vitro*.	CA 5.8.1/024, Report no. IPL-R 020625
Similar to OECD 482, GLP Deviations: no HCD included, OECD 482 was deleted in 2014 and the UDS assay is no longer a standard method.	AMPA, Batch: HET-9001-1463-T, Purity: 94.38%	*In vitro* unscheduled DNA synthesis (UDS) in primary rat hepatocytes, 5-5000 µg/mL (1st and 2nd experiment)	Mean number of net nuclear grain counts of the positive and negative control were within the expected range of the HCD (although no HCD were included in the study report). Under the conditions of this test, AMPA did not induce unscheduled DNA synthesis in primary rat hepatocytes *in vitro*.	CA 5.8.1/025, Report no. SR-91-234

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations /Results	Reference
Study not acceptable				
Open literature studies (refer to Vol 3 CA B.6.4)				
Non-guideline study Non-GLP (literature study) Study supportive	Glyphosate and AMPA (purities not reported)	*In vitro*: induction of DNA double strand breaks (immunofluorescence of phosphorylated H2AX foci); induction of proteins involved in DNA recombination (Western blot; glyphosate only) Exposure of human peripheral blood lymphocytes to 0.4-50 µM glyphosate for 1.5 h. Assays without metabolic activation only.	Induction of DNA double strand breaks: negative for AMPA. Limitations: Purity not reported, HCD not reported, test substance stability and test concentration not analytically verified. In addition, as no guideline or validation of this assay is available the results are difficult to interpret.	Suárez-Larios, K. *et al.*, 2017 KCA 5.8.1/048 (Evaluated at KCA 5.4/009)
Non-guideline (although MN assay similar to OECD GL 487) Non-GLP (literature study) Study supportive	Glyphosate and AMPA (purities not reported)	*In vitro* micronucleus assay (±S9 and after photoactivation); intracellular ROS determination Exposure of CHO-K1 cells to 5-100 µg/mL glyphosate (±S9, +irradiation) and 0.005-0.01 µg/mL (-S9), 0.1-5 µg/mL (+S9), and 0.00005-0.001 µg/mL (+irradiation) AMPA for 3 h.	Micronucleus assay (AMPA): positive (-S9; ≥ 0.01 µg/mL); positive (+S9; ≥ 1 µg/mL); positive (+irradiation ≥ 0.0005 µg/mL) ROS formation (AMPA): elevated Main deviations from OECD GL 487: no continuous treatment schedule, test chemicals not characterized, no positive control or HCD (lab proficiency not proven), test substance stability and test concentration not analytically verified.	Roustan *et al.,* 2014 KCA 5.8.1/049 (Evaluated at KCA 5.4/011)

Table 2.6.8.1.1-8 Summary table of genotoxicity/mutagenicity tests in mammalian somatic or germ cells *in vivo* of the metabolite AMPA

Method, guideline, deviations if any	Test substance	Relevant information about the study (as applicable)	Observations/Results	Reference
OECD 474, GLP Multiple deviations, a.o.: only 1000 PCE per animal were scored, % of PCE among total erythrocytes scored for 200 erythrocytes only, no HCD included	AMPA, Batch: 286-JRJ-73-4, Purity: 99.2%	*In vivo* bone marrow micronucleus test, mice (m/f), 5000 mg/kg bw, single oral dose	Incidence of micronuclei in controls in accordance with the HCD (although HCD were not included in the study report). No statistically significant increase in the frequency of micronucleated PCEs (mPCEs) of test item-treated animals compared to the negative control. Bone marrow exposure sufficiently demonstrated. Under the conditions of this test, AMPA was negative for clastogenic effects.	CA 5.8.1/026, Report no. 13268

Method, guideline, deviations if any	Test substance	Relevant information about the study (as applicable)	Observations/Results	Reference
Study acceptable but with restrictions				
Similar to OECD 474, GLP Deviations, a.o.: i.p. treatment, only 1000 PCE per animal were scored, no HCD included for the positive control Study acceptable	AMPA, Batch: HET-9001-1463T (Test sample T900031), Purity: 94.38%	*In vivo* bone marrow micronucleus test, mice (m/f), 100, 500, and 1000 mg/kg bw, single i.p. dose	Incidence of micronuclei in negative control in accordance with the HCD. No statistically significant increase in the frequency of micronucleated PCEs (mPCEs) of test item-treated animals compared to the negative control.	CA 5.8.1/027, Report no. [redacted]-13243
Open literature studies (refer to Vol 3 CA B.6.4)				
Non-guideline (although comet assay shows some similarity to OECD GL 489) Non-GLP (literature study) Study supportive	Glyphosate and AMPA (Purity 96% and 99%, respectively)	*In vivo* comet assay (blood, liver) and determination of oxidative stress parameters (TBARs, SOD and CAT activity in liver, kidney, lung, and heart) 14-Day exposure of Balb C mice (sex unknown, 6 animals/ group) to 40 or 400 mg/kg bw/day glyphosate and 100 mg/kg bw/day AMPA via drinking water.	Comet assay: positive in blood and liver for AMPA at 100 mg/kg bw. Oxidative stress parameters: A non-statistically significant decrease in SOD activity was observed in all tissues of animals treated with 100 mg/kg bw AMPA but no effect of AMPA was found on CAT activity. Deviations/limitations: Description of the method very limited, sex of animals unknown, number of doses and scored nucleoids not in line with OECD TG 489 (only one dose tested), no positive controls or HCD (lab proficiency not proven).	Mañas *et al.*, 2013 KCA 5.8.1/050 (Evaluated at B.6.4.4.12)

Table 2.6.8.1.1-9 Summary table of animal studies on adverse effects on development of the metabolite AMPA

Method, guideline, deviations[1] if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results - NOAEL/LOAEL (for parent, offspring and for developmental effects) - adverse effects	Reference
Performed according to OECD 414 GLP Deviations: Shorter treatment duration, endocrine disrupting parameters not investigated Study acceptable Rat, Sprague-Dawley, 25 mated females/dose.	AMPA, batch 286-JRJ-73-4, purity 99.2% Vehicle: 0.5% carboxymethylcellulose in distilled water Dosed GD 6-16 by gavage, Sacrifice GD 20 Doses of 100, 350, 1000 mg/kg bw/day.	100, 350, 1000 mg/kg bw/day No adverse effects observed NOAEL (parent, offspring, dev effects): ≥ 1000 mg/kg bw/day	CA 5.8.1/028, Report No. 7891
No guideline followed GLP Deviations: Preliminary study Acceptable as preliminary study only	AMPA, batch HET-9001-1463T, purity 94.38% Vehicle: corn oil	125, 250, 500, 750, 1000 mg/kg bw/day No adverse effects observed NOAEL (parent, offspring, dev effects): ≥ 1000 mg/kg bw/day	CA 5.8.1/029, Report No. [redacted]-50146

Method, guideline, deviations[1] if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results - NOAEL/LOAEL (for parent, offspring and for developmental effects) - adverse effects	Reference
Rat, Sprague-Dawley, 8 mated females/dose	Dosed GD 6-15 by gavage, Sacrifice GD 20 Doses of 125, 250, 500, 750, 1000 mg/kg bw/day.		
OECD 414 (1981) GLP Deviations: Shorter treatment duration, endocrine disrupting parameters not investigated Study acceptable Rat, Sprague-Dawley, 25 mated females/dose	AMPA, batch HET-9001-1463T, purity 94.38% Vehicle: corn oil Dosed GD 6-15 by gavage, Sacrifice GD 20 Doses of 150, 400, 1000 mg/kg bw/day	150 mg/kg bw/day No adverse effects observed 400 mg/kg bw/day Increased mucoid faeces, hair loss, soft stool. 1000 mg/kg bw/day Increased mucoid faeces, hair loss, soft stool. Decreased bodyweight gain GD 12-16 Decreased food consumption. Reduction in foetal weight (-6%) maternal NOAEL: 150 mg/kg bw/day offspring NOAEL: 400 mg/kg bw/day developmental NOAEL: ≥ 1000 mg/kg bw/day	CA 5.8.1/030, Report No. [redacted]-50159

Short summary on toxicological information on AMPA:

A non-GLP, non-guideline ADME study which showed significant limitations, including a lack of detail on the analytical methods used and limited details on the results. Overall, the study was considered to unacceptable but showed that oral absorption was limited based on urinary excretion (20%) and that AMPA is excreted unchanged.

Five acute oral toxicity studies were provided. Of these studies four were considered to be acceptable. All studies indicated a low acute oral toxicity with LD_{50} values above 5000 mg/kg bw/day. Clinical signs were noted in the studies consisting of piloerection, diarrhoea, subdued behaviour, hunched appearance and soiled anal, perigenital areas, signs of urinary incontinence, ungroomed, reduced splay reflex and pinched abdomen.

The acute dermal toxicity of AMPA was assessed in two studies which were both considered to be acceptable. The dermal LD_{50} values of all four studies were >2000 mg/kg bw/day.

A skin irritation and an eye irritation study are available on AMPA but neither study is acceptable. However, such studies are not required for metabolites and therefore no further information is requested for these endpoints.

Two skin sensitisation studies were submitted of which were both considered to be acceptable. Under the test conditions AMPA did not show a sensitising potential.

Four Ames tests are available for AMPA of which one was concluded to be unacceptable and the other three as acceptable but with restrictions. One new additional study was submitted by the applicant (study report no. 8442150). In three of the four acceptable studies, including the recent fully guideline-compliant study, AMPA was considered to be non-mutagenic. The other study was concluded to be equivocal as some statistically significant increases were observed without a dose response which were difficult to assess due to the lack of historical control data. AMPA was negative in two *in vitro* mammalian gene mutation studies with and without metabolic activation. The first study was an mouse lymphoma assay, which was considered acceptable but with restrictions due to several deficiencies. The second study was a new, fully guideline-compliant and acceptable gene mutation study (HPRT assay in Chinese hamster V76 cells; study report no. 8441963). Overall, AMPA is concluded to be negative for gene mutations *in vitro*.
Two *in vitro* UDS studies were available which were both negative. However, these studies are no longer considered acceptable due to sensitivity issues of the study method.
The applicant submitted a new, fully guideline-compliant and acceptable *in vitro* micronucleus study (study report no. 8442149) which was negative. AMPA was also negative for clastogenic and aneugenic effects in two *in vivo*

micronucleus studies. In both studies, the number of scored PCE was too low compared to the current OECD test guideline although the study was in line with the OECD test guideline valid at the time of conduct of the study (1983). In the first study bone marrow exposure was proven as a decrease in PCE/NCE ratios was observed. In the second study at slightly lower dose levels (up to 1000 mg/kg bw), no increase in the frequency of micronucleated PCEs was observed. However, no direct evidence of bone marrow exposure was available as no effect on PCE/NCE ratio was observed. Systemic toxicity was however observed in the study including clinical signs and bodyweight losses. Higher dose levels could not be tested due to mortality observed in the dose range-finding study. Considering the systemic toxicity observed and the bone marrow toxicity at higher dose levels in the first study the RMS considers that the bone marrow was sufficiently exposed.
Based on the available information AMPA is concluded to be non-genotoxic.

Several short-term toxicity studies are available for AMPA.

The first was a 14-day study in rats (Report No. 401-026) which was considered to be unacceptable due to the severe limitations of the study. No adverse effects were noted up to 2000 mg/kg bw/day, but it should be noted that only a limited number of parameters were investigated.

In a 28-day range finding study in rats (Report No. 148-GLY) increased kidney weight was observed at 350 mg/kg bw/day and above and at 1000 a decreased body weight gain was seen leading to a NOAEL of 100 mg/kg bw/day.

A month dog study (Report No [redacted]-11127) also showed numerous limitations including a number of animals that was too low (2/sex/dose) and a lack of histopathological investigation. At 300 mg/kg bw/day and higher haematological effects were noted. In addition at 1000 mg/kg bw/day, diarrhoea was observed. No adverse effects were observed at 10, 30 and 100 mg/kg bw/day.

In a 90-day study in rats (Report No. 7866) groups of 10 male and 10 female Sprague-Dawley rats received AMPA at dose levels of 0, 10, 100 and 1000 mg/kg bw/day. No adverse effects were noted and the NOAEL was concluded to be 1000 mg/kg bw/day, the highest dose tested.

In a second 90-day study in rats (Report No. 401-050) groups of 20 male and 20 female Charles River CD rat were treated with AMPA at doses of 400, 1200 and 4800 mg/kg bw/day. Based on increased urothelial hyperplasia of the urinary bladder of both sexes at 1200 mg/kg bw/day, the NOAEL was concluded to be 400 mg/kg bw/day.

In a 90-day dog study groups of 5 male and 5 female Beagle dogs were treated with AMPA at 0, 10, 30, 100 and 300 mg/kg bw/day. No adverse effects were noted up to the highest dose level (achieved dose level of 263 mg/kg bw/day).

In a developmental toxicity study (Report No. 7891), groups of 25 female Sprague-Dawley rat received a daily gavage dose of AMPA at 0, 100, 350 and 1000 mg/kg bw/day from GD6 to GD16. No adverse effects were reported in maternal animals or foetuses up to highest dose tested.

In a second developmental toxicity study (Report No. [redacted]-50159), groups of 25 female Charles River Crl:CD BR rats were treated with an oral gavage dose of glyphosate at 0, 150, 400 and 1000 mg/kg bw/day from GD6 to GD15. Clinical findings which appeared related to test item administration occurred at 400 and 1000 mg/kg bw/day and included mucoid faeces, hair loss and soft stool. During gestation Days 12-16, mean body weight gain at 1000 mg/kg bw/day was slightly reduced and food consumption was reduced during GD6-9. The mean foetal body weight at 1000 mg/kg bw/day group was slightly decreased. No other indication of a developmental effect was apparent at any dose level. The maternal NOAEL was concluded to be 150 mg/kg bw/day while the developmental NOAEL was concluded to be 400 mg/kg bw/day.

Overall toxicity assessment of AMPA
The metabolite AMPA was extensively investigated for acute and sub-chronic effects, for skin sensitization, mutagenicity and developmental toxicity. In acute oral rodent studies the median lethal dose had been identified with signs of no toxicity as greater than 2000 mg/kg bw/day in rats. Non-sensitizing potential had been demonstrated with guinea pigs in a Magnusson and Kligman Maximization test. Sub-acute studies had been evaluated with rats and dogs. The lowest sub-acute NOAEL value of 100 mg/kg bw/day based on kidney weight increase in male rats and decreased bw gain in female animals. In addition, two 90-day studies are available in rats and one 90-day study in dogs. In the first rat study and in the dog study, no adverse effects were noted up to the highest dose tested and the NOAEL was concluded to be 1000 and 300 mg/kg bw/day, respectively. In the second rat study, based on increased urothelial hyperplasia of the urinary bladder of both sexes observed at 1200 mg/kg bw/day, the NOAEL

was concluded to be 400 mg/kg bw/day.
Two developmental toxicity studies are available. In the first developmental study, no adverse effects were reported in maternal animals or foetuses up to highest dose tested. In the second study, a maternal NOAEL value of 150 mg/kg bw/day was based on clinical signs of decreased food consumption and decreased body weight gain. The developmental NOAEL of 400 mg/kg bw/day was derived on mean foetal weight decrease.

Overall, it can be concluded that AMPA is of similar toxicity as glyphosate and the same reference values can be applied. This conclusion is in line with the previous EU evaluation. The RMS notes that in contrast to the previous evaluation, the reference values of glyphosate have been lowered based on salivary gland findings after repeated oral dosing in rats. In order to determine whether or not AMPA shares this effect with parent glyphosate and whether or not (higher) substance-specific reference values might be set for AMPA, special attention was paid to salivary gland findings in the studies with AMPA. However, based on the available sub-chronic data package, it cannot be excluded that AMPA would case the same effect in the salivary gland. In rats, a 28-day and a 90-day study are available in which the salivary glands including the parotid gland were investigated (study report no. 148-GLY and No. 7866). In these studies no treatment-related histopathological findings in the salivary glands were reported. However, the RMS notes that the route of administration in these studies was by gavage, thus bypassing the mouth. Therefore, it is questioned whether or not these studies covers the salivary gland findings reported after oral administration of glyphosate in rats. One 90-day study in rats is available (study report no 401-050) in which AMPA was administered through diet and also a 90-day study in dogs using oral (capsule) administration, however, these studies did only investigate the submandibular (submaxillary) salivary gland and did not include the parotid salivary gland. Therefore, no conclusion can be drawn based on these studies. Overall, as based on the available toxicity studies it cannot be excluded that AMPA causes similar histopathological changes in the salivary gland as parent glyphosate, the same reference values should be applied for both parent glyphosate and metabolite AMPA.

2.6.8.1.2 Studies with N-acetyl AMPA

Table 2.6.8.1.2-1 Summary table of animal studies on acute oral toxicity of the metabolite N-acetyl AMPA

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Value LD_{50}	Reference
OECD 425, GLP No deviations Study acceptable	Rat, Crl:CD(SD), Females, 3/dose	N-Acetyl AMPA, Batch: IN-EY252-003, Purity: 79%	5000 mg/kg bw/d, Single oral dose	> 5000 mg/kg bw/d (females)	CA 5.8.1/032; Report no. [redacted]-22229

Table 2.6.8.1.2-2 Summary table of animal studies on repeated dose toxicity (short-term and long-term toxicity) STOT RE (specific target organ toxicity - repeated exposure) of the metabolite N-acetyl AMPA

Method, guideline, deviations[1] if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL**	**Reference**
OECD 408 (1998) GLP Deviations: absence of LDL, HDL, T3, T4, TSH, organ weight of prostate, thyroid and pituitary gland, histopathology of coagulating glands, gall bladder and male mammary glands Study acceptable Rat, Crl:CD (SD), male and female, 10/sex/dose	N-Acetyl AMPA, batch (IN-EY252-)003, purity 79% 90-day, dietary dose Doses of 900, 6000 and 18000 ppm (equivalent to 55, 374 and 1163 mg/kg bw/day in males and 68, 455 and 1400 mg/kg bw/day in females)	900 and 6000 ppm No adverse effects observed 18000 ppm - Abnormal excreta in 10/10 males and 7/10 females (0/10 in control group) - Decreased body weight gain in males (-12%)	CA 5.8.1/033, Report No. [redacted]-23316

Table 2.6.8.1.2-3 Summary table of genotoxicity/germ cell mutagenicity tests *in vitro* of the metabolite N-acetyl AMPA

Method, guideline, deviations if any	**Test substance**	**Relevant information about the study including rationale for dose selection (as applicable)**	**Observations /Results**	**Reference**
OECD 471, GLP No deviations Study acceptable	N-acetyl AMPA Batch: IN-EY252-001, Purity: 76%	Ames test, ±S9 mix for metabolic activation, 1.5-5000 µg/plate, standard plate assay, Strains: *S. typhimurium* TA 98, TA 100, TA 1535 and TA 1537 and *E. coli* WP2 uvrA	Number of revertants of the positive and negative control was in the expected range. No relevant increase observed in any experiment with the tested concentrations neither in the presence nor absence of metabolic activation. N-acetyl AMPA is considered non-mutagenic under the conditions of this assay.	CA 5.8.1/034, Report no. DuPont-22227
OECD 473, GLP Deviations: 200 instead of 300 metaphases were scored. Study acceptable but with restrictions	N-acetyl AMPA, Batch: IN-EY252-001, Purity: 76%	*In vitro* mammalian chromosome aberration assay in human peripheral blood lymphocytes, ±S9 mix, 382.5-1530 µg/mL doses scored for aberrations	Number of aberrations of the positive and negative control were within the expected range. No significant increase in aberrant cells observed upon treatment with N-acetyl AMPA at any of the tested concentrations, neither in the presence, nor in the absence of metabolic activation. N-acetyl AMPA is considered negative for chromosome aberrations under the conditions of this assay.	CA 5.8.1/035, Report no. DuPont-22225
OECD 476, GLP Deviations: cytotoxicity based on cloning efficiencies, not on RTG/SG/RSG; number of treated	N-acetyl AMPA, Batch: IN-EY252-002, Purity: 72%	*In vitro* mammalian cell gene mutation test (CHO K_1 cells; HPRT locus), ±S9 mix, 100-1531 µg/mL	Number of mutants of the positive and negative control were within the expected range. No statistically significant increase in mutants observed upon treatment with N-acetyl AMPA at any of the tested concentrations, neither in the presence, nor in the absence of metabolic activation.	CA 5.8.1/036, Report no. DuPont-22224

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations /Results	Reference
cells too low; evaluation and acceptability criteria not completely in line with OECD 476. Study acceptable but with restrictions			N-acetyl AMPA is considered non-mutagenic under the conditions of this assay.	

Table 2.6.8.1.2-4 Summary table of genotoxicity/mutagenicity tests in mammalian somatic or germ cells *in vivo* of the metabolite N-acetyl AMPA

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations /Results	Reference
OECD 474, GLP Deviations: only 2000 PCE per animal were scored, no proof of bone marrow exposure Study acceptable but with restrictions	AMPA, Batch: IN-EY252-002, Purity: 72%	*In vivo* bone marrow micronucleus test, mice (m/f), 500-2000 mg/kg bw, single oral dose	Incidence of micronuclei in controls in accordance with the HCD. No statistically significant increase in the frequency of micronucleated PCEs (mPCEs) of test item-treated animals compared to the negative control. No proof of bone marrow exposure. Under the conditions of this test, AMPA was negative for clastogenic effects.	CA 5.8.1/037, Report no. [redacted] 22226

Short summary on toxicological information on N-acetyl AMPA

N-acetyl AMPA was found to be of low acute oral toxicity (LD_{50} > 5000 mg/kg bw).

Genotoxicity studies were provided consisting of an Ames test, an *in vitro* chromosomal aberration study, an *in vitro* mammalian genotoxicity study and an *in vivo* micronucleus study. All genotoxicity studies were negative. However, it is noted that no proof of bone marrow exposure was provided for the *in vivo* micronucleus study as no effect on PCE/NCE ratio was observed and no systemic toxicity occurred in the study. Moreover, the *in vitro* chromosomal aberration study does not cover aneugenicity. Therefore, no final conclusion can be made on the genotoxic potential of the metabolite N-acetyl AMPA. **The applicant is requested to provide an *in vitro* micronucleus study to address aneugenicity.**

In a 90-day toxicity study, groups of 10 male and 10 female Sprague-Dawley rats received N-acetyl AMPA at dietary concentrations of 900, 6000, and 18000 ppm (equivalent to 0, 55, 374 and 1163 mg/kg bw/day for males and 0, 68, 455 and 1400 mg/kg bw/day for females). At the highest dose level of 18000 ppm, abnormal excreta both sexes and a decreased body weight gain in males (-12%) were considered treatment-related and adverse. Based on these observations, the NOAEL is 6000 ppm, which is equivalent to 374 mg/kg bw/day in males and 455 mg/kg bw/day in females.

Overall toxicity assessment of N-acetyl AMPA
Based on the 90-day study it appears that N-acetyl AMPA **is not of greater toxicity** than glyphosate. However, due to the data gap on genotoxicity no conclusion can be made regarding reference values.

2.6.8.1.3 Studies with N-acetyl glyphosate

Table 2.6.8.1.3-1 Summary table of toxicokinetic studies of the metabolite N-acetyl glyphosate

Method	Results	Remarks	Reference
Guideline OPPTS 870.7485, 40 CFR 160 GLP Deviations: not full range, males only, only one dose Study acceptable but with restrictions Sprague-Dawley rats, male, 5 (faeces, urine, cage wash, carcass) and 4 per blood sampling time point N-Acetyl glyphosate, batch 123K5012, purity 84.3% sodium salt Single oral dose at 15 mg/kg bw.	Fast and almost complete excretion within 7 days (66.1% in urine, 26.4% in faeces, 2.79% in cage wash and 0.23% in residual carcass). Most excreted within 48 hours. Fast absorption. Peak blood and plasma concentrations at 1 and 2 hours respectively. $t_{1/2}$ is 20.1 and 15.6 hours in blood and plasma. Almost no metabolization. Glyphosate formed as faecal metabolite by N-deacetylation (0.25%).	As no information was provided for females no conclusion can be drawn on possible sex differences in kinetic properties	CA 5.8.1/038; Report no. [redacted] 7535-100 (Amendment Report No. [redacted] 56245A)

Table 2.6.8.1.3-2 Summary table of animal studies on acute oral toxicity of the metabolite N-acetyl glyphosate

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Value LD_{50}	Reference
OECD 423, GLP No deviations Study acceptable	Rat, Crl:CD(SD)IGS BR, Males and females, 5/dose	N-Acetyl glyphosate, Batch: 123K5012, Purity: 84.3% sodium salt; 67.4% free acid	5000 mg/kg bw/d, Single oral dose	> 5000 mg/kg bw/d (females) Clinical signs noted in all animals; mortality and findings at necroscopy in one male and two females	CA 5.8.1/039; Report no. 7535-103

Table 2.6.8.1.3-3 Summary table of animal studies on repeated dose toxicity (short-term and long-term toxicity) STOT RE (specific target organ toxicity - repeated exposure) of the metabolite N-acetyl glyphosate

Table 2.6.8.1.3-3 Summary table of animal studies on repeated dose toxicity (short-term and long-term toxicity) STOT RE (specific target organ toxicity - repeated exposure) of the metabolite N-acetyl glyphosate

Method, guideline, deviations[1] if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL	Reference
OECD 408 (1998) GLP Deviations: absence of LDL, HDL, T3, T4, TSH, organ weight of prostate, thyroid, pituitary gland, histopathology of cervix, coagulating glands, gall bladder and male mammary glands Study acceptable Rat, Crl:CD (SD), male and female, 10/sex/dose	N-acetyl glyphosate, batch IN-MCX20, purity 63% 90-day, dietary dose Doses of 180, 900, 4500 and 18000 ppm (equivalent to 11.3, 55.7, 283 and 1157 mg/kg bw/day in males and 13.9, 67.8, 360 and 1461 mg/kg bw/day in females)	180, 900 and 4500 ppm No adverse effects observed 18000 ppm: Decreased body weight gain in males (-14%)	CA 5.8.1/040, Report No. [redacted]-19008

Table 2.6.8.1.3-4 Summary table of genotoxicity/germ cell mutagenicity tests *in vitro* of the metabolite N-acetyl glyphosate

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations /Results	Reference
OECD 471, GLP No deviations Study acceptable	N-acetyl glyphosate, Batch: 123K5012, Purity: 84.3% sodium salt; 67.4% free acid	Ames test, ±S9 mix for metabolic activation, 100-5000 µg/plate, standard plate assay, Strains: *S. typhimurium* TA 98, TA 100, TA 1535 and TA 1537 and *E. coli* WP2 uvrA	Number of revertants of the positive and negative control was in the expected range. No relevant increase observed in any experiment with the tested concentrations neither in the presence nor absence of metabolic activation. N-acetyl glyphosate is considered non-mutagenic under the conditions of this assay.	CA 5.8.1/040, Report no. 7353-101
OECD 473, GLP Deviations: 200 instead of 300 metaphases were scored; cytotoxicity based on MI, not RPD or RICC Study acceptable but with restrictions	N-acetyl glyphosate, Batch: 123K5012, Purity: 84.3% sodium salt; 67.4% free acid	*In vitro* mammalian chromosome aberration assay in CHO cells, ±S9 mix, 960-2800 µg/mL doses scored for aberrations	Number of aberrations of the positive and negative control were within the expected range. No significant increase in aberrant cells observed upon treatment with N-acetyl glyphosate at any of the tested concentrations, neither in the presence, nor in the absence of metabolic activation. N-acetyl glyphosate is considered negative for chromosome aberrations under the conditions of this assay.	CA 5.8.1/041, Report no. 7535-102
OECD 476, GLP Deviations: cytotoxicity based on cloning efficiencies, not on RS; number of treated cells too low.	N-acetyl-N-(phosphonomethyl)glycine; Batch: IN-MCX20-002; Purity: 63%	*In vitro* mammalian cell gene mutation test (CHO K_1 cells; HPRT locus), ±S9 mix, 250-2091 µg/mL (1.2-10 mM)	Number of mutants of the positive and negative control were within the expected range. No statistically significant increase in mutants observed upon treatment with N-acetyl glyphosate at any of the tested concentrations, neither in the presence, nor in the absence of metabolic activation.	CA 5.8.1/043, Report no. DuPont-20155

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations /Results	Reference
Study acceptable but with restrictions			N-acetyl glyphosate is considered non-mutagenic under the conditions of this assay.	

Table 2.6.8.1.3-5 Summary table of genotoxicity/mutagenicity tests in mammalian somatic or germ cells *in vivo* of the metabolite N-acetyl glyphosate

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations /Results	Reference
OECD 474, GLP Deviations: only 2000 PCE per animal were scored, no proof of bone marrow exposure Study acceptable but with restrictions	N-acetyl-N-(phosphonomethyl)glycine; Batch: IN-MCX20-002; Purity: 63%	*In vivo* bone marrow micronucleus test, mice (5/sex/sampling time point), 500-2000 mg/kg bw, single oral dose	Incidence of micronuclei in controls in accordance with the HCD. No statistically significant increase in the frequency of micronucleated PCEs (mPCEs) of test item-treated animals compared to the negative control. No proof of bone marrow exposure. Under the conditions of this test, AMPA was negative for clastogenic effects.	CA 5.8.1/044, Report no. [redacted]-20154

Short summary on toxicological information on N-acetyl glyphosate

In an ADME study in rats with N-acetyl glyphosate it was shown that the metabolite is mainly excreted via urine (66.1%) with over 90% over radioactivity excreted within 48 hours. Radioactivity was eliminated from blood and plasma with half-life values of 20.1 and 15.6 hours, respectively. The metabolite was mainly excreted unchanged (99%). Glyphosate was found as a metabolite in faeces at 0.25% of the administered dose.

In an acute oral toxicity study the LD50 value of N-acetyl glyphosate was concluded to be >5000 mg/kg bw.

In a 90-day study, groups of 10 male and 10 female Sprague-Dawley rats were administered N-acetyl glyphosate at dietary doses of contained 0, 180, 900, 4500 or 18000 ppm IN-MCX20 (equivalent to 0, 11.3, 55.7, 283 and 1157 mg/kg bw/day for males and 0, 13.9, 67.8, 360 and 1461 mg/kg bw/day for females) for approximately 90 days (95 days in males and 96 days in females). In males dosed at 180000 ppm, a statistically significant decrease in body weight gain (day 0-91) of 14% compared with controls was seen, which is considered treatment-related and adverse. This effect was associated with an 8% decrease in final body weight compared to controls. This dose level of 18000 ppm (1157 mg/kg bw/day) in males is considered the LOAEL and the NOAEL is 4500 ppm (283 mg/kg bw/day).

N-acetyl glyphosate was negative in a bacterial gene mutation study, an *in vitro* chromosome aberration study and an *in vitro* mammalian gene mutation study. Furthermore, N-acetyl glyphosate was negative in an *in vivo* micronucleus study in mice. However, no direct proof of bone exposure was available and no systemic toxicity was observed in the study. The repeated dose toxicity also does not provide indirect evidence of bone marrow exposure as no toxicity was observed in that study. In the ADME study N-acetyl glyphosate was observed in blood and plasma with a half-life of 20.1 and 15.6 hours respectively indicated systemic exposure. However, this study was conducted with the rat while the *in vivo* micronucleus study was conducted in mice. Overall, the RMS concludes that there is insufficient evidence of bone marrow exposure. Although the *in vitro* data package was fully negative it is noted that the *in vitro* chromosome aberration study does not cover aneugenicity. Therefore, no final conclusion can be

made on the genotoxic potential of the metabolite N-acetyl glyphosate. **The applicant is requested to provide an in vitro micronucleus study to address aneugenicity**.

Overall toxicity assessment of N-acetyl glyphosate
Based on the 90-day study it appears that N-acetyl glyphosate **is not of greater toxicity** than glyphosate. However, due to the data gap on genotoxicity no conclusion can be made regarding reference values.

2.6.8.1.4 Studies with other metabolites

The notifier provided a (Q)SAR and read-across for the genotoxicity evaluation of glyphosate and seven metabolites (AMPA (M02), *N*-methyl AMPA (M03), *N*-acetyl glyphosate (M04), *N*-acetyl AMPA (M05), *N*-glyceryl AMPA (M06), *N*-malonyl AMPA (M07), methyl phosphonic acid (M08) and N-methyl glyphosate (M09)). The QSAR analysis did not provide a concern for genotoxicity for glyphosate and its metabolites. However, a QSAR analysis alone is not considered sufficient to fully address genotoxicity of the metabolites. For AMPA sufficient data is available to conclude that it is not genotoxic (refer to 2.6.8.1.1). For *N*-acetyl glyphosate (M04) and *N*-acetyl AMPA (M05) a data gap for aneugenicity is identified (refer to 2.6.8.1.2 and -3). The applicant proposed a grouping approach for read-across for the other metabolites, however, **this approach was not accepted by the RMS** (refer to Vol 3 B.6.8.1.1.11).

2.6.8.2 Supplementary studies on the active substance

Table 2.6.8.2-1 Supplementary studies on the active substance

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
In vivo and *ex vivo* pharmacology screening in rat and guinea pig No OECD guideline. GLP Study acceptable but with restrictions Limitations: the stability of the test material was not reported and the dose level tested was not analytically verified	Glyphosate technical (batch H95D161A, purity 95.3%)	In the *in vivo* study five male and five female SD rats were dosed at 5000 mg/kg bw with similar sized control groups receiving vehicle only. Approximately one hour after dosing control and treated animals were examined for either haematological changes, electrocardiographic changes or behavioural/functional changes. In the *ex vivo* study on the isolated guinea pig ileum and isolated rat gastrocnemius muscle were performed using saturated solutions of the test material	The *in vivo* study does not give an indication of a pharmacological effect on the parameters evaluated. In the *ex vivo* study glyphosate did not cause neuromuscular blocking activity up to the highest concentration tested. In the *ex vivo* study on intestinal contractibility glyphosate did induce a contractile response. However, this effect occurred only at fairly high concentrations (12 mg/L) and therefore the *in vivo* relevance of this finding is questionable.	Report no. 434/021, 1996 (KCA 5.8.2/004)
In vivo pharmacology screening in mice and rabbits No OECD guideline. GLP Study acceptable but with restrictions	Ammonium salt of glyphosate (MON-8750), (batch RUD-9201-3544F, purity 94.78%) The test substance was	A pharmacological study on the toxicity of the ammonium salt of glyphosate was conducted in mice and male rabbits. Different doses up to a top dose level of 5000 mg/kg bw was administered to mice by single	At the top dose levels, all mice died within 0.5 hours and all anesthetized rabbits within a few minutes after injection. Non-anesthetized rabbits survived intravenous application of 500 mg/kg bw. They showed transient clinical abnormalities, but fully recovered within 3 hours. At	Report no. [redacted] 90-0149/ET-92-15, 1992 (KCA 5.8.2/005)

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
Limitations: the stability of the test material was not reported and the dose level tested was not analytically verified	dissolved in physiological saline and adjusted to pH 5 with sodium hydroxide	intraperitoneal injection. In rabbits the top dose level administered by single intravenous injection was 500 mg/kg bw, which reflected the highest soluble concentration.	the next lower dose levels transient symptoms like a decrease in blood pressure, reduced activity and neuromuscular signs were observed but cleared to normal values or behaviour within some hours at the latest. At doses up to 125 mg/kg bw in mice and 7.8 mg/kg bw in rabbits no abnormalities were noted. Adverse effects were noted such as clinical signs, decreased respiratory rate, decrease blood pressure and a decrease in QRS complex. However, the effects occurred at high intravenous and intraperitoneal doses and therefore the study does not impact the overall risk assessment of glyphosate.	
In vivo toxicodynamic study in rats No OECD guideline Non-GLP (not compulsory) Study acceptable but with restrictions due to reporting limitations on the study design	Glyphosate technical (batch 72390788, purity 96%)	The toxicodynamics of glyphosate were examined in Wistar rats by the measurement of heart rate, ECG, venous- and arterial blood pressure and body temperature	30 min after anesthetising with a chloralose-urethane mixture, male Wistar rats were administered a single dose of 5000 mg/kg bw by oral gavage. All animals died within 2 to 7 hours. Treatment was followed by a marked decrease in arterial pressure by approximately 50% two hours after treatment as compared to the initial values. There was no clear impact on heart rate and respiratory rate. ECG and venous pressure changes, if occurring in some animals, were considered incidental and body temperature was not affected.	Report no not stated, 1988 (KCA 5.8.2/006)
In vivo single oral dose in rats No OECD guideline Non-GLP (not compulsory) Study supplementary Limitations: No batch number, stability or purity of the test material is reported.	Glyphosate technical (batch and purity not stated) in combination with 2,4-D sodium salt, isoproturon and metolachlor	Groups of 10 male Wistar rats were administered 5000 mg/kg bw of glyphosate as a single oral dose at a constant dose volume of 10 mL/kg bw in corn oil. Three compounds were administered in combination with 5000 mg glyphosate per kg bw: a) 2,4-D sodium salt (at six	LD_{50} values determined for the three substances, 2,4-D sodium salt, isoproturon and metalochlor were 750, 3900, and 2700 mg/kg bw, respectively. When administered after previous administration of 5000 mg glyphosate/kg bw, LD_{50} values of the three substances were reduced to 585, 3600, and 2450 mg/kg bw, respectively. The differences in LD_{50} values	Report no not stated, 1988 (KCA 5.8.2/007)

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
		dose intervals between 380 and 1200 mg/kg bw), b) isoproturon (at five dose intervals between 3200 and 5000 mg/kg bw), and c) metolachlor (at six dose intervals between 2100 and 3800 mg/kg bw). Prior to simultaneous dosing, each of the three compounds were administered to groups of rats individually.	of the three substances were marginal and at times within the confidence interval of the LD_{50} for the compounds alone.	
Acute toxicity study in goats No OECD guideline Non-GLP (not compulsory) Study supportive Limitations: Wide age range (8 months to 4 years) with a wide bodyweight range (13.37 - 44 kg). No environmental conditions during housing reported. Fairly high dosing volume (500 ml/goat). One control and one goat in the 4620 mg/kg bw gave birth during the study while one control goat gave birth one hour prior to treatment. It is unclear if other female goats were pregnant during the study. Pneumonia was observed in a number of animals independent of dose indicating that some kind of infection occurred in the animals. The clinical chemistry parameters were compared based on time of death instead of based on dose level.	Glyphosate (batch XHJ-64, NBP1494248, purity 98.7%)	Four groups consisting of five female (Spanish) goats were administered single oral gavage doses of glyphosate (via stomach tube) at doses of 1980, 3090, 4620, and 10000 mg/kg bw. Water was used as a vehicle in four negative control groups of five goats each. Mortality, clinical signs, body weight, haematology, clinical biochemistry, gross necropsy, histopathology were assessed during a post-treatment observation period of 14 days.	The acute oral LD_{50} was calculated to be 3530 mg/kg bw. Due to the small number of animals investigated and the heterogeneous study population (refer to limitations), the relevance of the biochemical and haematological findings is questionable and not reported here.	Report no. 80006, 1987 (KCA 5.8.2/008)
Acute toxicity study in goats	Glyphosate as isopropylamine salt (MON-	Four groups consisting of five female (Spanish)	The acute oral LD_{50} was calculated to be 5700 mg/kg bw.	Report no. 80007 [redacted], 1987

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No OECD guideline Non-GLP (not compulsory) Study supportive Limitations: Wide age range (8 months to 4 years) with a wide weight range (14.7-47.0 kg). No environmental conditions during housing reported. Fairly high dosing volume (500 ml/goat). Abortion was noted in a few animals. It is unclear from the study report if other animals were pregnant.	0139) (batch LURT 08020, purity 62.5% (46.2% for glyphosate))	goats were administered single oral gavage doses of glyphosate as isopropylamine salt (via rumen intubation) at doses of 1400, 4290, 5360, 6700 and 10000 mg/kg bw. Water was used as a vehicle in four negative control groups of five goats each. Mortality, clinical signs, body weight, haematology, clinical biochemistry, gross necropsy, histopathology were assessed during a post-treatment observation period of 14 days.	Due to the small number of animals investigated and the heterogeneous study population (refer to limitations), the relevance of the biochemical and haematological findings is questionable and not reported here.	(KCA 5.8.2/009)
Subacute toxicity study in cattle No OECD guideline Non-GLP (not compulsory) Study **not** acceptable Limitations: Environmental conditions were not reported. Number of animals used (n = 2 to 3). Considered too low to derive an NOAEL	Glyphosate as isopropylamine salt (MON-0139) (batch LBRT 08023, purity 62.4% (46.2% for glyphosate))	The test substance was administered on seven consecutive days by ruminal intubation to four groups of three female cattle (heifers) at 0, 540, 830, 1290 and 2000 mg/kg bw/day. Water was used as a vehicle to give a constant dosing volume of 500 mL. Four concurrent negative controls groups of two heifers each were sham treated with water. Mortality, clinical signs, body weight, haematology, clinical biochemistry, gross necropsy, histopathology were assessed during a post-treatment observation period of 14 days. Histopathology was confined to liver, kidney and tissues with gross lesions obtained from animals	Loss of appetite and diarrhoea at 830 mg/kg bw/day and above. Mortality at doses of 1290 and 2000 mg/kg bw/day. Nervous system effects (head tremors, convulsions, ataxia, etc.) were observed at 2000 mg/kg bw/day prior to death. At the two upper dose levels, haematological changes (haemoconcentration) probably resulted from diarrhoea. Serum chemistry changes and histopathologic lesions indicative of renal damage at 1290 mg/kg bw/day. Dehydration and gastrointestinal irritation at the upper dosages. Increased kidney weight at 1290 mg/kg bw/day and above, increased liver weight at 2000 mg/kg bw/day. At 1290 mg/kg bw/day, mild to marked tubular vacuolization (primarily proximal convoluted tubules) and nuclear pyknosis in many tubular epithelial cells was observed in kidneys, but not at 830 mg/kg/bw/day.	Report no. 80002, 1987 (KCA 5.8.2/010)

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
		receiving doses of 1290 and 830 mg/kg bw/day.		
In vivo/ex vivo irritating effect on stomach and small intestine No OECD guideline Non-GLP (not compulsory) Study acceptable but with restrictions Limitations: No information on purity and stability of the test substance.	isopropylamine (IPA) salt of glyphosate (batch Oda B6E20 88.10)	Undiluted Roundup (41% IPA, 15% MON-0818 surfactant), IPA salt of glyphosate (41%), the undiluted surfactant MON-0818, and 0.25 N hydrochloric acid solution (control) were directly administered on the gastric and small intestinal mucosa of fasted male beagle dogs. The specimens were examined microscopically and evaluated for mucosal damage in comparison with normal gastric and intestinal tissues.	Direct application of Roundup herbicide, and the surfactant caused mild mucosal damage in the stomach and intestine. These effects were more severe with the Roundup formulation than with either the IPA salt or the surfactant. The intestine appeared to be more affected than the stomach. The severity of the damage was equivalent to that caused be 0.25 N hydrochloric acid.	Report no. 2309496, 1987 (KCA 5.8.2/011)
OECD 428, GLP Deviations: receptor fluid solubility not tested The study is considered supplementary only (not relevant for risk assessment).	Radiolabelled test substance: ^{14}C-glyphosate (as glyphosate acid); Radiochemical purity: 96.7% Non-radiolabelled test substance: Glyphosate acid; Purity: 95.93%	*In vitro* dermal absorption study on abraded rat skin. Exposure to 48.3 mg glyphosate/cm² for 6 hours. Sampling from a static receptor chamber for 24 hours.	Total potentially absorbable (Amount in receptor fluid + remaining dermis after heat separation): 2.66% Study setup considered acceptable, however, the regulatory value of the study is limited. Dermal absorptions need to be determined for active substances present in a certain formulation.	Report no. JV2182-REG (KCA 5.8.2/014)
No guideline, non-GLP Deviations/shortcomings: test substance not characterised, no positive controls The study is considered supplementary only (not relevant for risk assessment).	Glyphosate (purchased from Sigma Aldrich); no additional information available	Toxicity of glyphosate (25 µM-25mM) on liver and cardiac organoids was investigated. Endpoints: cell viability, ATP activity, beating rate of the cardiac organoid.	Cell viability: reduced organoid integrity and viability at doses from 250 µM to 2.5 mM. ATP activity (IC50): 10.53 mM (liver); 10.85 mM (cardiac) Beating rate: non-statistically significant effect on beating rate at 0.25 mM. Exposure to 2.5 mM for 2 days resulted in all organoids stopping beating.	Forsythe, S.D. et al., 2018 (KCA 5.8.2/015)

Acute toxicity via i.p. injection

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Value LD_{50}	Reference
Acute toxicity following i.p. injection. No guideline available, GLP not specified No guideline available, but several deviations from other guidelines addressing acute toxicity. Study not acceptable	Rat, Wistar; 10 females/dose	N-phosphono-methyl-glycine, Batch: unknown, Purity: unknown	620, 760, 940, 1040, 1260 mg/kg bw Single i.p. dose	Not determined due to unacceptability of the study Multiple clinical signs observed during conduct of the study. Multiple findings at necroscopy in dead and surviving animals.	CA 5.8.2/012; Report no. unknown.
Acute toxicity following i.p. injection. No guideline available, non-GLP No guideline available, but several deviations from other guidelines addressing acute toxicity. Study not acceptable	Rat, Wistar-Imamichi; Males and females; 10/sex/dose	CP67573, Batch: unknown, Purity: 98.4%	Males: 182, 255, 357, 422, 500 mg/kg bw Females: 255, 357, 500, 700 mg/kg bw	Not determined due to unacceptability of the study Multiple clinical signs observed during conduct of the study. Multiple findings at necroscopy in dead and surviving animals.	CA 5.8.2/013; Report no. [redacted]-79-109

Immunotoxicity:

The potential immunotoxicity of glyphosate was evaluated after repeated dietary administration to B6C3F1 mice (report number [redacted]-50393). The study was performed according to EPA OPPTS 870.7800 and conducted under GLP. The study was considered acceptable. Four groups of 10 female mice were offered diets containing glyphosate (batch GLP-0807-19475-T, purity 95.11%) at concentrations of 0, 500, 1500 or 5000 ppm (equivalent to 0, 150, 449, and 1448 mg/kg bw/day) and for 28 consecutive days. A further group of 10 females were used as positive immunosuppressive control group. These mice received basal diet for 28 days and were treated with an intraperitoneal (i.p.) injection of 50 mg/kg bw/day cyclophosphamide monohydrate (CPS) once daily for four consecutive days (study days 24 - 27).

There were no test substance-related effects on survival, clinical observations, body weight, food consumption, as well as any gross pathological changes. There were no test substance-related effects on spleen or thymus weights (absolute or relative to final body weight), spleen cellularity, or the T-cell dependent antibody response (TDAR), as measured by the splenic antibody-forming cell (AFC) IgM Specific Activity (AFC/10^6 spleen cells) and Total Spleen Activity (AFC/spleen), at any dosage level tested.

The NOAEL of study was 5000 ppm (equal to 1448 mg/kg bw/day), the highest dose tested.

Effects on the salivary glands:

Two additional studies were conducted to further investigate the effects on salivary glands which were observed in some of the repeated dose toxicity studies, long term studies and reproduction toxicity studies in rats and occasionally in mice. It was hypothesized that the effect might be due to the low pH of glyphosate technical acid in the diet caused local irritation in the oral cavity leading to the observed salivary gland effects.

To investigate this a study was conducted with citric acid which was considered an appropriate surrogate for glyphosate, having both a similar pH-dilution curve and low toxicity. Citric acid was presented in the diet (14000 ppm) and compared with a typical pH basal diet control group (report number [redacted]-50361). A higher pH diet group fed basal diet with trisodium citrate dihydrate (21400 ppm, an equivalent citrate ion concentration to the citric acid group) was also compared with the typical pH basal diet control group. In addition, low pH aqueous citric acid was administered by gavage and compared to a control deionised water gavage group to evaluate potential systemic effects of the citrate ion on the parotid salivary glands. These five test groups, each consisting of 10 male Sprague-Dawley (Crl:CD) rats, were dosed for eight weeks (minimum of 56 days).

Test substance-related effects on organ weights consisted of statistically significantly higher parotid salivary gland weights in the low pH diet group only (citric acid) when compared to the respective control group. Non-statistically significantly higher parotid salivary gland weights were noted in the gavage citric acid and high pH dietary (trisodium citrate dihydrate) groups when compared to their respective control group. There were no statistically significant test substance-related effects on the fused mandibular/sublingual salivary gland weights when the respective control and test substance-treated groups were compared; however, a non-statistically significantly higher fused mandibular/sublingual salivary gland weight was noted in the low pH diet group (14000 ppm citric acid). Histological effects consisted of cytoplasmic alterations in the parotid salivary glands characterized by the presence of hypertrophied acinar cells with basophilic granular cytoplasm. Although the overall incidence of affected animals was similar in all control and citric acid or trisodium citrate dihydrate-treated groups, these effects were clearly most severe in the low pH diet group (14000 ppm citric acid in basal diet). With the absence of microscopic findings such as cytotoxicity and hyperplasia, the observed effects are considered to be an adaptive response to local irritation of the low pH diet in the oral cavity rather than an adverse effect.

It was concluded that citric acid administered orally via gavage or diet and trisodium citrate dihydrate administered via the diet to Sprague Dawley rats for 56 days resulted in higher parotid salivary gland weights and a generally correlative increase in severity of background cytoplasmic alterations in the parotid salivary glands in all dose groups (gavage citric acid, diet citric acid, and diet trisodium citrate dihydrate). The magnitude of change in parotid gland weight and severity of the cytoplasmic alteration in the parotid salivary glands was most severe in the low pH diet citric acid group. **While this study shows that the effect on salivary glands observed in the glyphosate studies is likely due to administration of a low pH diet, it does not exclude the possibility of other mode of actions being behind the observed effects**.

In the second study (report number [redacted]/P/5160), the sensitivity of different strains for the observed effect on salivary glands was investigated. Study groups of 24 male Alpk:AP_fSD (Wistar-derived; AP), Sprague-Dawley (Charles River CD; CD) and Fischer 344 (F344) rats received 0 or 20000 ppm glyphosate acid. Eight animals from each group were killed on Day 29 and the remaining animals were retained without treatment for a further 4 (8 rats/group) or 13 weeks (8 rats/group). Clinical observations, bodyweights and food consumption were measured and at the end of the scheduled periods, the animals were killed and subjected to a necropsy. Salivary glands were weighed and taken for subsequent histopathology examination.

Salivary gland weight was unaffected in the CD rat but was increased in both AP and F344 rats at the end of the administration period. Microscopic examination of the salivary glands showed the most pronounced effect occurred in the F344 strain where there was diffuse cytoplasmic basophilia and enlargement of the parotid acinar cells. Similar but slight effects involving small foci of cells only occurred in the AP and CD strains. Recovery of effects was apparent in all strains during the recovery periods. Bodyweight and food consumption returned to control values in both AP and CD strains. After four weeks on control diet, significant recovery of the salivary gland changes, in terms of both weight and histopathology, was evident in the F344 strain and the AP and CD rats were indistinguishable from their corresponding controls. After 13 weeks on control diet slightly more treated F344 rats showed minor focal changes in the salivary gland compared to the contemporaneous controls and group mean salivary gland weights were increased slightly.

A third mechanistic study is available, which is part of an NTP study performed in rat and mice (Chan and Mahler, 1992). **The applicant is requested to submit this study together with a full evaluation.** This study in F344 rats shows that glyphosate fed during 14 days at 50000 ppm induces parotid and submandibular/sublingual salivary gland weight changes and lesions consisting of cytoplasmic basophilic change, fine vacuolation, and swelling of acinar cells, diagnosed collectively as cytoplasmic alteration. Similar effects were found when the adrenergic agonist isoproterenol was give alone and less severe effects were noted when glyphosate was given together with the adrenergic antagonist propranolol which indicates that at least partially an adrenergic effect may have contributed to the salivary gland findings.

In the previous RAR is was concluded that although there is no evidence of necrosis, apoptosis or inflammation or that the cellular alterations would progress with time to preneoplastic or neoplastic lesions, the organ weight increase and histological alterations in salivary gland are considered clearly treatment-related. **And despite any**

strain differences in sensitivity and a possible role of a low pH of the test substance, at a sufficiently high exposure similar effects in humans cannot be excluded. Therefore these effects should be taken into account for setting NOAELs/LOAELs in individual studies.

In addition, several studies found in the public literature search were classified by the applicant as "relevant but supplementary after detailed assessment of full-text article". Upon review of the titles and abstracts of articles assigned to this category, study summaries were requested by AGG for the studies listed in the table below to further justify the categorization of the information. The study summaries and justification provided by the applicant were reviewed by the RMS and can be found in Volume 3 CA B.6.8.2.

Table 2.6.8.2-2: Summary table of other studies on mechanistic data which are not considered further

Data requirement	Author	Year	Title
CA 5.8.2	Alleva R. Et al.	2018	Mechanism underlying the effect of long-term exposure of pesticides on DNA integrity.
CA 5.8.2	Ren X. Et al.	2018	Effects of glyphosate on the ovarian function of pregnant mice, the secretion of hormones and the sex ratio of their fetuses.

The study by Alleva et al. (2018) is considered to be supportive. Bronchial epithelial cells (BEAS-2B) and neuronal cell line (SHSY-5Y) were used as models to evaluate *in vitro* pesticide-induced DNA damage response (DDR) by exposing them to pure glyphosate and chlorpyrifos ethyl. Under the study conditions glyphosate induces DNA damage by mitochondrial ROS formation in BEAS-2B and SHSY-5Y cells. Increased OGG1-dependent DNA repair activity, associated with gene and protein upregulation of the DNA glycosylase OGG1, was found in cells after 3 h of pesticide treatment, then declining at prolonged time of incubation. The study has the following limitations: purity and source of the test substance were not reported, no positive control was included, only one or two concentrations of glyphosate were tested, negative controls were untreated instead of treated with vehicle, lack of HCD.

In the study by Ren et al. (2018), glyphosate (GLP) and a unknown Roundup formulation (RU) were administered to pregnant mice during GD 1-19 via drinking water. RMS considers this study to be unreliable because of the following reasons: the study was not conducted according to any international guideline, no GLP status, the test substances are not sufficiently characterised, unknown dose of exposure following administration via drinking water, small group size (n=5), exposure during GD1-19 without justification for this window of exposure, only one dose tested, individual data missing, no historical control data or positive control.

In addition the literature search found several studies related to the microbiome. The applicant classified these studies as Category C “unclear relevance after detailed assessment of full-text article”. Upon review of the titles and abstracts of articles assigned to this category, study summaries were requested by AGG for the studies listed in the table below to further justify the categorization of the information. The study summaries and justification provided by the applicant were reviewed by the RMS and can be found in Volume 3 CA B.6.8.2.
Investigation of the gut microbiota is currently not part of the European assessment framework for pesticides. These studies are not considered further for the assessment.

Table 2.6.8.2-3: Summary table of other studies on microbiome which are not considered further

Data requirement	Author	Year	Title
n.a.	Aitbali Y. et al.	2018	Glyphosate based herbicide exposure affect gut microbiota, anxiety and depression-like behaviors in mice
n.a.	Bote K. et al	2019	Minimum inhibitory concentration of glyphosate and of a glyphosate containing herbicide formulation for *Escherichia coli* isolates – Differences between pathogenic and non-pathogenic isolates between host species.
n.a.	Kruger M. et al.	2013	Glyphosate suppresses the antagonistic effect of enterococcus spp. on Clostridium botulinum.

n.a.	Good P.	2018	Evidence by the U.S. autism epidemic initiated by acetaminophen (Tylonel) is aggravated by oral antibiotic amoxicillin/clavulanate (Augmentin) and now exponentially by herbicide glyphosate (Roundup).
n.a.	Lozano V.L. et al.	2018	Sex-dependent impact of Roundup on the rat gut microbiome.
n.a.	Mao Q. Et al.	2018	The Ramazzine Institute 13-week pilot study on glyphosate and Roundup administered at human-equivalent dose to Sprague-Dawley rats: effects on the microbiome.

2.6.8.3 ED studies on the active substance

Endocrine disruption

The overall evaluation for endocrine disruption is reported in chapter 2.10. However, some specific studies were conducted on potential ED properties which are summarized in the table below.

Table 2.6.8.3-1 ED studies on the active substance

Method, guideline, deviations if any	**Test substance**	**Relevant information about the study**	**Observations /Results**	**Reference**
AR binding study US OPPTS/OCSPP 890.1150, GLP Study acceptable	Glyphosate acid, batch GLP-1103-21149-T, purity 95.93% Positive controls: dexamethasone (weak), R1881 (strong)	Deviations: None	Glyphosate was classified as a non-binder in three independent runs.	6500V-100334ARB
ER transactivation study US OPPTS/OCSPP 890.1300, GLP Study not acceptable	Glyphosate acid, batch GLP-1103-21149-T, purity 95.93% Controls: 17β-estradiol, 17α-estradiol, corticosterone, 17α-methyltestosterone	The Log PC_{50} value for 17α-methyltestosterone was not reached which may indicate a decreased sensitivity for the study.	Glyphosate had no effect on the human estrogen receptor alpha.	6500V-100334ERTA
ER binding study US OPPTS/OCSPP 890.1250, GLP Study acceptable	Glyphosate acid, batch GLP-1103-21149-T, purity 95.93% Controls: 17β-estradiol,, 19-norethindrone, octyltriethoxysilane	Deviations: None	Glyphosate was classified as non-interacting for binding to the estrogen receptor.	6500V-100334ERB
Aromatase activity US OPPTS/OCSPP 890.1200, GLP Study acceptable	Glyphosate acid, batch GLP-1103-21149-T, purity 95.93% Positive control: 4-hydroxyandrostendione	Deviations: None	Glyphosate did not inhibit aromatase inhibition	6500V-100334AROM
Uterotrophic assay, OECD 440, GLP Study acceptable	Glyphosate acid, batch GLP-1103-21149-T, purity 95.93%	Deviations: none	Glyphosate did not demonstrate an estrogenic effect.	[redacted]-843002

Method, guideline, deviations if any	Test substance	Relevant information about the study	Observations /Results	Reference
Ovariectomized Crl:CD(SD) rats, 6/females/dose	0, 100, 300 and 1000 mg/kg bw/day for three days; by gavage at PND 66-67 Positive control: 17α-ethinyl estradiol			
Hershberger assay, OECD 441, GLP Study acceptable Castrated Crl:CD(SD) rats, 6/males/dose	Glyphosate acid, batch GLP-1103-21149-T, purity 95.93% 0, 100, 300 and 1000 mg/kg bw/day for ten days; by gavage at PND 54-55 Positive controls: testosterone propionate, flutamide	Deviations: none	Glyphosate did not demonstrate an androgenic or anti-androgenic effect.	[redacted]-843003
Male pubertal assay, US EPA OPPTS/OCSPP 890.1500, GLP Study acceptable Sprague-Dawley rats, 15/males/dose	Glyphosate acid, batch GLP-1103-21149-T, purity 95.93% 0, 100, 300 or 1000 mg/kg bw/day from PND 23 to 53	Slight deviations from the guideline performance criteria range which were not considered to affect the interpretation of the results.	Glyphosate did not demonstrate an androgenic or anti-androgenic effect and did not have an effect on pubertal development or thyroid function.	[redacted]-843005
Female pubertal assay, US EPA OPPTS/OCSPP 890.1450, GLP Study acceptable Sprague-Dawley rats, 15 females/dose	Glyphosate acid, batch GLP-1103-21149-T, purity 95.93% 0, 100, 300 or 1000 mg/kg bw/day from PND 22 to 42	Slight deviations from the guideline performance criteria range which were not considered to affect the interpretation of the results.	Result equivocal A slight but not statistically significant increase in age at first estrus was observed. In addition, a decrease in estrus regularity was observed at 300 and 1000 mg/kg bw/day but this was based on a low number of animals and without dose response.	[redacted]-843007
QSAR analysis QSAR models used: OECD QSAR Toolbox, Vega, Endocrine Disruptome, Danish QSAR database and ToxCast COMPARA/CERAPP consensus models	Glyphosate	Investigated receptors: estrogen receptor (ER), androgen receptor (AR), thyroid receptor (TR), glycocorticoid receptor (GR), mineralocorticoid receptor (MR), liver X receptor (LXR), peroxisome proliferator-activated receptor (PPAR), retinoid X receptor (RXR), aryl hydrocarbon receptor (AhR), pregnane X receptor (PXR), and CYP3A4 receptor	The results of the QSAR analysis did not indicated an endocrine potential. It should be noted that there was a general lack of models for endocrine activity other than estrogen, androgen, steroid and thyroid.	110517-1
In vitro steroidogenesis, public literature study	Glyphosate, purity not reported 0.0001-100 µM	Glyphosate was tested as part of a validation study for OECD 456	Glyphosate had no effect in the *in vitro* steroidogenesis assay.	Hecker, 2011

Method, guideline, deviations if any	Test substance	Relevant information about the study	Observations /Results	Reference
Study reliable (Klimisch Score 1)				
In vitro study on effect of glyphosate on swine granulosa and adipose stromal cells, public literature study Study reliable (Klimisch Score 1)	Glyphosate, purity not reported 0, 0.2, 4 or16 µg/mL	-	Glyphosate decreased cell proliferation, cell viability, estrogen production and ferric reducing capacity and increased progesterone and NO production in granulosa cells. Glyphosate significantly decreased the viability of adipose stromal cells and inhibited their adipogenic differentiation, however, this was only tested at one concentration (4 µg/mL).	Gigante, 2018
In vitro study on TM4 Sertoli cells, public literature study Study reliable (Klimisch Score 1)	Glyphosate, purity not reported Formulations: Glyphogan and Roundup Bioforce 10 to 10,000 ppm glyphosate	-	Glyphosate did not impact cell viability while the formulations did. Glyphosate reduced succinate dehydrogenase but without dose-response. Very high concentrations of glyphosate (2,500 and 5,000 ppm) resulted in an increase in cytoplasmic lipid droplets.	Vanlaeys, 2018
In vitro study on ERalpha, public literature study Study reliable (Klimisch Score 1)	Glyphosate, >98% Four commercial formulations and polyethoxylated tallowamine	-	Glyphosate, but not the other components in glyphosate-based formulations, activated the ERα in breast cancer cells however only at very high concentrations not relevant for the *in vivo* situation.	Mesnage, 2017
In vitro study on ER transactivation, public literature study Study acceptable but with restrictions	Glyphosate, >98%	Study was referred to in publication by Mesnage, 2017. A striking difference was observed between the two studies where in the study by Thongprakaisang effect on the ER started at very low doses of 10^{-12} M while in the study Mesange effect were only observed at very high *in vitro* concentrations.	Glyphosate activated the ER-α and ER-β in breast cancer cells.	Thongprakaisang, 2013
In vitro study on ERα and ERβ, public literature study Study acceptable but with restrictions (Klimisch score 2).	Glyphosate, purity not reported.	Limitations noted: unclear which concentrations were tested, only limited results were provided (no individual data shown)	Glyphosate had no effect on ERα and ERβ.	Brennan, 2016
In vitro aromatase inhibition, public literature study	Glyphosate, purity not reported. Several formulations and co-formulants.	-	Glyphosate only affected aromatase inhibition at very high concentrations (17.7 mM) while the co-formulants	Defarge, 2016

Method, guideline, deviations if any	Test substance	Relevant information about the study	Observations /Results	Reference
Study reliable (Klimisch Score 1)			and formulations showed stronger effects.	
In vivo study on folliculogenesis and steroidogenesis in mice following exposure to glyphosate, public literature study Study reliable (Klimisch Score 1)	Glyphosate, batch and purity not reported	Oral treatment of C57BL/6 J female mice (PND 42) with 2 mg/kg bw glyphosate for 5 or 10 weeks (10 animals /treatment)	No effect observed compared to the negative control (saline) on parameters representative for folliculogenesis and steroidogenesis (body weight, cyclicity, follicle number, circulating ovarian steroid hormone levels and ovarian intracellular signalling parameters)	Ganesan, 2020
In vitro study on cell migration, invasion and DNA expression of Ishikawa endometrial cancer cells following exposure to glyphosate, public literature study Study reliable (Klimisch Score 1)	Glyphosate, batch and purity not reported	Treatment of Ishikawa endometrial cancer cells with 0.2 and 2 µM glyphosate and testing of cell migration (scratch-wound healing assay), cell invasion (transwell invasion assay), and quantification of E-cadherin and vimentin expression.	Induction of cell migration and invasion, and downregulation of E-cadherin mRNA expression following exposure to glyphosate and 17β-estradiol (positive control). Observations are reversible when co-treating cells with an ER antagonist (fulvestrant). In conclusion, results indicate *in vitro* epithelial-mesenchymal-transition-related changes upon treatment with glyphosate.	Gastiazoro, 2020
In vitro study on the effects of glyphosate on testosterone secretion and the role of endoplasmic reticulum (ER) stress in the process in TM3 cells (murine Leydig cell line endoplasmic reticulum (ER), public literature study Study acceptable but with restrictions (negative control unknown)	Glyphosate, batch and purity not reported	Treatment of TM3 cells (murine Leydig cell line) with 0.01-2000 mg/L glyphosate (cell viability and testosterone secretion assay) or 5 mg/L glyphosate (western blot and immunofluorescence analyses)	Cell viability significantly decreased ≥ 500 mg/L, significant decrease in testosterone secretion at ≥ 0.5 mg/L. Glyphosate (5 mg/L) inhibited expression of testosterone synthases (StAR and CYP17A1), induces ER stress, and activated PERK/eIF2α signalling pathway. These observations were not seen when cells were pre-treated with an inhibitor of ER stress (PBA) or PERK inhibitor (GSK2606414).	Xia, 2020
In vivo study in male rats analysing the possible effect of a formulation on adrenal gland; public literature study Study reliable with restrictions	Unknown Roundup formulation that might contain POAE, batch and purity not reported	Exposure once daily by oral gavage for 14 days at doses of 10 and 50 mg/kg bw/day. The effect of adrenocorticotropic hormone (ACTH) on adrenal gland steroidogenesis was examined. Body	The final doses selected for analysis (10 and 50 mg/kg bw/day) were too high for the purpose of this study, as a significant decrease in body weight was observed (-19 to -25% decrease compared to control). A decrease was seen in circulatory corticosterone levels; no change in total	Pandey, A. et al, 2015

Method, guideline, deviations if any	Test substance	Relevant information about the study	Observations /Results	Reference
(unknown formulation, no HCD or positive control, no GLP, low and varying number of animals in test groups, no individual data, clinical observations not given)		weight and food consumption were recorded; adrenal glands were weighed; plasma corticosterone levels were determined; cholesterol was determined in adrenal gland tissue lysate and plasma; mRNA expression of key regulatory receptors, enzymes and carrier proteins involved in cholesterol homeostasis and steroidogenesis were determined by qPCR.	cholesterol levels was found in the circulation but was moderately higher in adrenal gland after treatment with Roundup. There was a downregulation of genes associated with cholesterol intake and de novo synthesis. Higher levels of esterified or stored form of cholesterol in adrenal gland of treated rats. The responsiveness of adrenal gland to external ACTH was similar or higher compared to vehicle control animals suggesting the process of steroidogenesis in adrenal gland appears intact after Roundup treatment.	
In vitro study on effect of glyphosate on estrogen signalling pathway involved in the induction of CCA cells growth; public literature study Reliable with restrictions (limited info on glyphosate used, no GLP, no HCD)	Glyphosate (>98%), batch not reported	Cholangiocarcinoma cells (CCA) treated with glyphosate. Cell cycle analysis was performed; qRT-PCR to determine mRNA expression of ERα, ERβ1, ERβ2, ps2, progesterone receptor (PR) and β-actin ; Western blotting and immunofluorescent staining.	Estradiol induced cell proliferation in HuCCA-1 cells but not in RMCCA-1 and MMNK-1 cells. Glyphosate induced huCCA-1 cell proliferation. After treatment with glyphosate or estradiol, S-phase cell cuycle and protein levels of cyclin family increased. Both compounds induced expression proliferative singaling-related proteins. Effects of glyphosate and estradiol were abolished by ER-antagonist 4-hydroxytamoxifen.	Sritana, N. et al, 2018
Effect glyphosate on testosterone synthesis in male SD rats; public literature study Reliable with restrictions (no batch info, high dose levels without justification, general toxicity not considered, no GLP, missing details methods, no HCD, no positive control)	Glyphosate (95% purity), batch not reported.	Four groups: control (CMC), 50, 200 and 800 mg/kg bw/day for 4, 8 or 12 weeks. Serum hormone levels (FSH, LH, T, E2) measured by RIA; expression levels 17β-HSD, 3β-HSD, StAR and P450scc were determined; histpathology testis	Glyphosate decreased the testis to body weight ratio (high dose 12 weeks) with histopathological findings. FSH and testosterone levels were decreased and expression levels of StAR and P450cc protein were decreased in the mid- and high-dose groups. High doses were tested without justification and no information on general toxicity is given, therefore, it cannot be determined if	Zhao, H. et al., 2018

Method, guideline, deviations if any	Test substance	Relevant information about the study	Observations /Results	Reference
			effects seen might be secondary to general toxicity.	

2.6.9 Summary of medical data and information

Reports on medical surveillance on manufacturing plant personnel

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

Direct observations (reports on clinical cases and poisoning incidents)

As reported in the previous RAR (2015): For better understanding of the following, it must be emphasised that all the poisoning or irritation incidents resulted from exposure to glyphosate-containing plant protection products but not to the active ingredient. Thus, in principle, it is not possible to distinguish if they were due to the active substance or rather to co-formulants. From animal experiments, however, it is known that glyphosate acid was irritating to the eyes (i.e., a sign that is frequently associated also with mucosal irritation) but of low toxicity via all relevant routes. Even eye irritation was less pronounced in studies with glyphosate salts as compared to the acid. These salts are used for formulation of commercial products. Accordingly, one might assume that (frequently irritant or even corrosive) co-formulants will have contributed the most to intoxications following systemic intake and perhaps also to the irritation in cases of eye contact with glyphosate-based herbicides.

The extensive use of glyphosate as an active ingredient in herbicides worldwide, rather than the (low) toxicity of this compound, may explain the relatively large number of poisoning incidents that happened and were published. Extensive reviews of clinical cases were published by Bradberry *et al.* (2004, B.6.9.8.16) and by Lee *et al.* (2000, B.6.9.8.23). The applicant provided an overview of the glyphosate-related case reports of the National Chemical Emergency Centre (NCEC) in the UK over the years 2017-2019. Most calls were related to incidents that occurred after normal use and involved exposure to the skin, eyes, orally, through inhalation or on clothes. These calls concerned mostly small amounts accidentally sprayed in face or eyes or spilled on the body and were characterized by the emergency responders as of low severity. Most incidents were in male adults, whereas only few incidents with children or animals were reported. Two high severity calls both likely involved intentional overexposure to the product.

Most reported non-suicidal exposures in public literature involve skin and/or eye irritation or irritation of the respiratory tract by inhalation of spray mist (Goldstein *et al*., 2002 (B.6.9.8.19)). Systemic symptoms are rare following non-suicidal exposures to glyphosate products. The reported systemic symptoms appear unlikely to be causally related to exposure or it is not possible to distinguish if they were due to the active substance or rather to co-formulants (Goldstein *et al*., 2002 (B.6.9.8.19)).

What is known on the course of clinical cases, signs and symptoms is summarised below (copied from the summary provided in the previous RAR (2015) and additional information added were necessary). The studies are presented in greater detail in Volume 3 CA B.6.9.

<u>Clinical signs and symptoms of poisoning – skin and eye contact</u>
The vast majority of reported clinical signs following exposures (apart from attempted suicides or rare accidents) comprise skin and/or eye irritation or irritation of the respiratory tract by inhalation of spray mist. Contact with the skin may produce a dermatitis similar to that caused by detergents (Bradberry *et al.,* 2004 (B.6.9.8.16)) although the

active ingredient was not irritating to the skin in laboratory animals. Phototoxic reactions [sunlight or ultraviolet (UV) light induced skin reactions] have been reported. The study authors believed this might be caused by an antimicrobial additive (benzisothiazolone) which is a known skin sensitizer and is present in certain residential use (*i.e.*, non-agricultural) products containing 10% glyphosate or less (Bradberry *et al.*, 2004 (B.6.9.8.16)). The RMS notes that according to an evaluation by the Scientific Committee on Consumer Safety[24] in 2012, benzisothiazolone is not considered phototoxic. Further, it is further noted that dermal absorption of glyphosate is low (<1% for the concentrate and dilution).

Eye exposures have generally resulted in temporary conjunctival irritation, clearing either after irrigation or within 1-2 days. A review of ocular exposures to US glyphosate-surfactant formulations (1513 exposures over a 5-year period), showed no permanent eye injury (Bradberry *et al.*, 2004 (B.6.9.8.16); Acquavella *et al.*, 1999 (B.6.9.8.12)). Eye contact is not expected to cause systemic effects or serious ocular injury.

Clinical signs and symptoms of poisoning – oral intake

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

Minor gastrointestinal exposures are likely to be asymptomatic but the patient may experience an unpleasant taste, tingling, mild self-limiting nausea and vomiting. Self-limiting diarrhoea may also occur. After significant exposures, a burning sensation in the mouth and throat, salivation, oral erythema, sore throat, dysphonia, dysphagia, epigastric pain, nausea, spontaneous vomiting, abdominal pain and diarrhoea are common and may last up to a week. Serum amylase may be elevated and isoenzyme analysis done in a few cases identified a salivary gland origin (Tominack *et al.*, 1989 (B.6.9.8.27)). In severe cases with large ingested doses, hematemesis, GI bleeding, melena and hematochezia may occur. Paralytic ileus has been reported as a rare event. Endoscopy has noted erosions of the pharynx and larynx, esophagitis and gastritis with mucosal oedema, erosions and haemorrhage. Transmural injury and perforation have not been noted on panendoscopy (Chang *et al.*, 1999 (B.6.9.8.18)). In fatal cases, autopsy notes mucosal or transmural oedema and necrosis throughout the small bowel with erosion and haemorrhage; in the large bowel, mucosal oedema and focal haemorrhage was noted (Tominack *et al.*, 1989 (B.6.9.8.27)).

Hypotension is common after ingestion of a mouthful or more of the concentrated product (not the diluted forms) and will usually favourably respond to intravenous administration of fluids and pressor amines. If not responsive to this treatment, however, hypovolemic shock may result in oliguria, anuria, organic failure and ultimately in death. Severe or prolonged vomiting and diarrhoea may induce fluid and electrolyte imbalance. Tachypnea, dyspnea, cough and bronchospasm including cyanosis have been seen in severe ingestions. Transient hypertension may also occur. In laboratory analysis, abrupt rises in BUN and serum creatinine may be seen. Hemoconcentration can result result from intravascular volume depletion and could possibly indicate severe capillary fluid leakage ((Tominack *et al.*, 1989 (B.6.9.8.27)); (Bradberry *et al.*, 2004 (B.6.9.8.16)). Several case reports indicate clinically significant hyperkalemia following ingestion of large amounts of glyphosate-potassium salt concentrate solutions (Bando *et al.*, 2010 (B.6.9.8.14)); Kamijo *et al.*, 2012 (B.6.9.8.21)) resulting in electrocardiographic changes consistent with hyperkalemia. In both cases, patients had a concomitant severe metabolic acidosis.

No direct hepatotoxic effects have been noted; however, minor elevations in transaminases and bilirubin are reported ((Tominack *et al.*, 1989 (B.6.9.8.27)); (Bradberry *et al.*, 2004 (B.6.9.8.16)). A 2018 case report describes a patient who developed fulminant hepatic failure after opening a bottle of formulated glyphosate with his mouth and accidentally ingesting a mouthful of the product (Khot *et al.*, 2018 (B.6.9.8.22)). However, based on other case reports of relatively small ingestions showing no liver damage and based on the fact that no acute effects are observed on the liver in the guideline animal studies, it is not likely that a small ingestion would cause fulminant hepatic failure.

Metabolic acidosis is often seen in a severely poisoned patient (Bradberry *et al.*, 2004, (B.6.9.8.16)) and may fail to respond to bicarbonate therapy. Although the exact aetiology is unknown, a lactic acidosis is suspected. There have been no reports of primary convulsions after ingestion and most patients are present with a clear sensorium unless another substance, such as alcohol, has been co-ingested or severe hypoxemia has occurred (Tominack *et al.*, 1989 (B.6.9.8.27)). However, in other cases, "moderate disorders of consciousness" have been reported within 48 hours

[24] https://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_099.pdf

after ingestions of the concentrate with suicidal intention (Sawada and Nagai, 1987 (B.6.9.8.25); Sawada *et al.*, 1988 (B.6.9.8.26)). Aspiration pneumonia, pulmonary oedema and respiratory failure have been seen although the exact role of aspiration has not been fully investigated. Mild fever may occur even in the absence of infection. In addition, leukocytosis without evidence of bacterial infection has been noted in peripheral blood after ingestion of the concentrate (Bradberry *et al.*, 2004, (B.6.9.8.16)). No direct hepatotoxic effects have been noted; however, minor elevations in transaminases and bilirubin were reported (Tominack *et al.*, 1989 (B.6.9.8.27); Bradberry *et al.*, 2004 (B.6.9.8.16)). Respiratory distress requiring intubation, pulmonary oedema, shock (systolic BP < 90 mm Hg), altered consciousness, abnormal chest X-ray, ingestion of over 200 cc concentrate (41 %), or renal failure making dialysis necessary have been associated with a higher risk of poor clinical outcomes including mortality (Lee *et al.*, 2000 (B.6.9.8.23)). These authors also developed a prognostic index based upon these factors. However, as onset of symptoms may be delayed, early use of such prognostic indicators and too much reliance on them may lead to an under-estimate of clinical severity.

Cardiovascular effects are not expected from minor exposures. A recent case report describes a patient who presented with syncope and a wide-complex tachycardia where the authors claim that there is a prolonged QTc while measuring a wide complex beat (Brunetti *et al.*, 2020 (B.6.9.8.17)). The patient, when pressed repeatedly about pesticide exposure, said that she had spilled a small amount of formulated glyphosate on her hand the previous evening. The authors attribute her syncope and arrythmia to this minor exposure. Since significant absorption through the skin does not occur (generally less than 1%), it is not possible to conclude on causality

Clinical signs and symptoms of poisoning – Inhalation

An isolated case report from Israel suggests the development of acute pneumonitis in a worker (smoker) shortly after he had repaired a spraying device (not in operation). From ”occupational history”, the occupational physicians concluded that he had been exposed to Roundup herbicide and suspected a polyoxyethylene amine surfactant in the product as the possibly responsible agent (Pushnoy *et al.*, 1998 (B.6.9.8.24)). However, actual exposure and its extent could not be really substantiated in this case. Accordingly, the occurrence of pneumonitis in this individual is more likely to be coincidental by nature although a (different) occupational origin seems plausible (Goldstein *et al.*, 1999 (B.6.9.8.19)).

However, Burger *et al.* (2009, publication not submitted as publication date is >10 years before submission of the current dossier, the assessment is copied from the previous RAR (2015)) also reported severe acute dyspnoea, rise in body temperature and histological lung changes (acute alveolitis and bronchiolitis) in a 59-year old German farmer who had sprayed a herbicide containing glyphosate on a warm day for three hours without respiratory protection. First clinical symptoms occurred seven hours after spraying. The patient was given i.v. steroids at high doses and antibiotic cover. This therapy was successful but six months later, he still complained of moderate breathing difficulties under conditions of exercise. It was suspected that the combination of glyphosate with the tallowamine surfactant in the formulation might have caused this incident. In addition, in the same reference, 20 cases of inhalative exposure among a total of 60 reports on confirmed or presumed poisoning incidents with glyphosate herbicides from Germany (since 1990) were mentioned with breathing difficulties occurring in 50 % of the affected people. No more details on clinical courses or outcomes were given but it was emphasised by the authors as ”striking” that the involved products nearly always contained tallowamines.

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

In Volume 3 in section B.6.9.8.30 an overview of case reports from public literature over the period 2010-2019 is presented. No additional findings arose from these case studies.

First aid measures and therapeutic regimes

First aid measures and therapeutic regimes have been proposed by the applicant and presented in Volume 3 CA B6.9 and may be found in Volume 3 CA B.6.9.6, but were not evaluated by the RMS.

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

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

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

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

- Following minor or incidental ingestions, or ingestion of fully diluted formulations, gastrointestinal upset with nausea, vomiting, and diarrhoea may occur. Nausea and vomiting usually resolve within a few hours of ingestion. Diarrhoea may last for several days but is generally not severe. Following ingestion of a larger amount, the onset of systemic symptoms may be delayed by several hours. For serious ingestions having major electrolyte disturbances or life threatening alterations of cardiovascular performance, medical intervention may be life saving. Fatalities due to cardiovascular failure are generally delayed by 12 – 36 hours. For serious but non-fatal cases, primary clinical injury generally is manifest within 72 hours but secondary complications such as infection or respiratory distress syndrome may supervene. The majority of serious but surviving cases will fully recover within 7-10 days of ingestion. Individuals with complicate clinical courses can require a more extended and highly variable time to recover.

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

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

Observations on exposure of the general population, human biomonitoring and exposure assessment

Urinary concentrations of glyphosate in humans

In the previous assessment by RMS DE (RAR 2015) seven studies from Europe and US were described in which in which urine samples obtained from humans following either occupational or dietary exposure (sometimes perhaps overlapping) had been analysed for glyphosate (Acquavella et al. (2004, ASB2012-11528); Mage (2006, ASB2012-11888); Curwin et al. (2006, ASB2012-11597); Mesnage et al. (2012, ASB2014-3846); Hoppe (2013, ASB2013-8037); Markard (2014; ASB2014-2057); Krüger et al. (2014; ASB2014-5024)).
Based on these urinary monitoring studies, it was concluded in the RAR (2015) that:
" • Current analytical techniques allow the detection and determination of much lower amounts of glyphosate in human urine than in the past. The results obtained with different methods are not that much different and, to some extent, confirm each other.
• Positive glyphosate findings in human urine are quite common and may result from occupational or residential exposure, from dietary intake or from both. The origin may often not be clearly distinguished and will probably overlap sometimes.
• Urinary concentrations in operators after application of plant protection products tend to be higher than those resulting from dietary intake of glyphosate by consumers.
• The by far highest concentrations were measured in the urine of one operator and his son and may indicate that the recommended protective measures were not properly taken.
• Even though the data is not representative, mean urine concentrations in consumers in the U.S. appear higher than those found in Europe. This result is likely to reflect differences in the agricultural use of glyphosate-based herbicides and the plantation of glyphosate-resistant, genetically modified crops in North America.
• There is a trend towards increasing glyphosate concentrations in measured urine samples also in Europe, probably reflecting more sensitive analytical techniques, more frequent use in agricultural practice in Europe or higher residues in imported foodstuffs.
• All measured values, even the highest, were of no health concern. The calculated human exposures were at least one order but mainly two or more orders of magnitude lower than the ADI and AOEL.
• The same holds true if urine concentrations of AMPA are taken into account. However, correlation between glyphosate and AMPA in urine is poor suggesting that other sources of AMPA than metabolism of glyphosate in plants should be considered."

In the current assessment, five additional studies were submitted which investigated human urinary glyphosate levels. All studies were in line with the previous conclusions.

A newly submitted biomonitoring survey involved the collection and analysis of 20 ml spot urine samples from 50 Irish adults in a non-occupational setting (Connolly *et al*., 2018b (B.6.9.8.4)). The LC-MC/MS analyses of urinary samples revealed that 20% of the samples analysed contained detectable levels of glyphosate (the limit of detection (LOD) was set at 0.5 µg/L (signal to noise ratio of ≥ 3:1)). The urinary glyphosate levels ranged from 0.80 to 1.35 µg/L, which is around or below the levels that were reported in the publications that were assessed in the previous RAR (max mean urinary level of 3.2 µg/L). The relatively low proportion of detectable glyphosate levels could be due to lower localised use of pesticides, or due to a small sample size or due to the higher analytical detection limit used in this study (0.5 µg/L) compared to other studies.

The study by Conrad *et al.* (2017; B.6.9.8.6) reported on the internal exposure of the general German population to glyphosate and its metabolite AMPA and the change over time. The study shows that from 2001 – 2015 31.8% of analysed samples contained detectable levels of glyphosate above the LOQ of 0.1 µg/L with a peak levels in 2012 and 2013. The 95th percentiles of glyphosate concentrations in 24 h-urine were substantially higher in 2013 (1.25 µg/L) and 2014 (0.80 µg/L) compared to all other years. Also the maximum concentrations of glyphosate peaked in these two years (2013: 2.80 µg/L, 2014: 1.78 µg/L). According to the study authors these highest urinary levels are a factor 1000 below the excretion that is expected when exposed at the ADI, which is in line to what was previously reported (RAR, 2015). Urinary levels of AMPA were correlated to glyphosate levels.

In the study by McGuire *et al.* (2016; B.6.9.8.8) reported on glyphosate and AMPA concentrations in urine of 0.28 and 0.30 µg/L, respectively in 41 lactating women (also refer to next paragraph). The LOD and LOQ for glyphosate in urine were 0.02 and 0.10 µg/L, respectively, and those for AMPA in urine were 0.03 and 0.10 µg/L, respectively. No difference was found in urine glyphosate and AMPA concentrations between subjects consuming organic compared with conventionally grown foods or between women living on or near a farm/ranch and those living in an urban or suburban non-farming area.

In a comparative cross-sectional study (Sierra-Diaz *et al.*, 2019 (B.6.9.8.9) using the urine of children living in two agricultural communities in Mexico, the presence of glyphosate was detected in more than 70% of the cases (LOD and LOQ not reported). The mean urinary level of glyphosate were 0.363 ng/mL in Agua Caliente and 0.606 ng/mL in Ahuacapán, detected in 73% and 100% of their respective total samples.

The study by Trasande *et al.* (2020; B.6.9.8.11) showed detectability of glyphosate in 8 to 30% of samples of children's urine obtained from children at various ages (LOD for glyphosate was 0.1 ng/mL, and LOQ was 0.33 ng/mL). The mean urinary glyphosate concentration was 0.278 ng/mL, with a range of 0.105-2.125 ng/mL. There was no evidence for renal injury in children exposed to low levels of glyphosate.

Glyphosate in human breast milk

In the previous assessment RAR (2015) glyphosate findings in human breast milk had been reported, but were considered not reliable (for discussion, refer to RAR (2015) Vol. 3, B.6.9.3). Even if the measured values were true, no health concern for breast-fed infants would have resulted.

In the current assessment, two additional studies were presented which showed that glyphosate levels in human breast milk samples were below the LOD/LOQ. In the first study by McGuire *et al.* (2016; B.6.9.8.8) breast-milk samples of 41 lactating women were analysed in order to determine whether glyphosate and its metabolite AMPA could be detected. In parallel, urinary samples were obtained from the same women. In none of the breast milk samples glyphosate or AMPA was detected (LOD of 1 µg/L and LOQ of 10 µg/L for both analytes). Because of the complex nature of milk matrixes, these samples required more dilution before analysis than did urine, thus decreasing the sensitivity of the assay in milk compared with urine. In a second study, human breast milk samples of 114 German lactating women were analysed. All samples the glyphosate concentrations were at or below the LOQ of 1 ng/mL (Steinborn *et al.*, 2016 (B.6.9.8.10)).

The RMS noted that in Volume 3 section B.6.10 one additional study was reported investigating glyphosate in human breast milk samples (Abdel-Halim, 2019). The applicant reported that the reason for not submitting this study was that this study was considered supplementary due to several limitations. AGG disagrees and requests the applicant to submit this publications and to provide an assessment of the findings in order to evaluate the findings.

Other general public exposure studies

Kongtip *et al.* (2017; B.6.9.8.7) reported on a longitudinal study in which determined glyphosate concentrations in maternal and umbilical cord serum in 82 pregnant women who gave birth in three provinces of Thailand. Through questionnaires and biological samples collected at childbirth, factors such as personal characteristics, family members occupation, agricultural activities, and herbicide use in agricultural work were evaluated as predictors of glyphosate levels in the pregnant women. The glyphosate concentrations in the pregnant women's serum at childbirth (median: 17.5, range: 0.2–189.1 ng/mL) were significantly higher than those in the umbilical cord serum (median: 0.2, range: 0.2–94.9 ng/mL). Women with glyphosate levels > LOD in serum at childbirth were 12 times more likely to report work as an agriculturist ($p < 0.001$), 4 times more likely to live near agricultural areas ($p = 0.006$), and 6 times more likely to have a family member who worked in agriculture ($p < 0.001$).

Occupational exposure studies

The publication by Connolly *et al*. (2018a, B.6.9.8.1) describes an operator exposure study in amenity horticulturalists in which glyphosate exposure was monitored by urinary sampling after three types of applications (manual knapsack, pressurized handheld lance and one using a controlled droplet applicator) of glyphosate-based products. Glyphosate concentrations were below LOQ in 27% of the urinary samples, of which 38% were pre-task samples and 38% were following morning void samples. The mean peak glyphosate urine concentrations reported were 1.9 µg/L with a maximum of 7.4 µg/L in contrast to a mean of 0.68 µg/L for pre-task samples and 0.83 µg/L for following morning void samples. Of the workers, 100% wore gloves, 90% a Tyvec suit and 97% used RPE. However, 55% of workers reported to reuse PPE. Based on the same dataset of urinary samples gathered in amenity horticulturalists after glyphosate application, Connolly *et al*. (2019a, B.6.9.8.2) derived an average glyphosate half-life of approximately 5.5 to 10 hours. The same authors also published another occupational exposure study in amenity horticulturalists using glyphosate by urinary biomonitoring (Connolly *et al*. (2017, B.6.9.8.1). In this study, urinary glyphosate concentrations in post-work samples had a geometric mean of 0.66 µg/L. In a third publication by the same authors (Connolly *et al*., 2019b (B.6.9.8.5)), the perioral exposure the and potential and actual hand exposure to glyphosate with the use of different types of gloves was determined. The combined hand and perioral

region glyphosate concentrations explained 40% of the variance in the urinary (μg/L) biomonitoring data in amenity horticulturalists after application of glyphosate-based products.

Epidemiological studies

In the study by Avgerinou et al, (2017), a hospital-based myelodysplastic syndrome (MDS) case-control study was conducted in two public hospitals in Greece. A total of 228 individuals (126 cases, 102 controls) were interviewed based on a questionnaire regarding demographics, occupational exposures, smoking, alcohol intake, dietary and domestic factors. In the study an increased risk of MDS in subjects exposed to glyphosate (Roundup) was reported, although not statistically significant. Exposure to pesticides was significantly associated with the risk of MDS in a multivariate analysis. The study is considered to be unreliable. When participants reported exposure to pesticides, they were asked to recall brand names of the products they had been exposed to, therefore, effects caused by co-formulants cannot be excluded, nor can the effects of recall bias be excluded. This was reported as one of the limitations of the present study together with the non-blind interviewers. In addition, the sample size of the study (126 cases and 102 controls) is considered to be too small. Overall, the major drawback in this study is a selection bias.

Caballero *et al.* (2018) examined the relationship between assumed residential exposure to agricultural chemicals and premature mortality from Parkinson's disease (PD) in Washington State. In the multivariable-adjusted models used in this study, the residential exposure to pesticide that was associated with all cropland was not significantly related to premature death by PD, but the OR was in the hypothesized direction (OR=1.19, 95%-CI = 0.98-1.44). The residential exposure to agricultural land associated with glyphosate had a statistically significant OR for premature mortality associated with PD (OR=1.33, 95%-CI = 1.06-1.67). However the study design does have its limitations, including no exposure data are available (exposure is assumed based on residential proximity at time of death to agricultural land), unclear to what extent glyphosate based herbicides were applied and no information on previous exposures and confounding factors such as lifestyle factors were not included. The study is considered to be reliable with restrictions.

Cremonese *et al.* (2017) performed a cross-sectional study of potential effects of exposure to pesticides and reproductive hormones, semen quality and genital measures among young men in the south of Brazil. This study concluded that chronic occupational exposure to pesticides might affect reproductive outcomes in young men. The study specifically reports a statistically significant association between glyphosate use for 6 or more years with reduced LH levels and lowered sperm morphology. The study is considered to be reliable with restrictions, as the study has a low sample size with limited age categories (18-20, 21-23) and the study design does not allow to identify effects of specific active substances; the statistically significant effects are found on groupes categories of pesticides (e.g. fungicides, herbicides).

2.6.10 Toxicological end points for risk assessment (reference values)

Table 67: Overview of relevant studies for derivation of reference values for risk assessment

Species	Study (method/type, length, route of exposure)	Test substance	Critical effect	NOAEL	LOAEL	Cross reference
Sub-chronic studies						
Rat	OECD407, repeated dose 28-day oral (diet)	Glyphosate 0, 200, 2000, or 20000 ppm	No adverse effects observed.	20000 ppm (equivalent to 1895 mg/kg bw/day in males 2251 mg/kg/day in females)	-	CA 5.3.1/001 CA 5.3.1/002 CA 5.3.1/003 [redacted].881.28 DDR (1991)
Rat	OECD407, repeated dose 28-day oral (diet)	Glyphosate 0, 50, 250, 1000 and 2500 mg/kg bw/day	Decreased body weight gain in females, increased alkaline phosphatase in males and females, increased bilirubin in females and soft stool in males.	1000 mg/kg bw/day	2500 mg/kg bw/day	CA 5.3.1/004 5626 (1991)
Rat	OECD407, repeated dose, dose-range finding study 28-day oral (diet)	Glyphosate 0, 30000, 40000 or 50000 ppm	Reduced body weight gain (all dose levels), reduced food consumption (at mid and high dose), soft stool (all dose levels) and diarrhoea (mid and high dose levels).	-	30000 ppm (equivalent to 1921 mg/kg bw/day for males and 0, 2311 mg/kg bw/day for females	CA 5.3.1/005 [redacted]-8921 (1989)
Rat	No guideline, repeated dose, dose-range finding study 28-day oral (diet)	Glyphosate 80, 235 or 800 mg/kg bw/day	No adverse effects observed.	No NOAEL derived due to limited reporting	-	CA 5.3.1/006 77-2110 (1978)
Rat	OECD408, repeated dose 90-day oral (diet)	Glyphosate 0, 1000, 5000 or 20000 ppm	Decreased body weight gain in males and increased alkaline phosphatase in males and females.	5000 ppm (equivalent to 413.5 mg/kg bw/day in males and 446.9 mg/kg bw/day in females	20000 ppm (equivalent to 1612 mg/kg bw/day in males and 1821 mg/kg bw/day in females	CA 5.3.2/001 CA 5.3.2/002 [redacted]/P/1599 (1996)
Rat	OECD408, repeated dose 90-day oral (diet)	Glyphosate	Increased alkaline phosphatase in females. Caecum atrophy in males and females.	1000 ppm (equivalent to 79 mg/kg bw/day for males and	10000 ppm (equivalent to 730 mg/kg bw/day for males and 844	CA 5.3.2/003

Species	Study (method/type, length, route of exposure)	Test substance	Critical effect	NOAEL	LOAEL	Cross reference
		0, 1000, 10000 or 50000 ppm		90 mg/kg bw/day for females)	mg/kg bw/day for females)	434/016 (1996)
Rat	OECD408, repeated dose 13-week oral (diet)	Glyphosate 0, 3000, 10000 or 30000 ppm	Distension of the caecum in males and increased absolute/relative weight of caecum in males and in females.	3000 ppm (equivalent to 168.4 for males and 195.2 mg/kg bw/day for females)	10000 ppm (equivalent to 569 for males and 637 mg/kg bw/day for females)	CA 5.3.2/004 [redacted] 94-0138 (1995)
Rat	OECD408, repeated dose 13-week oral (diet)	Glyphosate 0, 2000, 6000 or 20000 ppm	Diarrhoea in both sexes, blood in urine, decreased body weight gain in both sexes, decreased absolute and relative adrenal weights in males, increased absolute and relative spleen weight in females.	6000 ppm (equivalent to 371.9 for males and 481.2 for females)	20000 ppm (equivalent to 1262 mg/kg bw/day for males and 1687 mg/kg bw/day for females)	CA 5.3.2/005 CA 5.3.2/006 CA 5.3.2/007 011-0001 (1993)
Rat	OECD408, repeated dose 90-day oral (diet)	Glyphosate 0, 200, 2000 and 20000 ppm	Decreased body weight in females, increased alkaline phosphatase in males and increased blood glucose in females.	2000 ppm (equivalent to 147.3 mg/kg bw/day for males and 195.7 mg/kg bw/day for females)	20000 ppm (equivalent to 1359 mg/kg bw/day for males and 2012 mg/kg bw/day for females)	CA 5.3.2/008 CA 5.3.2/009 CA 5.3.2/010 [redacted]882.90 OR (1992)
Rat	OECD408, repeated dose 13-week oral (diet)	Glyphosate 0, 30, 300 and 1000 mg/kg bw/day	Increased incidence of (minor) parotid cellular alteration in females.	-	30 mg/kg bw/day	CA 5.3.2/011 7136 (1991)
Rat	OECD408, repeated dose 90-day oral (diet)	Glyphosate 0, 2000, 3000, 5000, or 7500 ppm	No adverse effects observed.	7500 ppm (equivalent to 375 mg/kg bw/day for males and females)	-	CA 5.3.2/013 [redacted]-891002 (1989)
Rat	OECD408, repeated dose 90-day oral (diet)	Glyphosate 0, 950, 4600 and 19000 ppm	No adverse effects observed.	19000 ppm (equivalent to 1267 mg/kg bw/day for males and 1623 mg/kg bw/day for females)	-	CA 5.3.2/014 [redacted]-7375 (1987)
Mouse	OECD408, repeated dose	Glyphosate	Decreased food consumption in first week in males,	10000 ppm	50000 ppm	CA 5.3.2/017

Species	Study (method/type, length, route of exposure)	Test substance	Critical effect	NOAEL	LOAEL	Cross reference
	90-day oral (diet)	0, 5000, 10000 or 50000 ppm	increased alkaline phosphatase in both sexes, increased blood phosphorus in females, increased creatinine phosphokinase in females, distension of the caecum and increased absolute/relative caecum weight in both sexes, and an increased incidence of cystitis in the urinary bladder in males.	(equivalent to 1221 mg/kg bw/day for males and 1486 mg/kg bw/day for females)	(equivalent to 6295 mg/kg bw/day for males and 7435 mg/kg bw/day for females)	[redacted] 94-0136 (1995)
Mouse	OECD408, repeated dose 13-week oral (diet)	Glyphosate 0, 200, 1000 or 4500 mg/kg bw/day	No adverse effects. However, limited clinical chemistry parameters investigated.	4500 mg/kg bw/day (NOAEL of limited value)	-	CA 5.3.2/018 7024 (1991)
Mouse	OECD408, repeated dose 13-week oral (diet)	Glyphosate 0, 5000, 10000 or 50000 ppm	Decreased body weight and body weight gain in males and females. Limited haematology and clinical chemistry parameters investigated.	10000 ppm (equivalent to 1867 mg/kg bw/day for males and 2735 mg/kg bw/day for females) (NOAEL of limited value)	50000 ppm (equivalent to 9707 mg/kg bw/day for males and 14858 mg/kg bw/day for females) (LOAEL of limited value)	CA 5.3.2/019 77-2111 (1979)
Dog	OECD409, repeated dose 13-week oral (capsule)	Glyphosate 0, 30, 300 or 1000 mg/kg bw/day	Clinical signs; early sacrifice of two moribund animals and termination of high dose groups after 11 weeks for humane reason; decreased final body weight and body weight gain in males and females; reduced food consumption in both sexes; clinical chemistry alterations and urine parameters alterations, atrophy of prostate and uterus; histological lesions in many	300 mg/kg bw/day	1000 mg/kg bw/day	CA 5.3.2/020 29646[redacted] (2007)

Species	Study (method/type, length, route of exposure)	Test substance	Critical effect	NOAEL	LOAEL	Cross reference
			organs (such as kidney liver, bone marrow).			
Dog	OECD409, repeated dose 13-week oral (diet)	Glyphosate 0, 200, 2000 or 10000 ppm	Decreased food consumption in week 2 in both sexes, increased GGT in males and females, alkaline phosphatase in males, and bilirubin in males and females.	2000 ppm (equivalent to 54.2 mg/kg bw/day in males and 52.8 mg/kg bw/day in females)	10000 ppm (equivalent to 252.4 mg/kg bw/day in males and 252.7 mg/kg bw/day in females)	CA 5.3.2/021 CA 5.3.2/022 CA 5.3.2/023 CA 5.3.2/024 1816 (1999)
Dog	OECD409, repeated dose 13-week oral (diet)	Glyphosate 0, 2000, 10000 or 50000 ppm	Decreased body weight gain in males and females, decreased plasma calcium in males.	10000 ppm (equivalent to 323 mg/kg bw/day for males and 334 mg/kg bw/day for females)	50000 ppm (equivalent to 1680 mg/kg bw/day for males and 1750 mg/kg bw/day for females)	CA 5.3.2/025 CA 5.3.2/026 [redacted]/P/1802 (1996)
Dog	OECD409, repeated dose 13-week oral (diet)	Glyphosate 0, 1600, 8000 or 40000 ppm	No adverse effects observed.	40000 ppm (equivalent to 1015 mg/kg bw/day for males and 1014 mg/kg bw/day for females)	-	CA 5.3.2/027 [redacted] 94-0158 (1996)
Dog	OECD409, repeated dose 6-month oral (capsule)	MON 0139 (Isopropylamine salt of glyphosate) 0, 10, 60 or 300 mg/kg bw/day	Decreased body weight in males.	60 mg/kg bw/day	300 mg/kg bw/day	CA 5.3.2/029 810166 (1983)
Dog	OECD452, repeated dose 1-year oral (capsule)	Glyphosate 0, 30, 125 or 500 mg/kg bw/day	Decreased body weight gain in males.	125 mg/kg bw/day	500 mg/kg bw/day	CA 5.3.2/031 29647[redacted] (2007)
Dog	OECD452, repeated dose 52-week oral (capsule)	Glyphosate 0, 1600, 8000 or 50000 ppm	Loose stool in males and females, decreased body weight gain in males and females, decreased final body weight in females, lower urinary pH in both sexes, slight	8000 ppm (equivalent to 182 mg/kg bw/day for males and 184 mg/kg bw/day for females)	50000 ppm (equivalent to 1203 mg/kg bw/day for males and 1259 mg/kg bw/day for females)	CA 5.3.2/032 [redacted] 94-0157 (1997)

Species	Study (method/type, length, route of exposure)	Test substance	Critical effect	NOAEL	LOAEL	Cross reference
			anaemia in females, changes in blood electrolytes in females, increased frequency of slight focal pneumonia in females, higher thyroid weight accompanied by c-cell hyperplasia in males.			
Dog	OECD452, repeated dose 52-week oral (diet)	Glyphosate 0, 3000, 15000 or 30000 ppm	Decreased body weight in females.	15000 ppm (equivalent to 440.3 mg/kg bw/day for males and 447.8 mg/kg bw/day for females)	30000 ppm (equivalent 906.5 mg/kg bw/day for males and 926.2 mg/kg bw/day for females)	CA 5.3.2/033 CA 5.3.2/034 [redacted]/P/5079 (1996)
Dog	OECD452, repeated dose 52-week oral (capsule)	Glyphosate 0, 30, 300 or 1000 mg/kg bw/day	Changes in faecal consistency and decreased body weight gain in males and females.	300 mg/kg bw/day	1000 mg/kg bw/day	CA 5.3.2/035 7502 (1990)
Dog	OECD452, repeated dose 1-year oral (capsule)	Glyphosate 0, 20, 100 or 500 mg/kg bw/day	No adverse effects observed.	500 mg/kg bw/day	-	CA 5.3.2/036 [redacted]-4965 (1985)
Rat	OECD410, repeated dose 21-day dermal	Glyphosate 0, 250, 500 or 1000 mg/kg bw/day	No adverse effects observed.	$NOAEL_{local\ and\ systemic}$: 1000 mg/kg bw/day (highest dose tested)	-	CA 5.3.3/001 CA 5.3.3/002 [redacted]/P/4985 (1996)
Rat	OECD410, repeated dose 21-day dermal	Glyphosate 0 and 1000 mg/kg bw/day (limit test)	No systemic effects; mild skin irritation.	$NOAEL_{systemic}$: 1000 mg/kg bw/day (highest dose tested) $NOAEL_{local}$: < 1000 mg/kg bw/day	$LOAEL_{systemic}$: - $LOAEL_{local}$: 1000 mg/kg bw/day	CA 5.3.3/003 7839 (1993)
Rabbit	OECD410, repeated dose 28-day dermal	Glyphosate 0, 500, 1000 or 2000 mg/kg bw/day	No adverse systemic findings; slight skin irritation.	$NOAEL_{systemic}$: 2000 mg/kg bw/day (highest dose tested) $NOAEL_{local}$: 1000 mg/kg bw/day	$LOAEL_{systemic}$: - $LOAEL_{local}$: 2000 mg/kg bw/day	CA 5.3.3/004 CA 5.3.3/005 CA 5.3.3/006 [redacted] 214/94 (1994)

Species	Study (method/type, length, route of exposure)	Test substance	Critical effect	NOAEL	LOAEL	Cross reference
Rat	OECD424, repeated dose Sub-chronic 90-day neurotoxicity study	Glyphosate 0, 1000, 5000 or 20000 ppm	Reduced body weight and body weight gain in males; reduced food consumption in males during week 1-3.	5000 ppm (equivalent to 395 mg/kg bw/day in males and 404 mg/kg bw/day in females)	20000 ppm (equivalent to 1499 mg/kg bw/day in males and 1555 mg/kg bw/day in females)	Report no. 2060-0010, 2006
Rat	OECD424, repeated dose Sub-chronic 13-week neurotoxicity study	Glyphosate 0, 2000, 8000 or 20000 ppm	Reduced body weight gain in males.	8000 ppm (equivalent to 617.1 mg/kg bw/day for males and 672.1 mg/kg bw/day for females)	20000 ppm (equivalent to 1547 mg/kg bw/day for males and 1631 mg/kg bw/day for females)	Report no. [redacted]/P/4867, 1996
Rat	Two generation reproduction study (dietary) OECD 416	Glyphosate at 0, 1500, 5000, 15000 ppm	Adults: liver weight, kidney weight, changes in sperm parameters Offspring: delayed sexual maturation	Parental, offspring and reproductive toxicity: 5000 ppm (351 mg/kg bw/day)	Parental, offspring and reproductive toxicity: 15000 ppm (1063 mg/kg bw/day)	Report No. 2060/0013
Rat	Two generation reproduction study (dietary) OECD 416	Glyphosate acid at 0, 1000, 3000, 10000 ppm	Adults: none Offspring: reduced body weight	Parental and reproductive toxicity: 10000 ppm (985 mg/kg bw/day) Offspring toxicity: 3000 ppm (293 mg/kg bw/day)	Offspring toxicity: 10000 ppm (985 mg/kg bw/day)	Report No. [redacted]/P/6332
Rat	Two generation reproduction study (dietary) OECD 416	Glyphosate at 0, 1200, 6000, 30000 ppm	Adults: clinical signs (loose stool), organ weight changes, lower fertility indices, distension of caecum Offspring: pup weights, distension of caecum	Parental, offspring and reproductive toxicity: 6000 ppm (417 mg/kg bw/day)	Parental, offspring and reproductive toxicity: 30000 ppm (2151 mg/kg bw/day)	Report No. [redacted] 96-0031
Rat	Two generation reproduction study (dietary) OECD 416 (1983)	Glyphosate at 0, 1000, 3000, 10000 ppm	Adults: histopathological changes in salivary gland Offspring: none	Parental toxicity: 1000 ppm (66 mg/kg bw/day) Offspring and reproductive toxicity: 10000 ppm (668 mg/kg bw/day).	Parental toxicity: 3000 ppm (197 mg/kg bw/day)	Report No. [redacted] 47/911129

Species	Study (method/type, length, route of exposure)	Test substance	Critical effect	NOAEL	LOAEL	Cross reference
Rat	Two generation reproduction study (dietary)	Glyphosate at 0, 2000, 10000, 30000 ppm	Adults: clinical signs (soft stool), reduced bodyweight, reduced litter size Pups: reduced pup weight	Parental, offspring and reproductive toxicity: 10000 ppm (666 mg/kg bw/day)	Parental, offspring and reproductive toxicity: 30000 ppm (1983 mg/kg bw/day)	Report No. [redacted]-10387
Rat	Developmental OECD 414	Glyphosate acid at 0, 250, 500, 1000 mg/kg bw/d	None	Maternal and developmental: 1000 mg/kg bw/d	-	Report No. [redacted]/P/4819
Rat	Developmental OECD 414	Glyphosate acid at 0, 30, 300, 1000 mg/kg bw/d	Maternal: loose faeces Developmental: none	Maternal: 300 mg/kg bw/d Developmental: 1000 mg/kg bw/d	Maternal: 1000 mg/kg bw/d	Report No. [redacted] 94-0152
Rat	Developmental OECD 414	Glyphosate at 0, 300, 1000, 3500 mg/kg bw/d	Maternal: noisy respiration Developmental: reduced ossification, skeletal variations	Maternal: 300 mg/kg bw/d Developmental: 300 mg/kg bw/d	Maternal: 1000 mg/kg bw/d Developmental: 1000 mg/kg bw/d	Report No. [redacted] 43 & 41/90716
Rat	Developmental OECD 414	Glyphosate at 0, 300, 1000, 3500 mg/kg bw/d	Maternal: mortality, soft stool/diarrhoea, reduced body weight gain Developmental: decrease in implantations, increased post-implantation loss, reduced mean foetal body weight, decrease in mean number of viable foetuses, increased visceral and skeletal malformations/variations	Maternal and developmental: 1000 mg/kg bw/d	Maternal and developmental: 3500 mg/kg bw/d	Report No. 401-054
Rabbit	Developmental OECD 414	Glyphosate at 0, 100, 175, 300 mg/kg bw/d	Maternal: diarrhoea, reduced food intake Developmental: reduced foetal weight	Maternal: 100 mg/kg bw/d; Developmental: 175 mg/kg bw/d	Maternal: 175 mg/kg bw/d; Developmental: 300 mg/kg bw/d	Report No. [redacted]/P/5009
Rabbit	Developmental OECD 414	Glyphosate at 0, 50, 200, 400 mg/kg bw/d	Maternal: reduced body weight gain Developmental: none	Maternal: 50 mg/kg bw/d Developmental: >400 mg/kg bw/day	Maternal: 200 mg/kg bw/d Developmental: >400 mg/kg bw/day	Report No. 434/020
Rabbit	Developmental OECD 414	Glyphosate at	Maternal: loose faeces, abortions	Maternal: 100 mg/kg bw/d	Maternal: 300 mg/kg bw/d	Report No. [redacted] 94-0153

Species	Study (method/type, length, route of exposure)	Test substance	Critical effect	NOAEL	LOAEL	Cross reference
		0, 10, 100, 300 mg/kg bw/d		Developmental: >300 mg/kg bw/d	Developmental: >300 mg/kg bw/d	
Rabbit	Developmental OECD 414	Glyphosate at 0, 50, 150, 450 mg/kg bw/d	Maternal: soft/liquid stool, reduced food consumption, reduced body weight gain Developmental: late embryonic deaths, post-implantation loss, cardiac malformations	Maternal: 50 mg/kg bw/d Developmental: 150 mg/kg bw/d	Maternal: 150 mg/kg bw/d Developmental: 450 mg/kg bw/d	Report No. [redacted] 45 & 39 and 40/901303
Chronic studies						
Rat	OECD453, chronic toxicity/carcinogenicity 2-year, oral	Glyphosate 0, 1500, 5000 and 15000 ppm	Increased ALP, changes in plasma electrolytes, mineral deposition kidney, skin effects	5000 ppm (285.2 mg/kg bw/day in males and 348.6 mg/kg bw/day in females)	15000 ppm (1077.4 mg/kg bw/day in males and 1381.9 mg/kg bw/day in females)	Report No. 2060-0012
Rat	OECD453, chronic toxicity/carcinogenicity 2-year, oral	Glyphosate at 0, 2000, 6000 and 20000 ppm	Clinical chemistry changes, histopathological changes liver, kidney and prostate	6000 ppm (361 mg/kg bw/day in males and 437 mg/kg bw/day in females)	20000 ppm (1214 mg/kg bw/day in males and 1498 mg/kg bw/day in females)	Report No. [redacted]/PR1111
Rat	OECD453, chronic toxicity/carcinogenicity 2-year, oral	Glyphosate at 0, 3000, 10000 and 30000 ppm	Increased caecum weight	3000 ppm (104 mg/kg bw/day in males and 115 mg/kg bw/day in females)	10000 ppm (354 mg/kg bw/day in males and 393 mg/kg bw/day in females)	Report No. [redacted] 94-0150
Rat	OECD453, chronic toxicity/carcinogenicity 2-year, oral	Glyphosate at 0, 100, 1000 and 1000 ppm	Increased ALP, increased incidence of cataract	1000 ppm (59.4 mg/kg bw/day in males and 88.5 mg/kg bw/day in females)	10000 ppm (595.2 mg/kg bw/day in males and 886.0 mg/kg bw/day in females)	Report No. 886.C.C-R
Rat	OECD 452, chronic toxicity 1-year, oral	Glyphosate at 0, 2000, 8000 and 20000 ppm	Increased ALP, increase in focal basophilia of the acinar cells of the parotid salivary glands in females	2000 ppm (167 mg/kg bw/day in females)	8000 ppm (560 mg/kg bw/day in males and 671 mg/kg bw/day for females)	Report No. [redacted]/P/5143

Species	Study (method/type, length, route of exposure)	Test substance	Critical effect	NOAEL	LOAEL	Cross reference
Rat	OECD 453, chronic toxicity/carcinogenicity 2-year, oral	Glyphosate at 0, 10, 100, 300 and 1000 mg/kg bw/day	Increased salivary gland weight and cellular alteration of salivary gland	10 mg/kg bw/day	100 mg/kg bw/day	Report No. 7867
Rat	OECD 453, chronic toxicity/carcinogenicity 2-year, oral	Glyphosate at 2000, 8000 and 20000 ppm	Increased inflammation and hyperplasia of stomach squamous mucosa	2000 ppm (89 mg/kg bw/day in males and 113 mg/kg bw/day in females)	8000 ppm (362 mg/kg bw/day in males and 457 mg/kg bw/day in females)	Report No. [redacted]-10495
Mouse	OECD 451, carcinogenicity study 18-month, oral	Glyphosate at 0, 500, 1500 and 5000 ppm	None	5000 ppm (810 mg/kg bw/day in males, 1081.2 mg/kg bw/day in females)	-	Report No. 2060-0011
Mouse	OECD 451, carcinogenicity study 18-month, oral	Glyphosate at 0, 100, 1000 and 10000 ppm	None	10000 ppm (1454 mg/kg bw/day for males and 1467 mg/kg bw/day for females)	-	Report No. Toxi: 1559.CARCI-M
Mouse	OECD 451, carcinogenicity study 18-month, oral	Glyphosate at 0, 1600, 8000 and 40000 ppm	Reduced body weight (gain)	1600 ppm (165.0 mg/kg bw/day in males and 153.2 mg/kg bw/day in females)	8000 ppm (838.1 mg/kg bw/day in males and 786.8 mg/kg bw/day in females)	Report No. [redacted] 94-0151
Mouse	OECD 451, carcinogenicity study 18-month, oral	Glyphosate at 0, 100, 300 and 1000 mg/kg bw/day	None	1000 mg/kg bw/day	-	Report No. 7793
Mouse	OECD 451, carcinogenicity study 2-year, oral	Glyphosate at 0, 1000, 5000 and 30000 ppm	Urinary bladder epithelium hyperplasia (slight to mild) in males	1000 ppm (157 mg/kg bw/day in males)	5000 ppm (814 mg/kg bw/day in males)	Report No. 77-2061

Overall NOAEL short-term studies:

Most of the studies in rats demonstrated a low toxicity of glyphosate in different rat strains upon sub-chronic repeated oral administration. Several studies showed no adverse effects up and above the limit dose of 1000 mg/kg bw/day. Toxicological effects attributed to glyphosate exposure were soft stool, diarrhoea, decreased body weight gain and food consumption, which might suggest some irritation of the gastrointestinal tract by glyphosate. Further, a decrease in urinary pH was frequently reported. Other effects reported in rats are increased liver weight and changes in blood chemistry (increase in alkaline phosphatase, AST and ALT, increase in blood glucose). At dose levels above the limit dose of 1000 mg/kg bw/day, one study reported increased kidney weights. Further, the caecum was identified as a target organ because of certain findings (distention, elevated weight of this part of the intestines and its contents, mucosal atrophy). At much lower dose levels, one study reported histopathological changes in the parotid salivary gland which comprised deep basophilic staining and enlargement of cytoplasm at the lowest dose level (30 mg/kg b/day) and above (report number 7136, 1991). The RMS considers this a treatment-related effect for which human relevance cannot be excluded (refer to Vol 1 section 2.6.8.2). However, for the interpretation of effects on salivary glands several aspects are considered in order to decide if the effect is adverse or not. The RMS considers salivary gland weight changes and histopathology (severity grade and incidence) along with dose-response and statistical significance. A histopathological finding which is statistically significant is not considered adverse if the severity grade of the finding is minor and there are no salivary gland weight changes. In the case there are no data for salivary gland weights, the effect on histopathology might be considered as potentially adverse in the absence of such data as a precautionary approach. **As for this 90-day study no data is available on the parotid gland weight, the RMS proposes to set the LOAEL at the lowest dose level of 30 mg/kg bw/day as a precautionary approach although the severity grade of findings observed at this dose level was minimal (very mild).** Based on this approach, the LOAEL of 30 mg/kg bw/day is the most critical value relevant for reference dose setting.

Toxicity of glyphosate to mice was investigated in a relatively small number of sub-chronic studies. At very high doses (>6000 mg/kg bw/day) a reduction in body weight (gain), food consumption and alterations in some haematological and clinical chemistry parameters with the latter findings pointing to liver toxicity. Gross necropsy revealed caecum distention that was supported by a higher organ weight but not accompanied by histological lesions. Cystitis of urinary bladder became histologically apparent in some high dose males. Urinary pH (most likely due to acidic properties of the test substance) was noted in all treated male groups, but this was not considered adverse as this was attributed to the acidity of the test substance. The first study (Report no [redacted] 94-0136, 1995) is considered the only study relevant for "overall" NOAEL setting in mice as the NOAELs of the other studies were of limited value due to missing or only partial haematology and clinical chemistry investigation. Therefore the NOAEL for sub-chronic exposure to glyphosate is considered 600 mg/kg bw/day. However, it should be noted that in the previous assessment a NOAEL of 500 mg/kg bw/day was proposed based on salivary gland findings in the NTP study in mice. **However, for this study has not been submitted (data gap) and therefore the NOAEL of 600 mg/kg bw/day should be considered a provisional NOAEL.**

In dogs, a large number of 90-day and 1-year studies are available. The results show that the dog is of similar sensitivity as the rat when the NOAELs/LOAELs are considered. However, high dose effects may be more severe in dogs than in rats or mice, but appear somehow inconsistent among the studies. In the previous assessment, an overall NOAEL for the dog was set at 300 mg/kg bw/day. This NOAEL is no longer considered valid as in the current assessment for two 90-day dog studies a LOAEL has been set at or around this dose level (LOAELs between 252 to 300 mg/kg bw/day). For these studies a NOAEL was set at 54.2/52.8 and 60 mg/kg bw/day (report numbers 1816 (1999) and 810166 (1983), respectively). Based on these two studies, an **overall NOAEL of 60 mg/kg bw/day (the highest dose level at which no adverse effects were noted) is proposed for sub-chronic toxicity in the dog**. It is noted that this overall NOAEL is below the NOAEL set in the one-year repeated oral exposure studies in dogs. These studies resulted in NOAELs between 125 and 500 mg/kg bw/day.

Reproductive toxicity

The potential of glyphosate to cause effects on sexual function and fertility was examined in several 2-generational studies in the rat. Parental toxicity observed in these studies indicated effects on salivary gland, gastrointestinal disturbances, reduced body weight and organ weight changes. The lowest parental NOAEL of 66 mg/kg bw/day was observed in study report [redacted] 47/911129, where effects on salivary glands were found at 197 mg/kg bw/day. The findings in salivary glands consisted of histopathological changes in the parotid (both sexes) and submaxillary glands (females only), manifested as minimal hypertrophy of acinar cells with prominent granular cytoplasm. The histopathological findings were statistically significant using Cohran-Armitage trend test. As no data is available on the salivary gland weight, the RMS proposes to set the NOAEL in study at 66 mg/kg bw/day, although the severity grade of findings observed in study was minimal. Based on this approach the parental NOAEL for reproductive

toxicity is proposed to be set at lowest NOAEL of 66 mg/kg bw/day. In previous RAR (2015) an overall NOAEL for parental toxicity was set at 300-400 mg/kg bw/day.

Offspring toxicity consisted of reduced body weight, delayed preputial separation and distension of caecum. The NOAEL for offspring toxicity is proposed to be set at the lowest NOAEL of 293 mg/kg bw/day based on reduced body weight observed at 985 mg/kg bw/day in study report [redacted]/P6332. In previous RAR (2015) an overall NOAEL for offspring toxicity was set at 300-400 mg/kg bw/day.

Regarding reproductive toxicity, equivocal effects on litter size was observed at 2000 mg/kg bw/day (study report [redacted]-10387). Also, at a very high dose level (2151 mg/kg bw/day) lower fertility index (not statistically significant) was observed (study report [redacted] 96-0031). In another study, a significant decrease in homogenisation resistant spermatid count in F0 males was observed at ca 1000 mg/kg bw/day (study report 2060/0013). Based on this latter effect, the NOAEL for reproductive toxicity was 351 mg/kg bw/day. Thus, the NOAEL for reproductive toxicity set in previous RAR 2015 remains.

Developmental toxicity

Rats: The overall maternal and developmental rat NOAELs set at 300 mg/kg bw/d in the previous evaluation based on the findings in study Study CA 5.6.2/003/ Report No. [redacted]43 & 41/90716 (clinical signs, reduced bodyweight gain in dams, reduced ossification, skeletal variations in foetuses) are not fully supported since the effect on bodyweight gain at 1000 mg/kg bw/day is considered mild (3%) and not an appropriate basis for the overall maternal NOAEL. Loose faeces was observed in almost all rats administered 1000 mg/kg bw/day in study Study CA 5.6.2/002/ Report No. [redacted] 94-0152 but not at the same dose level in study Study CA 5.6.2/003/ Report No. [redacted] 43 & 41/90716, also performed in CD rats, or at this dose level in the other rat studies considered acceptable. Despite this lack of consistency between studies and the mild nature of the effect, the maternal NOAEL in rats is proposed to remain at 300 mg/kg bw/day as established in the previous assessment since gastrointestinal irritation seems to be an inherent property of the substance. Skeletal variations were seen in study Study CA 5.6.2/003/ Report No. [redacted]43 & 41/90716 at 1000 mg/kg bw/d, delayed ossification of unclear significance was noted at 1000 mg/kg bw/d in another study of acceptable quality (Study CA 5.6.2/002/ Report No. [redacted] 94-0152) and in one supportive study (Study CA 5.6.2/004/ Report No. [redacted].883.TER-R). However, no developmental toxicity was observed in two other studies considered acceptable (6.2/001 and Study CA 5.6.2/008/ Report No. 401-054) at 1000 mg/kg bw d. The developmental NOAEL is proposed to be set at 300 mg/kg bw/day based on effects on the skeletal variations observed at 1000 mg/kg bw/d in study Study CA 5.6.2/003/ Report No. [redacted] 43 & 41/90716.

Rabbits: The critical effect for the lowest maternal LOAEL in the studies considered acceptable is reduced body weight gain during treatment. In study Study CA 5.6.2/010/ Report No. 434/020, the bodyweight gain (days 7-19) was reduced 24-29% in dams administered 200 mg/kg bw/day. Although not statistically significant at this dose level, the same effect but more severe was observed at the next higher dose along with reduced food consumption and mortality. This is further supported by similar findings in study Study CA 5.6.2/014/ Report No. [redacted] 45, 39 & 40/901303 at a LOAEL of 150 mg/kg bw/d. The NOAEL in both studies is 50 mg/kg bw/day. The critical effect for the developmental LOAEL is a reduced foetal weight observed in study Study CA 5.6.2/009/ Report No. [redacted]/P/5009 at 300 mg/kg bw/day and the increased incidence of cardiac malformations and statistically significant increase of post-implantation loss in study Study CA 5.6.2/014/ Report No. [redacted] 45, 39 & 40/901303 at 450 mg/kg bw/day. Due to the lack of a clear dose-response and similar effects at the same dose levels in other studies, the lowest adverse effect level for post-implantation loss is considered to be 450 mg/kg bw/d rather than 150 mg/kg bw/d since the latter was yet within the range for historical control data. The NOAEL for reduced foetal weight is 175 mg/kg bw/day and 150 mg/kg bw/day for increased incidences of cardiac malformations and post-implantation loss, respectively. A dose of 150 mg/kg bw/day to represent an overall developmental NOAEL.

The data on developmental toxicity clearly indicate a higher sensitivity of rabbits compared to rats. The LOAEL/NOAELs for maternal toxicity in both rabbits and rats are based on reduced body weight gain, there were no developmental effects observed up to the limit dose in rats whereas the LOAEL/NOAELS for developmental toxicity in rats and rabbits are set for different effects; skeletal variations in rats and increased incidences of post-implantation loss and cardiac malformations. The differences in sensitivity between pregnant rabbits and pregnant rats to glyphosate may be due to rabbits ingesting their caecotrophes which may either lead to an increased exposure to glyphosate as it is excreted unchanged in faeces or it may lead to an undernourishment due to soft stools and diarrhoea, observed in the studies, preventing the rabbits from ingesting their caecotrophs. It may thus be argued that effects in rabbits are due to this special behaviour leading to a repeated exposure to the substance or a malnutrition that would not exist in other species. Consequently, the rabbit would be a non-representative animal model for effects of glyphosate and the NOAEL for use in risk assessment should not be taken from this type of study.
According to the applicant, “It is likely that the bolus administration of low pH glyphosate acid stresses the does as well as leads to the irritation of mucosal membrane of the rabbit gastro-intestinal tract. Consequently the associated stress leads to gastro-intestinal stasis. The gross necropsy signs observed in maternal animals in the studies

5.6.2/011, CA 5.6.2/010 and 5.6.2/009, such as hair like boluses in the stomach, fluid filled large intestines and gas distension in the lower gastrointestinal tract are indicative of gastro-intestinal stasis. These findings appear to be relevant to only hindgut fermenters as it is not seen in rats or dogs following administration of an oral bolus dose." Furthermore, the applicant states published literature shows coprophagy to be vital to the rabbit accessing the necessary nutrition to thrive and survive. Due to the gastrointestinal disturbances in the studies the essential practice of coprophagy was not possible (soft pellets could not form due to diarrhoea), leading to nutritional compromise of the rabbits. Therefore, the applicant considers the rabbit maternal toxicity findings "clearly not relevant to humans for three simple reasons. Firstly, humans are not exposed to bolus doses of glyphosate acid in their diet, and therefore are not subjected to the irritating effects seen in rabbit gastrointestinal tracts. Secondly, the maternal toxicity in rabbit developmental toxicity studies is not due to sub-chronic or chronic exposures. Thirdly, humans are not coprophagic; we obtain our nutrients through a balanced diet rather than nutrient recycling via the consumption of faeces."
However, caecotrophes which are much higher in moisture than the regular hard faeces are often referred to as soft faeces[25] complicating the assessment of the clinical sign diarrhoea/soft faeces noted in the study reports. Moreover, it is clear from studies both in rabbits and rats, that the substance causes gastrointestinal irritation in both species although only at high doses in rats. In rabbits this was seen both as diarrhoea and histopathological changes (studies 5.6.2/010, 5.6.2/019) whereas only diarrhoea/loose faeces was observed in rats. According to Guidance for the setting and application of acceptable operator exposure levels (AOELs), SANCO 7531 - rev.10) the AOEL is based on "…the highest level at which no adverse effect is observed in tests in the most sensitive relevant animal species or, if appropriate data are available, in humans". Since the original study reports do not inform if rabbits were able to eat their caecotrophes or not, it is not considered safe to anticipate that the higher sensitivity in rabbits only results from a species-specific mechanism and thus dismiss these effect levels. Consequently, it is proposed to take the NOAELs for rabbits into consideration for the derivation of reference values for human risk assessment.

Overall NOAEL for the long-term studies:
During the previous EU evaluation, the overall NOAEL for the long-term rat studies was set at 100 mg/kg bw per day while the overall NOAEL for the long-term mice studies was set at 150 mg/kg bw/day. In the current assessment, however, for the rat studies a NOAEL of 10 mg/kg bw/day (Report No. 7867) was derived based on histopathological findings in the salivary gland at the LOAEL of 100 mg/kg bw/day. As for other rat studies with a dose level between 10 and 100 mg/kg bw/day either the parotid salivary gland was not investigated microscopically or was not specifically mentioned as being investigated and because a strain-specific sensitivity for this endpoint cannot be excluded (refer to Vol 1 section 2.6.8.2), it is not possible to derive a higher overall NOAEL.

For the long-term studies in mice, three studies resulted in a NOAEL of around 150 mg/kg bw/day. The LOAELs of these studies were at the level of the NOAEL of the other studies and therefore the NOAEL of the other studies cannot be used to set a higher overall NOAEL. The overall NOAEL for mice of 150 mg/kg bw/day which was set during the previous EU evaluation is still considered to be acceptable.

2.6.10.1 Toxicological end point for assessment of risk following long-term dietary exposure – ADI (acceptable daily intake)

In contrast to the previous assessment, no "overall" NOAEL could be set for the rat based on the long-term studies (refer to last paragraph above). In the current evaluation, the most critical endpoint was the histopathological findings in the salivary gland observed in a 2-year rat study (study report no. 7867). Based on these findings, a NOAEL of 10 mg/kg bw/day has been derived which is used as the point of departure for the ADI. With the standard assessment factor of 100, this results in an **ADI of 0.1 mg/kg bw/day**.

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

A low acute oral toxicity of glyphosate was proven in a large number of studies (refer to 2.6.2.1). In addition, in an acute neurotoxicity study, the NOAEL for systemic effects was 1000 mg/kg bw (i.e., the limit dose that might justify a need for an ARfD) and there was no evidence of neurotoxicity (refer to 2.6.7). Based on these studies, there is no need for setting an ARfD.

In the developmental toxicity studies, some adverse effects in rabbits were reported (i.e. mortality, post-implantation loss and cardiac malformations) which should be considered. Mortality was seen in rabbits at doses of 175 mg/kg bw/day and above in one teratogenicity study (KCA 5.6.2/019; report no. 401-056) and at 100 mg/kg bw/day and

[25] Caecotrophy in Rabbits, Amy E. Halls, M.Sc. – Monogastric Nutritionist Shur-Gain, Nutreco Canada Inc., January 2008 (http://www nutrecocanada.com/docs/shur-gain---specialty/caecotrophy-in-rabbits.pdf).

above in a second study (KCA 5.6.2/012/013; report no. TOXI: 884-TER-RB). Both studies were considered as "supplementary" or "supportive information". A single maternal death was observed in a third study at 450 mg/kg bw/day, a study considered acceptable (KCA 5.6.2/014; report no. [redacted] 45;39;40/901303). This may justify setting of an ARfD although it is recognised that the aetiology of death is unclear and this mortality may reflect a species-specific sensitivity that is of little relevance for humans. However, since the information available does not give clear evidence to conclude a species-specific mechanism, effects in rabbits are considered relevant for humans. Nevertheless, as also discussed during the previous evaluation of the substance, these deaths occurred after a number of daily administrations rather than following a single or a few doses.

Despite that it has been concluded in the previous assessment, as well as by RAC and confirmed in this assessment that there is no evidence for teratogenicity in terms of classification, the post-implantation loss and cardiac malformations observed in study (KCA 5.6.2/014; report no. [redacted]45;39;40/901303) are considered the basis for the overall NOAEL set at 150 mg/kg bw/d for developmental toxicity in the rabbit and thus to justify the setting of an ARfD. Using a standard assessment factor of 100, an **ARfD of 1.5 mg/kg bw can be derived**.

It is recognized that this ARfD is conservative since it is set to protect from effects that are not relevant for the entire population.

2.6.10.3 Toxicological end point for assessment of occupational, bystander and residents risks – AOEL (acceptable operator exposure level)

The point of departure for the AOEL is a LOAEL of 30 mg/kg bw/day obtained from a 90-day rat study (study report no. 7136, 1991). This LOAEL is based on very mild and mild cellular alterations in the parotid glands, which is considered potentially adverse. It is noted that this is based on a precautionary approach as data on the weight of the parotid salivary gland is missing for this study.

To derive the AOEL based on this LOAEL, an additional assessment factor of 2 is applied instead of 3 due to the low severity of the observed histopathological changes in the salivary gland. With a standard assessment factor of 100 and a correction for oral absorption of 20%, the resulting **AOEL is 30 / (2*100) * 0.2 = 0.03 mg/kg bw/day.**

2.6.10.4 Toxicological end point for assessment of occupational, bystander and residents risks – AAOEL (acute acceptable operator exposure level)

On the same basis as for setting the ARfD (refer to 2.6.10.2) and using a standard assessment factor a systemic **AAOEL of 0.3 mg/kg bw/d** can be derived (based on 20% oral absorption). Since the AAOEL is based on foetal effects, it is not applicable to exposure scenarios for infants, toddlers and young children. No AAOEL is needed for these scenarios as there are no other acute effects considered relevant (refer to section 2.6.10.2).

2.6.11 Summary of product exposure and risk assessment

The reference formulation MON 52276 exhibits low acute oral, dermal and inhalation toxicity, is slightly irritant to skin, slightly to moderately irritant to eyes and is not a skin sensitizer. No additional classification has to be adopted for MON 52276 due to known toxicological properties of the active substance or any of the co-formulants. Refer to Vol 3 CP B.6 for the evaluation of the studies.

Table 2.6.11-1: Summary of evaluation of the studies on acute toxicity including irritancy and skin sensitisation for MON 52276

Type of test, model system (Guideline)	Result	Acceptability	Classification (acc. to the criteria in Reg. 1272/2008)	Reference
LD_{50} oral, rat (OECD 401)	> 5000 mg/kg bw	Yes	None	CP 7.1.1/001 Report no 6097-91
LD_{50} dermal, rat (OECD 402)	> 5000 mg/kg bw	Yes	None	CP 7.1.2/001 Report no 6098-91
LC_{50} inhalation, rat (OECD 403)	>5.25 mg/L	Yes	None	CP 7.1.3/001 Report no 40830

Type of test, model system (Guideline)	Result	Acceptability	Classification (acc. to the criteria in Reg. 1272/2008)	Reference
		(new study for AIR 5)		
Skin irritation, rabbit (OECD 404)	Non-irritant	Yes	None	CP 7.1.4/001 Report no 6099-91
Eye irritation, rabbit (OECD 405)	Non-irritant	Yes	None	CP 7.1.5/001 Report no 5999-91
Skin sensitisation, guinea pig (OECD 406, Buehler (9 applications)	Non-sensitising	Yes	None	CP 7.1.6/001 Report no CI-2001-153
Skin sensitisation, guinea pig (OECD 406, Buehler	Non-sensitising	No; study unacceptable due to too low number of animals included in the study	N.a.	CP 7.1.6/002 Report no 6100-91

In addition, three *in vitro* mutagenicity assays have been performed with MON 52276. All studies were guideline-compliant and gave negative results. The results are shown in Table 2.6.11-2.

Table 2.6.11-2 *In vitro* mutagenicity assays performed with MON 52276

Method, guideline, deviations if any	Test substance	Relevant information about the study	Observations /Results	Reference
OECD 471 (1997) GLP Study acceptable	MON 52276 Batch: 11427995 Purity: 30.3 wt% glyphosate acid	TA98, TA100, TA1535, TA1537 and WP2 uvrA strains; 1.5 to 5000 µg/plate (initial assay; duplicate) and 15 to 5000 µg/plate (confirmatory assay, triplicate), both assays ± rat liver S9; adequate positive and negative controls	MON 52276 was negative for the ability to induce reverse mutations in this bacterial mutagenicity assay in the presence and absence of S9	CP 7.1.7/001; AE60YE-503-BTL; 2016 (new study for AIR 5)
OECD487 (2014) Study acceptable but with restrictions The concentration, homogeneity, and stability of the test substance in the vehicle were not analysed. However, the test substance was tested to the maximum appropriate concentration based	MON 52276 Batch: 11427995 Purity: 30.3 wt% glyphosate acid	Micronucleus test in Human Lymphocytes, ±S9, 2-2000 µg/mL	Treatment with MON 52276 did not induce a statistically significant increase of micronuclei in human peripheral blood lymphocytes in the presences or absence of metabolic activation under the conditions of this study. The test substance is considered non-	CP 7.1.7/002; AE60YE.348.BTL; 2016 (new study for AIR 5)

on records of formulation preparation (weigh tapes, etc.) and the preparation of test substance dilutions occurred immediately before usage. Therefore, lack of verification is not considered to impact the validity of the study.			clastogenic and non-aneugenic under the conditions of this study.	
OECD487 (2016) GLP Demecolcine (DC) used as positive control. Study acceptable	MON 52276 Batch: 0190A Purity: 30.8% w/w glyphosate acid (41.5% w/w isopropylamine glyphosate) tested, with no correction for purity	Micronucleus test in Human Lymphocytes, ±S9, 321.5-5000 µg/mL	Treatment with MON 52276 did not induce a statistically significant increase of micronuclei in human peripheral blood lymphocytes in the presences or absence of metabolic activation under the conditions of this study. The test substance is considered non-clastogenic and non-aneugenic under the conditions of this study.	CP 7.1.7/003 Report no. WC22PQ; 2020 (new study for AIR 5)

The percentages for dermal absorption used in the exposure assessment are in Table 2.6.11-3.

Table 2.6.11-3 Dermal absorption end-points for the risk assessment

	Concentration	**Adapted values used in calculations for risk assessment**	**Reference**
Concentrate	360 g/L	0.096%	[redacted] 2010 EFSA Guidance on Dermal Absorption (EFSA Journal 2017;15(6):4873)
Dilution (1:12.5)	28.8 g/L	0.23%	[redacted] 2010 EFSA Guidance on Dermal Absorption (EFSA Journal 2017;15(6):4873)
Dilution (1:150)	2.4 g/L	0.68%	[redacted] 2010 EFSA Guidance on Dermal Absorption (EFSA Journal 2017;15(6):4873)

MON 52276 is formulated as a soluble liquid (SL) containing nominal 360 g glyphosate acid/L as the active substance. The product is used as herbicide for the control of annual, perennial, and biennial weeds.

Applications are made pre-sowing, pre-planting and post-harvest of the crops, as well as post-emergence of weeds. The product is used on bare soil, on vegetables, orchard crops, vines, railroad tracks and on invasive species in non-agricultural and agricultural areas.

Usage information pertinent to operator exposure is summarized in the Table 2.6.11-4. All uses are for F = professional field use.

Table 2.6.11-4: Summary of representative uses (risk envelope approach)

Crop	Application method	Water volume [L/ha]	Number of applications	Application rate [L product / ha and year]	Application rate a) max. rate per appl b) max total application rate per year [kg a.s./ha]	Minimum application interval [days]	Application timing [e.g. BBCH]
All crops (pre-sowing, pre-planting)	Field spraying, tractor-mounted	100-400	1	4	a) 1.44 b) 1.44	Not applicable	Pre-emergence
Vegetables	Field spraying, tractor-mounted	100-400	1 and 2[1]	6	a) 1.08-1.44 b) 2.16	28	Post-harvest, pre-sowing, pre-planting
Orchards	Ground directed, shielded spray, band application[2]	100-400	2[1]	8	a) 1.44 b) 2.88	28	Post-emergence of weeds
Vines	Ground directed, shielded spray, band application[3]	100-400	2[1]		a) 1.44 b) 2.88	28	Post-emergence of weeds
Railroad tracks	Ground directed, spray	100-400	2	10	a) 1.8 b) 3.6	90	Post-emergence of weeds
Invasive species in agricultural and non-agricultural areas	Spot treatment (shielded)	5 - 400	1	5	a) 1.8 b) 1.8	Not applicable	Post-emergence of weeds

[1] 2 applications at higher rates are worst case compared to 3 application at a lower dose rate, hence the selection of the GAP with 2 applications for a risk envelope approach. However, for vegetables a calculation has also been done with one application at the highest dose (1.44 kg as /ha) as this scenario gave higher exposure values for operators and residents.
[2] Band application in the rows below the trees or as spot treatments. The treated area represents not more than 50 % of the total orchard area. The application rate with reference to the total orchard surface area is not more than 50 % of the stated dose rate.
[3] Band application in the rows below the vine stock or as spot treatments. The treated area represents not more than 50 % of the total vineyard area. The application rate with reference to the total vineyard surface area is not more than 50 % of the stated dose rate.

The endpoints used for the risk assessment were: AOEL 0.03 mg/kg bw/day, AAOEL 0.3 mg/kg bw only applicable to exposure scenarios for adults and not for children, dermal absorption rates 0.096 % (concentrate), 0.68 % (spray dilution at 1:150).

The results of the exposure calculations for operators, bystanders, residents and workers are summarized in Tables 2.6.11-5 to 2.6.11-10.

Table 2.6.11-5: Predicted systemic long term exposure of operator as a proportion of the AOEL using the EFSA calculator

Model data	Level of PPE	Total absorbed dose (mg/kg/day)	% of systemic AOEL
Pre-emergence of crops (bare soil) Tractor mounted boom spray application outdoors (downward spraying)			
Application rate		1.44 kg a.s./ha (4 L MON 52276/ha)	
Spray application (AOEM; 75th percentile) Body weight: 60 kg	Potential exposure	0.0055905	18.63
	Work wear – arms, body and legs covered (no gloves)	0.0037956	12.65
Vegetables Including: Root & tuber vegetables, Bulb vegetables, Fruiting vegetables, Brassica, Leafy vegetables, Stem vegetables, Sugar beet) Tractor mounted boom spray application outdoors (downward spraying)			
Application rate		1.44 kg a.s./ha (6 L MON 52276/ha)	
Spray application (AOEM; 75th percentile) Body weight: 60 kg	Potential exposure	0.0055905	18.63
	Work wear – arms, body and legs covered (no gloves)	0.0037956	12.65
Application rate		2 x 1.08 kg a.s./ha (6 L MON 52276/ha)	
Spray application (AOEM; 75th percentile) Body weight: 60 kg	Potential exposure	0.0044314	14.77
	Work wear – arms, body and legs covered (no gloves)	0.0030085	10.03
Orchard crops Including: stone and pome fruits, kiwi, tree nuts, banana, and table olives, citrus Outdoor, downward spraying, vehicle-mounted			
Application rate		2 x 1.44 kg a.s./ha (8 L MON 52276/ha)	
Spray application (AOEM; 75th percentile) Body weight: 60 kg	Potential exposure	0.0077576	25.86
	Work wear – arms, body and legs covered (no gloves)	0.0038067	12.69
Outdoor, downward spraying, manual hand-held			
Spray application (AOEM; 75th percentile) Body weight: 60 kg	Potential exposure	0.0416198	138.73
	Work wear – arms, body and legs covered (no gloves)	0.0066254	22.08
Outdoor, downward spraying, manual knapsack			
Spray application (AOEM; 75th percentile) Body weight: 60 kg	Potential exposure	0.0112629	37.54
	Work wear – arms, body and legs covered (no gloves)	0.0021878	7.29
Vines Ground directed, shielded spray Outdoor, downward spraying, vehicle-mounted			
Application rate		2 x 1.44 kg a.s./ha (8 L MON 52276/ha)	
Spray application (AOEM; 75th percentile) Body weight: 60 kg	Potential exposure	0.0077576	25.86
	Work wear – arms, body and legs covered (no gloves)	0.0038067	12.69

Model data	Level of PPE	Total absorbed dose (mg/kg/day)	% of systemic AOEL
Outdoor, downward spraying, manual hand-held			
Spray application (AOEM; 75th percentile) Body weight: 60 kg	Potential exposure	0.0416198	138.73
	Work wear – arms, body and legs covered (no gloves)	0.0066254	22.08
Outdoor, downward spraying, manual knapsack			
Spray application (AOEM; 75th percentile) Body weight: 60 kg	Potential exposure	0.0112629	37.54
	Work wear – arms, body and legs covered (no gloves)	0.0021878	7.29
Railroad tracks (bare soil) Ground directed, spray – application by spray train			
Application rate		2 x 1.8 kg a.s./ha (10 L MON 52276/ha)	
Spray application (AOEM; 75th percentile) Body weight: 60 kg	Potential exposure	0.0067055	22.35%
	Work wear – arms, body and legs covered (no gloves)	0.0045539	15.18%
Invasive species in non-agricultural areas – manual knapsack			
Application rate		1.8 kg a.s./ha (5 L MON 52276/ha) Here RMS has used the value 0.68 % for dermal absorption of in-use dilution (used in all other calculations) instead of 0.1 % that the applicant used. 0.68 % represents a worst-case assumption.	
Spray application (AOEM; 75th percentile) Body weight: 60 kg	Potential exposure	0.0135155	45.05
	Work wear – arms, body and legs covered (no gloves)	0.0026253	8.75
Invasive species in agricultural areas – manual knapsack			
Application rate		1.8 kg a.s./ha (5 L MON 52276/ha)	
Spray application (AOEM; 75th percentile) Body weight: 60 kg	Potential exposure	0.0137046	45.05
	Work wear – arms, body and legs covered (no gloves)	0.0026253	8.75

Table 2.6.11-6: Estimated acute operator exposure to Glyphosate

Model data	Level of PPE	Total absorbed dose (mg/kg/day)	% of systemic AAOEL
Pre-emergence of crops (bare soil) Tractor mounted boom spray application outdoors (downward spraying)			
Application rate		1.44 kg a.s./ha (4 L MON 52276/ha)	
Spray application (AOEM; 95th percentile) Body weight: 60 kg	Potential exposure	0.0229126	7.64
	Work wear – arms, body and legs covered (no gloves)	0.0156460	5.22
Vegetables Including: Root & tuber vegetables, Bulb vegetables, Fruiting vegetables, Brassica, Leafy vegetables, Stem vegetables, Sugar beet)			

Model data	Level of PPE	Total absorbed dose (mg/kg/day)	% of systemic AAOEL
Tractor mounted boom spray application outdoors (downward spraying)			
Application rate		1.44 kg a.s./ha (6 L MON 52276/ha)	
Spray application (AOEM; 95th percentile) Body weight: 60 kg	Potential exposure	0.0229126	7.64
	Work wear – arms, body and legs covered (no gloves)	0.0156460	5.22
Application rate		2 x 1.08 kg a.s./ha (6 L MON 52276/ha)	
Spray application (AOEM; 95th percentile) Body weight: 60 kg	Potential exposure	0.0187962	6.7
	Work wear – arms, body and legs covered (no gloves)	0.0126683	4.22
Orchard crops Including: stone and pome fruits, kiwi, tree nuts, banana, and table olives, citrus Outdoor, downward spraying, vehicle-mounted			
Application rate		2 x 1.44 kg a.s./ha (8 L MON 52276/ha)	
Spray application (AOEM; 95th percentile) Body weight: 60 kg	Potential exposure	0.0163224	5.44
	Work wear – arms, body and legs covered (no gloves)	0.0093113	3.10
Outdoor, downward spraying, manual hand-held			
Spray application (AOEM; 95th percentile) Body weight: 60 kg	Potential exposure	0.0667148	22.24
	Work wear – arms, body and legs covered (no gloves)	0.0324429	10.81
Outdoor, downward spraying, manual knapsack			
Spray application (AOEM; 95th percentile) Body weight: 60 kg	Potential exposure	0.0173337	5.78
	Work wear – arms, body and legs covered (no gloves)	0.0088614	2.95
Vines Ground directed, shielded spray Outdoor, downward spraying, vehicle-mounted			
Application rate		2 x 1.44 kg a.s./ha (8 L MON 52276/ha)	
Spray application (AOEM; 95th percentile) Body weight: 60 kg	Potential exposure	0.0163224	5.44
	Work wear – arms, body and legs covered (no gloves)	0.0093113	3.10
Outdoor, downward spraying, manual hand-held			
Spray application (AOEM; 95th percentile) Body weight: 60 kg	Potential exposure	0.0667148	22.24
	Work wear – arms, body and legs covered (no gloves)	0.0324429	10.81
Outdoor, downward spraying, manual knapsack			
Spray application (AOEM; 95th percentile) Body weight: 60 kg	Potential exposure	0.0173337	5.78
	Work wear – arms, body and legs covered (no gloves)	0.0088614	2.95
Railroad tracks (bare soil)			

Model data	Level of PPE	Total absorbed dose (mg/kg/day)	% of systemic AAOEL
Ground directed, spray – application by spray train			
Application rate		2 x 1.8 kg a.s./ha (10 L MON 52276/ha)	
Spray application (AOEM; 95th percentile) Body weight: 60 kg	Potential exposure	0.0268056	8.94
	Work wear – arms, body and legs covered (no gloves)	0.0184530	6.15
Invasive species in non-agricultural areas – manual knapsack			
Application rate		1.8 kg a.s./ha (5 L MON 52276/ha) Here RMS has used the value 0.68 % for dermal absorption of in-use dilution (used in all other calculations) instead of 0.1 % that the applicant used.	
Spray application (AOEM; 95th percentile) Body weight: 60 kg	Potential exposure	0.0208005	6.93
	Work wear – arms, body and legs covered (no gloves)	0.0106337	3.54
Invasive species in agricultural areas – manual knapsack			
Application rate		1.8 kg a.s./ha (5 L MON 52276/ha)	
Spray application (AOEM; 95th percentile) Body weight: 60 kg	Potential exposure	0.0208005	6.93
	Work wear – arms, body and legs covered (no gloves)	0.0106337	3.54

Table 2.6.11-7: Predicted systemic exposure to residents as a proportion of the AOEL using the EFSA calculator

		Glyphosate	
Model data		**Total absorbed dose (mg/kg bw/day)**	**% of systemic AOEL**
Pre-emergence of crops (bare soil) Tractor mounted boom spray application outdoors Buffer zone: 2-3 (m) Drift reduction technology: no DT_{50}: 30 days DFR: 4.32 µg/cm^2			
Number of applications and application rate		1.44 kg a.s./ha (4 L MON 52276/ha)	
Resident child Body weight: 10 kg	Drift (75th perc.)	0.0029424	9.81
	Vapour (75th perc.)	0.0010700	3.57
	Deposits (75th perc.)	0.0003764	1.25
	Re-entry (75th perc.)	0.0016524	5.51
	Sum (mean)	**0.0043532**	**14.51**
Resident adult Body weight: 60 kg	Drift (75th perc.)	0.0006530	2.18
	Vapour (75th perc.)	0.0002300	0.77
	Deposits (75th perc.)	0.0000667	0.22
	Re-entry (75th perc.)	0.0009180	3.06
	Sum (mean)	**0.001311**	**4.44**
Vegetables Including: Root & tuber vegetables, Bulb vegetables, Fruiting vegetables, Brassica, Leafy vegetables, Stem vegetables, Sugar beet) Tractor mounted boom spray application outdoors Buffer zone: 2-3 (m) Drift reduction technology: no DT_{50}: 30 days DFR: 4.32 µg/cm^2			

		Glyphosate	
Model data		**Total absorbed dose (mg/kg bw/day)**	**% of systemic AOEL**
Number of applications and application rate		**1.44 kg a.s./ha (6 L MON 52276/ha)**	
Resident child Body weight: 10 kg	Drift (75th perc.)	0.0029424	9.81
	Vapour (75th perc.)	0.0010700	3.57
	Deposits (75th perc.)	0.0003764	1.25
	Re-entry (75th perc.)	0.0016524	5.51
	Sum (mean)	**0.0043532**	**14.51**
Resident adult Body weight: 60 kg	Drift (75th perc.)	0.0006530	2.18
	Vapour (75th perc.)	0.0002300	0.77
	Deposits (75th perc.)	0.0000667	0.22
	Re-entry (75th perc.)	0.0009180	3.06
	Sum (mean)	**0.0013311**	**4.44**
Vegetables Including: Root & tuber vegetables, Bulb vegetables, Fruiting vegetables, Brassica, Leafy vegetables, Stem vegetables, Sugar beet) Tractor mounted boom spray application outdoors Buffer zone: 2-3 (m) Drift reduction technology: no DT_{50}: 30 days DFR: 3.24 µg/cm^2 28 days between applications			
Number of applications and application rate		**2 x 1.08 kg a.s./ha (6 L MON 52276/ha)**	
Resident child Body weight: 10 kg	Drift (75th perc.)	0.0220682	7.36
	Vapour (75th perc.)	0.0010700	3.57
	Deposits (75th perc.)	0.0004302	1.43
	Re-entry (75th perc.)	0.0018883	6.29
	Sum (mean)	**0.0041581**	**13.86**
Resident adult Body weight: 60 kg	Drift (75th perc.)	0.0004897	1.63
	Vapour (75th perc.)	0.0002300	0.77
	Deposits (75th perc.)	0.000762	0.25
	Re-entry (75th perc.)	0.0010490	3.50
	Sum (mean)	**0.0013624**	**4.54**
Orchard crops Including: stone and pome fruits, kiwi, tree nuts, banana, and table olives, citrus Ground directed, shielded spray, band application Buffer zone: 2-3(m) Drift reduction technology: no DT_{50}: 30 days DFR: 4.32 µg/cm^2 28 days between applications			
Number of applications and application rate	2 x 1.44 kg a.s./ha (8 L MON 52276/ha)		
Resident child Body weight: 10 kg	Drift (75th perc.)	0.0029424	9.81
	Vapour (75th perc.)	0.0010700	3.57
	Deposits (75th perc.)	0.0024539	8.18
	Re-entry (75th perc.)	0.0025177	8.39
	Sum (mean)	**0.0067094**	**22.36**
Resident adult Body weight: 60 kg	Drift (75th perc.)	0.0006530	2.18
	Vapour (75th perc.)	0.0002300	0.77
	Deposits (75th perc.)	0.0004349	1.45
	Re-entry (75th perc.)	0.0013987	4.66
	Sum (mean)	**0.0020097**	**6.70**
Vines Ground directed, shielded spray Buffer zone: 2-3 (m) Drift reduction technology: no			

		Glyphosate	
Model data		**Total absorbed dose (mg/kg bw/day)**	**% of systemic AOEL**
DT_{50}: 30 days DFR: 4.32 µg/cm^2 28 days between applications			
Number of applications and application rate		2 x 1.44 kg a.s./ha (8 L MON 52276/ha)	
Resident child Body weight: 10 kg	Drift (75th perc.)	0.0029424	9.81
	Vapour (75th perc.)	0.0010700	3.57
	Deposits (75th perc.)	0.0007067	2.36
	Re-entry (75th perc.)	0.0025177	8.39
	Sum (mean)	**0.0053052**	**17.68**
Resident adult Body weight: 60 kg	Drift (75th perc.)	0.0006530	2.18
	Vapour (75th perc.)	0.0002300	0.77
	Deposits (75th perc.)	0.0001252	0.42
	Re-entry (75th perc.)	0.0013987	4.66
	Sum (mean)	**0.0017608**	**5.87**
Railroad tracks (bare soil) Ground directed, spray Buffer zone: 2-3 (m) Drift reduction technology: no DT_{50}: 30 days DFR: 5.4 µg/cm^2 90 days between applications			
Number of applications and application rate		2 x 1.8 kg a.s./ha (10 L MON 52276/ha)	
Resident child Body weight: 10 kg	Drift (75th perc.)	0.0036780	12.26
	Vapour (75th perc.)	0.0010700	3.57
	Deposits (75th perc.)	0.0005294	1.76
	Re-entry (75th perc.)	0.0023237	7.75
	Sum (mean)	**0.0054229**	**18.08**
Resident adult Body weight: 60 kg	Drift (75th perc.)	0.0008162	2.72
	Vapour (75th perc.)	0.0002300	0.77
	Deposits (75th perc.)	0.0000938	0.31
	Re-entry (75th perc.)	0.0012909	4.30
	Sum (mean)	**0.0017283**	**5.76**
Invasive species in non-agricultural areas (golf course, turf or other sports lawns) Spot treatment (shielded)/spray application Buffer zone: 2-3 (m) Drift reduction technology: no DT_{50}: 30 days DFR: 5.4 µg/cm^2			
Number of applications and application rate		1.8 kg a.s./ha (5 L MON 52276/ha) Here RMS has used the value 0.68 % for dermal absorption of in-use dilution (used in all other calculations) instead of 0.1 % that the applicant used. 0.68 % represents a worst-case assumption.	
Resident child Body weight: 10 kg	Drift (75th perc.)	0.0735607	245.20
	Vapour (75th perc.)	0.0010700	3.57
	Deposits (75th perc.)	0.0004705	1.57
	Re-entry (75th perc.)	0.0026253	8.75
	Sum (mean)	**0.0440648**	**146.88**
Resident adult Body weight: 60 kg	Drift (75th perc.)	0.0163243	54.41
	Vapour (75th perc.)	0.0002300	0.77
	Deposits (75th perc.)	0.0000834	0.28
	Re-entry (75th perc.)	0.0001862	0.62
	Sum (mean)	**0.0084839**	**28.28**
Invasive species in agricultural areas			

		Glyphosate	
Model data		**Total absorbed dose (mg/kg bw/day)**	**% of systemic AOEL**
Spot treatment (shielded)/spray application Buffer zone: 2-3 (m) Drift reduction technology: no DT_{50}: 30 days DFR: 5.4 µg/cm^2			
Number of applications and application rate		1.8 kg a.s./ha (5 L MON 52276/ha)	
Resident child Body weight: 10 kg	Drift (75th perc.)	0.0735607	245.20
	Vapour (75th perc.)	0.0010700	3.57
	Deposits (75th perc.)	0.0004705	1.57
	Re-entry (75th perc.)	0.0020655	6.89
	Sum (mean)	**0.0453139**	**151.05**
Resident adult Body weight: 60 kg	Drift (75th perc.)	0.0163243	54.41
	Vapour (75th perc.)	0.0002300	0.77
	Deposits (75th perc.)	0.0000834	0.28
	Re-entry (75th perc.)	0.0011475	3.83
	Sum (mean)	**0.0092127**	**30.71**

Table 2.6.11-8: Predicted systemic exposure to adult bystanders as a proportion of the AAOEL using the EFSA calculator

		Glyphosate	
Model data		**Total absorbed dose (mg/kg bw/day)**	**% of systemic AAOEL**
Pre-emergence of crops (bare soil) Tractor mounted boom spray application outdoors Buffer zone: 2-3 (m) Drift reduction technology: no DT_{50}: 30 days DFR: 4.32 µg/cm^2			
Number of applications and application rate		1.44 kg a.s./ha (4 L MON 52276/ha)	
bystander adult Body weight: 60 kg	Drift (95th perc.)		0.58
	Vapour (95th perc.)		0.08
	Deposits (95th perc.)		0.07
	Re-entry (95th perc.)		0.31
Vegetables Including: Root & tuber vegetables, Bulb vegetables, Fruiting vegetables, Brassica, Leafy vegetables, Stem vegetables, Sugar beet) Tractor mounted boom spray application outdoors Buffer zone: 2-3 (m) Drift reduction technology: no DT_{50}: 30 days DFR: 4.32 µg/cm^2			
Number of applications and application rate		1.44 kg a.s./ha (6 L MON 52276/ha)	
bystander adult Body weight: 60 kg	Drift (95th perc.)		0.58
	Vapour (95th perc.)		0.08
	Deposits (95th perc.)		0.07
	Re-entry (95th perc.)		0.31
Vegetables Including: Root & tuber vegetables, Bulb vegetables, Fruiting vegetables, Brassica, Leafy vegetables, Stem vegetables, Sugar beet) Tractor mounted boom spray application outdoors Buffer zone: 2-3 (m) Drift reduction technology: no DT_{50}: 30 days DFR: 3.24 µg/cm^2 28 days between applications			

		Glyphosate	
Model data		**Total absorbed dose (mg/kg bw/day)**	**% of systemic AAOEL**
Number of applications and application rate		2 x 1.08 kg a.s./ha (6 L MON 52276/ha)	
bystander adult Body weight: 60 kg	Drift (95th perc.)		0.43
	Vapour (95th perc.)		0.08
	Deposits (95th perc.)		0.08
	Re-entry (95th perc.)		0.35
Orchard crops Including: stone and pome fruits, kiwi, tree nuts, banana, and table olives, citrus Ground directed, shielded spray, band application Buffer zone: 2-3(m) Drift reduction technology: no DT_{50}: 30 days DFR: 4.32 µg/cm^2 28 days between applications			
Number of applications and application rate	2 x 1.44 kg a.s./ha (8 L MON 52276/ha)		
bystander adult Body weight: 60 kg	Drift (95th perc.)		0.58
	Vapour (95th perc.)		0.08
	Deposits (95th perc.)		0.35
	Re-entry (95th perc.)		0.47
Vines Ground directed, shielded spray Buffer zone: 2-3 (m) Drift reduction technology: no DT_{50}: 30 days DFR: 4.32 µg/cm^2 28 days between applications			
Number of applications and application rate		2 x 1.44 kg a.s./ha (8 L MON 52276/ha)	
bystander adult Body weight: 60 kg	Drift (95th perc.)		0.58
	Vapour (95th perc.)		0.08
	Deposits (95th perc.)		0.10
	Re-entry (95th perc.)		0.47
Railroad tracks (bare soil) Ground directed, spray Buffer zone: 2-3 (m) Drift reduction technology: no DT_{50}: 30 days DFR: 5.4 µg/cm^2 90 days between applications			
Number of applications and application rate		2 x 1.8 kg a.s./ha (10 L MON 52276/ha)	
bystander adult Body weight: 60 kg	Drift (95th perc.)		0.72
	Vapour (95th perc.)		0.08
	Deposits (95th perc.)		0.09
	Re-entry (95th perc.)		0.43
Invasive species in non-agricultural areas (golf course, turf or other sports lawns) Spot treatment (shielded)/spray application Buffer zone: 2-3 (m) Drift reduction technology: no DT_{50}: 30 days DFR: 5.4 µg/cm^2			
Number of applications and application rate		1.8 kg a.s./ha (5 L MON 52276/ha) Here RMS has used the value 0.68 % for dermal absorption of in-use dilution (used in all other calculations) instead of 0.1 % that the applicant used. 0.68 % represents a worst-case assumption.	
bystander adult Body weight: 60 kg	Drift (95th perc.)		14.49
	Vapour (95th perc.)		0.08

		Glyphosate	
Model data		**Total absorbed dose (mg/kg bw/day)**	**% of systemic AAOEL**
	Deposits (95th perc.)		0.08
	Re-entry (95th perc.)		0.12
Invasive species in agricultural areas Spot treatment (shielded)/spray application Buffer zone: 2-3 (m) Drift reduction technology: no DT_{50}: 30 days DFR: 5.4 µg/cm^2			
Number of applications and application rate		1.8 kg a.s./ha (5 L MON 52276/ha)	
bystander adult Body weight: 60 kg	Drift (95th perc.)		14.49
	Vapour (95th perc.)		0.08
	Deposits (95th perc.)		0.08
	Re-entry (95th perc.)		0.38

Table 2.6.11-9: Predicted systemic recreational exposure as a proportion of the AOEL using the EFSA calculator

Model data		**Total absorbed dose (mg/kg/day)**	**% of systemic AOEL**
Invasive species in non-agricultural areas, knapsack sprayer application outdoors to low crops, (golf course, turf and other sports lawns)			
Application rate:		1.8 kg a.s./ha (5 L MON 52276/ha) Here RMS has used the value 0.68 % for dermal absorption of in-use dilution (used in all other calculations) instead of 0.1 % that the applicant used. Does AGG agree with RMS?	
Child Body weight: 10 kg	Recreational exposure	0.0084024	28.01
Adult Body weight: 60 kg	Recreational exposure	0.0014892	4.96

Table 2.6.11-10: Predicted systemic worker exposure as a proportion of the AOEL using the EFSA calculator

Model data	**Level of PPE**	**Total absorbed dose (mg/kg bw/day)**	**% of systemic AOEL**
Pre-emergence of crops (bare soil) No worker's tasks and therefore no calculation has been made			
Vegetables Including: Root & tuber vegetables, Bulb vegetables, Fruiting vegetables, Brassica, Leafy vegetables, Stem vegetables, Sugar beet) Reaching, picking Outdoor Work rate: 8 hours/day DT_{50}: 30 days DFR: 4.32 µg/cm^2 Dermal absorption 0.68 %			
Number of applications and application rate		**1.44 kg a.s./ha (6 L MON 52276/ha)**	
Body weight: 60 kg	Potential **TC: 5800 cm^2/person/h**	0.0227174	75.72
	Work wear (arms, body and legs covered) **TC: 2500 cm^2/person/h**	0.0097920	32.64
	Work wear (arms, body and legs covered) and gloves	0.0022717	7.57

Model data	Level of PPE		Total absorbed dose (mg/kg bw/day)	% of systemic AOEL
	TC: 580 cm^2/person/h			
Vegetables Including: Root & tuber vegetables, Bulb vegetables, Fruiting vegetables, Brassica, Leafy vegetables, Stem vegetables, Sugar beet) Reaching, picking Outdoor Work rate: 8 hours/day DT_{50}: 30 days DFR: 3.24 µg/cm^2 Dermal absorption 0.68 % 28 days between applications				
Number of applications and application rate			**2 x 1.08 kg a.s./ha (6 L MON 52276/ha)**	
Body weight: 60 kg	Potential **TC: 5800 cm^2/person/h**		0.0259600	86.53
	Work wear (arms, body and legs covered) **TC: 2500 cm^2/person/h**		0.0111897	37.30
	Work wear (arms, body and legs covered) and gloves **TC: 580 cm^2/person/h**		0.0025960	8.65
Orchards Including: stone and pome fruits, kiwi, tree nuts, banana, and table olives, citrus **Hand harvesting** Outdoor Work rate: 8 hours/day DT_{50}: 30 days DFR: 4.32 µg/cm^2 Dermal absorption 0.68 % 28 days between applications				
Number of applications and application rate			2 x 1.44 kg a.s./ha (8 L MON 52276/ha)	
Body weight: 60 kg	Potential **TC: 22500 cm^2/person/h**	8 hours/day	0.1342760	447.59
	Work wear (arms, body and legs covered) **TC: 4500 cm^2/person/h**	8 hours/day	0.0268552	89.52
	Work wear (arms, body and legs covered) and gloves **TC: 2250 cm^2/person/h**	8 hours/day	0.0134276	44.76
Orchards Including: stone and pome fruits, kiwi, tree nuts, banana, and table olives, citrus **inspection** Outdoor Work rate: 2 or 8 hours/day DT_{50}: 30 days				

Model data	**Level of PPE**		**Total absorbed dose (mg/kg bw/day)**	**% of systemic AOEL**
DFR: 4.32 µg/cm^2 Dermal absorption 0.68 % 28 days between applications				
Number of applications and application rate			2 x 1.44 kg a.s./ha (8 L MON 52276/ha)	
Body weight: 60 kg	Potential **TC: 12500 cm^2/person/h**	2 hours/day	0.0186494	62.16
		8 hours/day	0.0745976	248.64
	Work wear (arms, body and legs covered) **TC: 1400 cm^2/person/h**	2 hours/day	0.0020887	6.96
		8 hours/day	0.0083548	27.84
Vines **Hand harvesting** Outdoor Work rate: 8 hours/day DT_{50}: 30 days DFR: 4.32 µg/cm^2 Dermal absorption 0.68 % 28 days between applications				
Number of applications and application rate			2 x 1.44 kg a.s./ha (8 L MON 52276/ha)	
Body weight: 60 kg	Potential **TC: 30000 cm^2/person/h**	8 hours/day	0.1790346	596.78
	Work wear (arms, body and legs covered) **TC: 10100 cm^2/person/h**	8 hours/day	0.0602750	200.92
	Work wear (arms, body and legs covered) and gloves TC: **NA**		NA	NA
Using the decline calculator as a refinement				
Body weight: 60 kg	Work wear (arms, body and legs covered) **TC: 10100 cm^2/person/h**	8 hours/day	0.051	170.91 (using PHI=7 days) Comment: If instead a PHI value of 30 days is used the result would be 100.46 % of AOEL and therefore, based on agricultural practices and timing of application in vineyards and harvesting, it is unlikely that exposure of workers exceeds AOEL.
Vines **Inspection**				

Model data	Level of PPE		Total absorbed dose (mg/kg bw/day)	% of systemic AOEL
Outdoor Work rate: 2 or 8 hours/day DT_{50}: 30 days DFR: 4.32 µg/cm^2 Dermal absorption 0.68 % 28 days between applications				
Number of applications and application rate			2 x 1.44 kg a.s./ha (8 L MON 52276/ha)	
Body weight: 60 kg	Potential **TC: 12500 cm^2/person/h**	2 hours/day	0.0186494	62.16
		8 hours/day	0.0745976	248.64
	Work wear (arms, body and legs covered) **TC: 1400 cm^2/person/h**	2 hours/day	0.0020887	6.96
		8 hours/day	0.0083548	27.84
Railroad tracks (bare soil) No worker's tasks and therefore no calculation has been made				
Invasive species in non-agricultural areas Maintenance Outdoor Work rate: 8 hours/day, DT_{50}: 30 days DFR: 5.4 µg/cm^2 Dermal absorption 0.68 %				
Number of applications and application rate			1.8 kg a.s./ha (5 L MON 52276/ha)	
Body weight: 60 kg	Potential **TC: 5800 cm^2/person/h**		0.0283968	94.66
	Work wear (arms, body and legs covered) **TC: 2500 cm^2/person/h**		0.0122400	40.80
	Work wear (arms, body and legs covered) and gloves **TC: 580 cm^2/person/h**		0.0028397	9.47
Invasive species in agricultural areas Inspection, irrigation Outdoor Work rate: 8 hours/day, DT_{50}: 30 days DFR: 5.4 µg/cm^2 Dermal absorption 0.68 %				
Number of applications and application rate			1.8 kg a.s./ha (5 L MON 52276/ha)	
Body weight: 60 kg	Potential **TC: 12500 cm^2/person/h**		0.0153000	51.00
	Work wear (arms, body and legs covered) **TC: 1400 cm^2/person/h**		0.0017136	5.71
	Work wear (arms, body and legs covered) and gloves **TC: NA**		NA	NA

A safe use could be demonstrated for operators without PPE using MON 52276 for all proposed uses.

For the scenarios vegetables, orchards, vines, invasive species in agricultural and non-agricultural areas, there are no unacceptable risk anticipated for the worker without PPE, when re-entering crops treated with MON 52276. This is when TC values of crop inspection in cereals and grasslands and 8 hours inspection scenario are used for vines as glyphosate is applied on the ground and not on the foliage of vines.

The bystander exposure is acceptable for all uses.

The resident exposure is predicted to be within acceptable limits after application on bare soil, vegetables, orchards, vines and railroad tracks. The recreational exposure is also estimated to be acceptable.

The exposure for resident adults during exposure to invasive species in both agricultural and non-agricultural areas are acceptable. However due to the high spray drift exposure, the exposure is not acceptable for resident children.

2.7 RESIDUE

2.7.1 Summary of storage stability of residues

Storage stability of glyphosate, AMPA, *N*-acetyl-glyphosate and *N*-acetyl-AMPA was investigated in several plant and animal matrices. In one study ([redacted], 1991, CA 6.1/012), stability of exogenous and incurred residues were investigated. For this study, results from the incurred residues are also reported despite that fact that they were not accepted. In all other cases, exogenous residues only were investigated. The overview of the available data is presented in Table 2.7.1-1 below. Analytical methods used in the storage stability studies were considered acceptable and fit for purpose to address stability.

Table 2.7.1-1. Overview of storage stability of glyphosate and its metabolites in different matrices.

Characteristics of the matrix	Matrix	Demonstrated storage duration	Comment
Glyphosate			
Plant products			
High water content	Sugar beet leaves	18 months	[redacted] 2010 (CA 6.1/003)
	Maize forage	12 months	[redacted] 2007 (CA 6.1/006)
	Maize green plant	12 months	[redacted] 2007 (CA 6.1/004)
	Maize forage	12 months	[redacted] 2007 (CA 6.1/004)
	Soybean forage	12 months	[redacted] 2007 (CA 6.1/005)
	Banana (whole fruit)	12 months	[redacted] 1996 (CA 6.1/010)
	Tomato	31 months	[redacted] 1991 (CA 6.1/012)
	Soybean forage	Max. 24 months	[redacted] 1991 (CA 6.1/012)
	Soybean forage (incurred)	Not acceptable	[redacted] 1991 (CA 6.1/012)
	Clover	31 months	[redacted] 1991 (CA 6.1/012)
	Clover (incurred)	Not acceptable	[redacted] 1991 (CA 6.1/012)
High protein content	Dry beans	18 months	[redacted] 1997 (CA 6.1/007)
High starch content	Maize grain	18 months	[redacted] 2010 (CA 6.1/003)
	Maize grain	12 months	[redacted] 2007 (CA 6.1/006)
	Maize grain	12 months	[redacted] 2007 (CA 6.1/004)
	Maize grain	Not conclusive, but no longer than 24 months	[redacted] 1991 (CA 6.1/012)

Characteristics of the matrix	Matrix	Demonstrated storage duration	Comment
	Maize grain (incurred)	Not acceptable	[redacted] 1991 (CA 6.1/012)
	Barley grain	18 months	[redacted] 2010 (CA 6.1/003)
	Wheat/rye grain	45 months	[redacted] 1995 (CA 6.1/011)
	Wheat grain	24 months	[redacted] 1989 (CA 6.1/013)
	Sorghum grain	48 months	[redacted] 1989 (CA 6.1/013)
	Sugar beet roots	18 months	[redacted] 2010 (CA 6.1/003)
	Alfalfa seed (incurred)	Not acceptable	[redacted] 1991 (CA 6.1/012)
High oil content	Soybean seeds	12 months	[redacted] 2007 (CA 6.1/005)
	Soybean seeds	24 months	[redacted] 1989 (CA 6.1/013)
	Oilseed rape seeds/linseeds	18 months	[redacted] 1997 (CA 6.1/007)
High acid content	Orange	24 months	[redacted] 2012 (CA 6.1/002)
Other commodities [1]	Barley straw	18 months	[redacted] 2010 (CA 6.1/003)
	Wheat/rye straw	45 months	[redacted] 1995 (CA 6.1/011)
	Soybean straw	24 months	[redacted] 1989 (CA 6.1/013)
	Soybean hay	12 months	[redacted] 2007 (CA 6.1/005)
	Maize stover	12 months	[redacted] 2007(CA 6.1/006)
	Maize stover	23 months	[redacted] 2007 (CA 6.1/004)
	Sorghum stover	31 months	[redacted] 1991 (CA 6.1/012)
	Sorghum stover (incurred)	Not acceptable	[redacted] 1991 (CA 6.1/012)
Animal products			
Pig	Fat, muscle, liver, kidney	26 months	[redacted] 1988 (CA 6.1/014)
Ruminant	Fat, muscle, liver, kidney	24 months	[redacted] 1988 (CA 6.1/014)
Ruminant	Milk	16 months	[redacted] 1988 (CA 6.1/014)
Poultry	Fat, muscle, liver	25 months	[redacted] 1988 (CA 6.1/014)
Poultry	Kidney	13 months	[redacted], 1988 (CA 6.1/014)
Poultry	Egg	Max. 14 months	[redacted] 1988 (CA 6.1/014)
Poultry	Egg	23 months	[redacted] 1987 (CA 6.1/015)
Ruminant	Milk	22 months	[redacted] 1987 (CA 6.1/015)
Ruminant	Muscle, fat	23 months	[redacted] 1987(CA 6.1/015)
Bee	Honey	6 months	[redacted] 2020 (CA 6.1/001)
AMPA			
Plant products			
High water content	Sugar beet leaves	18 months	[redacted] 2010 (CA 6.1/003)
	Maize forage	12 months	[redacted] 2007 (6.1/006)

Characteristics of the matrix	Matrix	Demonstrated storage duration	Comment
	Maize green plant	12 months	[redacted] 2007 (6.1/004)
	Maize forage	12 months	[redacted] 2007 (6.1/004)
	Soybean forage	12 months	[redacted] 2007 (6.1/005)
	Tomato	31 months	[redacted] 1991 (CA 6.1/012)
	Soybean forage	24 months	[redacted] 1991 (CA 6.1/012)
	Clover	Max. 1 month	[redacted] 1991 (CA 6.1/012)
High starch content	Maize grain	18 months	[redacted] 2010 (CA 6.1/003)
	Maize grain	12 months	[redacted] 2007 (6.1/006)
	Maize grain	12 months	[redacted] 2007 (6.1/004)
	Maize grain	Not conclusive	[redacted] 1991 (CA 6.1/012)
	Barley grain	Max. 12 months	[redacted] 2010 (CA 6.1/003)
	Sugar beet roots	Max. 12 months	[redacted] 2010 (CA 6.1/003)
	Wheat/rye grain	Max. 10 months	[redacted] 1995 (CA 6.1/011)
	Wheat grain	24 months	[redacted] 1989 (CA 6.1/012)
	Sorghum grain	48 months	[redacted] 1989 (CA 6.1/012)
High oil content	Soybean seed	12 months	[redacted] 2007 (6.1/005)
	Soybean seed	24 months	[redacted] 1989 (CA 6.1/012)
High acid content	Orange	24 months	[redacted] 2012 (CA 6.1/002)
Other commodities [1]	Barley straw	Not conclusive	[redacted] 2010 (CA 6.1/003)
	Maize stover	Max. 6 months	[redacted] 2007 (CA 6.1/006)
	Maize stover	23 months	[redacted] 2007 (CA 6.1/004)
	Soybean hay	9 months	[redacted] 2007 (CA 6.1/005)
	Soybean straw	24 months	[redacted] 1989 (CA 6.1/012)
	Wheat/rye straw	Max. 6 months	[redacted] 1995 (CA 6.1/011)
	Sorghum stover	Max. 9 months	[redacted] 1991 (CA 6.1/012)
	Sorghum stover (incurred)	Not acceptable	[redacted] 1991 (CA 6.1/012)
Animal products			
Pig	Fat	Max. 15 months	[redacted] 1988 (CA 6.1/014)
Pig	Muscle, liver, kidney	26 months	[redacted] 1988 (CA 6.1/014)
Ruminant	Fat	24 months	[redacted] 1988 (CA 6.1/014)
Ruminant	Muscle, liver, kidney	24 months	[redacted] 1988 (CA 6.1/014)
Ruminant	Milk	16 months	[redacted] 1988 (CA 6.1/014)
Poultry	Fat	25 months	[redacted] 1988 (CA 6.1/014)
Poultry	Muscle, liver	25 months	[redacted] 1988

Characteristics of the matrix	Matrix	Demonstrated storage duration	Comment
			(CA 6.1/014)
Poultry	Kidney	13 months	[redacted] 1988 (CA 6.1/014)
Poultry	Egg	Max. 14 months	[redacted] 1988 (CA 6.1/014)
Poultry	Egg	Not conclusive	[redacted] 1987 (CA 6.1/015)
Ruminant	Milk	Not conclusive	[redacted] 1987 (CA 6.1/015)
Ruminant	Liver	Not conclusive	[redacted] 1987 (CA 6.1/015)
Ruminant	Muscle, fat	23 months	[redacted] 1987 (CA 6.1/015)
Bee	Honey	6 months	[redacted] 2020 (CA 6.1/001)
***N*-acetyl-glyphosate**			
Plant products			
High water content	Maize forage	12 months	[redacted] 2007 (CA 6.1/006)
	Maize green plant	12 months	[redacted] 2007 (CA 6.1/004)
	Maize forage	12 months	[redacted] 2007 (CA 6.1/004)
	Soybean forage	12 months	[redacted] 2007 (CA 6.1/005)
High starch content	Maize grain	12 months	[redacted] 2007 (CA 6.1/006)
	Maize grain	12 months	[redacted] 2007 (CA 6.1/004)
High oil content	Soybean seed	12 months	[redacted] 2007 (CA 6.1/005)
Other commodities [1]	Maize stover	12 months	[redacted] 2007 (CA 6.1/006)
	Maize stover	Max. 12 months	[redacted] 2007 (CA 6.1/004)
	Soybean hay	12 months	[redacted] 2007 (CA 6.1/005)
***N*-acetyl-AMPA**			
Plant products			
High water content	Maize green plant	23 months	[redacted] 2007 (CA 6.1/004)
	Maize forage	23 months	[redacted] 2007 (CA 6.1/004)
	Soybean forage	18 months	[redacted] 2007 (CA 6.1/005)
High starch content	Maize grain	23 months	[redacted] 2007 (CA 6.1/004)
High oil content	Soybean seed	18 months	[redacted] 2007 (AC 6.1/005)
Other commodities [1]	Maize stover	23 months	[redacted] 2007 (CA 6.1/004)
	Soybean hay	18 months	[redacted] 2007 (CA 6.1/005)

[1] In the OECD guideline 506 these commodities are not allocated to any of the five categories for storage stability.

Glyphosate

Glyphosate is demonstrated to be stable in dry beans (high protein matrix) for 18 months. This data can be extrapolated to the whole group high protein matrix crops.

Stability was demonstrated in orange (high acid matrix) for 24 months. Stability was investigated only in one crop from the high acid group, however, there is sufficient data available from five different crop groups to extrapolate to all plants (see conclusion below).

In high oil content matrices, stability was investigated in oilseed rape and soybean seeds. It can be concluded that glyphosate is stabile in high oil matrices for 18 months.

Stability in high water matrices was investigated in several categories of commodities: forage crops, fruiting vegetable, leaves of root vegetables. In general, it can be concluded that glyphosate was stable in high water matrix commodities for a maximum of 24 months, since in soybean forage a decline of stability was observed at later timepoints. In all other commodities stability was demonstrated during the maximum investigated storage time and no decline was observed. It should be noted that in tomato and clover, stability was demonstrated for 31 months.

Stability in high starch matrices was investigated in several crops. In general, it can be concluded that glyphosate was stable in high starch matrix commodities for a maximum of 24 months, since in some samples a decline of stability was observed in maize grain after 24 months. It should be noted that in two studies, stability was demonstrated for 45-48 months in wheat, rye and sorghum grain.

In other matrix commodities (straw/stover/hay), for glyphosate no decline of stability in all investigated matrices was observed. In general in can be concluded that glyphosate is considered to be stable in those matrices for 12 months. It should be noted that in some of the investigated dry plant parts, stability was demonstrated over a longer time and conclusion per commodity can be more suitable in those cases.

According to OECD Guideline 501, storage stability data from each of the five categories (high water content, high oil content, high protein content, high starch content, and high acid content) may be extrapolated to all plant commodities in case residues are shown to be stable in each of these matrices. Considering that glyphosate was shown to be stable for at least 18 months in each of the categories, it is concluded that glyphosate will be stable for at least 18 months in all plant commodities. It is noted that "other matrices" matrices are an exception since stability of residues was only demonstrated for 12 months in this group. However, those matrices do not belong to one of the five 'standard' categories as defined by OECD Guideline 501 and therefore the corresponding data are not considered for extrapolation.

In animal matrices, glyphosate is demonstrated to be stable in eggs for maximally 14 months, since a decline was observed after 25 and 28 months to 32%. Furthermore, stability of glyphosate was demonstrate for 22 months in milk, for 26 months in pigs tissues, for 24-25 months in ruminant and poultry tissues, except for poultry kidney, where stability was investigated up to 13 months.

Storage stability of glyphosate was also investigated in honey and stability was demonstrated for 6 months.

AMPA

AMPA is demonstrated to be stable in orange (high acid matrix) for 24 months and in soybean seed (high oil content matrix) for 24 months. Stability was investigated only in one crop from both categories, therefore, no extrapolation to the whole group can be made.

Stability of AMPA was investigated in several high water matrix commodities. In general, AMPA was stable in high water matrices for 18 months with the exception of clover, where decline was observed after 1 month.

Stability of AMPA was investigated in several crops of high starch matrices. In sugar beet roots, AMPA was stable for max. 12 months, since a decline was observed at later time points. In cereals grain, stability is demonstrated for 10-12 months, since a decline was observed at later time points. In one study, stability was demonstrated in wheat grain for 24 months and sorghum grain for 48 months. In general, it can be concluded that AMPA is stable in high starch matrix commodities for 10-12 months.

In other matrices (straw/stover/hay), stability seems to depend on the crop and no general conclusion can be drawn. For some matrices results were not conclusive. In wheat/rye straw and maize stover, stability was demonstrated for 6 months. In soybean hay and straw, stability was demonstrated for 9 and 24 months, respectively.

Storage stability data with AMPA cannot be extrapolated to all plant commodities, since no data on a commodity from the high protein category is available. It is noted, however, that such a study is currently ongoing. Furthermore, it is questionable whether an extrapolation to all plant commodities can be considered acceptable in the end, since stability of AMPA seems to be not straight-forward.

In animal matrices, AMPA is demonstrated to be stable in eggs for a maximum of 14 months, in milk for 16 months, in pig tissues for 26 months, except for fat where decline was observed after 15 months. In ruminant and poultry tissues, AMPA is stable for 24-25 months, except for poultry kidney, where stability was investigated up to 13 months.

Storage stability of AMPA was also investigated in honey and stability was demonstrated for 6 months.

***N*-acetyl-glyphosate**
N-acetyl-glyphosate is demonstrated to be stable for 12 months in forage (maize, soybean), maize grain, soybean seed, maize stover and soybean hay. Since only single crops were investigated per matrix, no general extrapolations can be made.

***N*-acetyl-AMPA**
N-acetyl-AMPA is stable in forage for 18 months, in maize for 23 months and soybean seed for 18 months. In dry matrices, *N*-acetyl-AMPA was stable for 23 months in maize stover and 18 months in soybean hay. Since only single crops were investigated per matrix (two crops in category high water), no general extrapolations can be made.

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

Within the framework of the current renewal of glyphosate, no new plant metabolism studies have been submitted. All existing and previously evaluated metabolism studies have been assessed again with the latest guidelines in Vol. 3, B.7.2.1. Data are summarized here in Vol. 1, 2.7.2, and they are also summarized in Appendix G.

Several metabolism studies are available for non-tolerant/conventional plants and tolerant/genetically modified plants. Also the application methods that were investigated are numerous, and include application to soil and hydroponic solutions, applications to stems and trunks, and foliar applications of glyphosate to conventional crops and pre-and post-emergence application of glyphosate to tolerant crops. Furthermore, the rotational crop metabolism studies have been evaluated in Vol. 1, 2.7.7.

Within the different plant metabolism studies glyphosate, the trimesium salt of glyphosate or its metabolite AMPA (aminomethylphosphonic acid) were used. Three different glyphosate labels are possible, the first one (which is used in the majority of metabolism studies) is *N*-(phosphono-^{14}C-methyl)glycine (^{14}C methane-glyphosate) where the methylene carbon is labelled. In addition, two labels in the glycine moiety are possible, the one labelled on the carbon of the carboxyl group, named N-(phosphonomethyl)- ^{14}C carboxy-glycine and the other one labelled on the other carbon of the glycine group, named N-(phosphonomethyl)- ^{14}C-methyl-glycine.

Non-tolerant/conventional plants

Fruit crops
Several metabolism studies are available investigating the fate and nature of glyphosate-derived residues in fruits. An overview on the studies is given in the following table.

Table 2.7.2-1: overview of metabolism studies in fruit crops

Plant	Application	Application rate	Reference and remark on acceptability
Citrus (calamondin citrus, lemon)	Soil application	Glyphosate or AMPA at 2.24 kg/ha	CA 6.2.1/001; [redacted] 1975; supportive only
	Hydroponic treatment	Glyphosate or AMPA at 10 mg/L hydroponic solution	
	Foliar application, dropping on leaves	4 mg glyphosate	
	Soil application	Glyphosate trimesium salt at 3.9 kg/ha (expressed in glyphosate equiv.)	CA 6.2.1/002; [redacted] 1987; acceptable
Tree nuts (walnut, almond, and pecan)	Soil application	Glyphosate at 5.07 kg/ha for pecan and walnut, and at 2.43 kg/ha for almonds	CA 6.2.1/003; [redacted] 1976; supportive only
	Foliar application	Glyphosate at 100 µg per leaf surface	
Apple	Soil application	Glyphosate at 3.36 kg/ha or AMPA at 1.68 kg/ha	CA 6.2.1/004; [redacted] 1974; supportive only
	Trunk application	Glyphosate at 92.4 µg/tree	
	Foliar application	Glyphosate at 10 µg/leaf or 10.7 mg/leaf	

Plant	Application	Application rate	Reference and remark on acceptability
Grapes	Soil application	Glyphosate trimesium salt at 8.1 (PMG-label) and 7.8 kg/ha (TMS-label) corresponding to 5.6 or 5.4 kg glyphosate equiv./ha, respectively	CA 6.2.1/005; [redacted] 1991; acceptable
	Overspray on bunches	Glyphosate trimesium salt at 14.3 mg per 10 bunches (PMG-label) and 13.2 mg per 10 bunches (TMS-label) respectively, 9.9 mg and 9.1 mg expressed as glyphosate equivalents	
	Soil application (drench)	Glyphosate trimesium salt at 8.3 kg/ha (PMG-label) (corresponding to 5.7 kg glyphosate equiv./ha) or 7.1 kg/ha (TMS label) (corresponding to 4.9 kg glyphosate equiv./ha)	CA 6.2.1/006; [redacted] 1990; acceptable
	Soil application	Glyphosate at 3.36 kg/ha or AMPA at 1.68 kg/ha	CA 6.2.1/007; [redacted] 1974; supportive only
	Trunk application	Glyphosate at 40 µg per tree (corresponding to 0.17 kg glyphosate/ha)	
	Hydroponic treatment	Glyphosate at 5, 10, 20 or 40 mg/kg hydroponic solution	
	Foliar application	Glyphosate at 20 µg per leaf (120 µg per plant)	

Two citrus metabolism studies have been submitted, of which the one with lemon is considered fully acceptable. The study with calamondin citrus can only be used to qualitatively describe metabolism. It shows that after soil application of both glyphosate as well as AMPA <0.1% of the applied activity is taken up by the plants into their leaves, stems or fruits, and <0.5% into their roots. Foliar treatment led to higher translocation of the applied activity into untreated leaves of the same plants (up to 2.6%), and the stems and fruits (up to 9.8%), while the treated leaves contained 76.6% of the applied activity after one week. During hydroponic treatment the percentage of radioactivity recovered was 1.3% or 1.8% in the leaves, 0.3% in the stems both for ^{14}C-glyphosate or ^{14}C-AMPA treatment, and 4.2% and 5.5% in the roots, for ^{14}C-glyphosate or ^{14}C-AMPA, respectively.

The lemon study has been conducted according to the type of application that is requested within the defended uses, i.e. soil application, and is considered overdosed compared to the cGAP (1.35N with regard to the max. application rate per year). The TRR in immature lemons harvested 3 days and 2 months after treatment, and in mature lemons harvested four months after treatment was very low (max. 0.019 mg/kg), although some residue was found in the leaves at that collection time (<0.05 mg/kg). Due to the low level of residue in mature lemons, characterization of metabolites was not pursued.

The metabolism study with tree nuts is considered as supportive only, as it only provides some qualitative information on glyphosate metabolism. The soil application experiments yielded low residues in comparison to radioactivity applied, demonstrating low plant uptake of ^{14}C-glyphosate from soil (max. 0.36%). After foliar treatment translocation occurred into untreated plant parts (other tops, roots), but most radioactivity remained in the treated leaves. Furthermore, mainly glyphosate was found, and to a lesser extent AMPA in leaves, tops and roots. However, no relevant food or feed items were investigated.

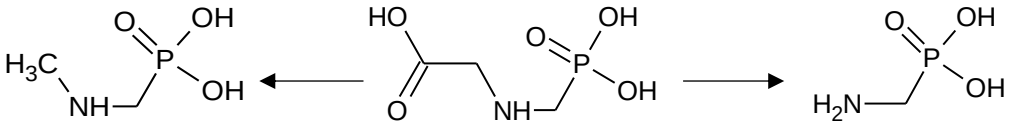
Also the apple metabolism study is considered supportive only, and can only be used for qualitative information on the metabolism of glyphosate. The uptake of ^{14}C-glyphosate or ^{14}C-AMPA in leaves, stems, branches and trunk was very low after soil treatment (maximum of 0.13% of the applied radioactivity at 12 weeks after treatment). After trunk treatment with glyphosate, uptake and translocation was also minimal with 0.08% of the applied activity recovered in leaves and stems and untreated trunk, 0.1% was recovered in roots, while 72.1% of the applied radioactivity was found in treated trunk. Foliar applied ^{14}C-glyphosate was rapidly and efficiently transported throughout the apple tree from the treated leaves. The highest amount was observed in the growing stem and leaves immediately above the treatment. Significant amounts of compound could also be found in other new growth, trunk and roots. Within samples taken after foliar application, the major residue in treated leaves, new growth above the treatment, other new growth, roots, and trunk of the apple trees was parent glyphosate (64–101% TRR). A maximum of 6% TRR (7.9 mg/kg) behaved in a manner chromatographically identical to AMPA/*N*-methyl AMPA in treated leaves, new growth above the treatment, other new growth of the apple trees. No other metabolites were identified. No relevant food or feed items were investigated.

Three reports containing investigation of glyphosate metabolism in grapes are available, of which the third one is considered supportive only. In the first one (CA 6.2.1/005), after soil treatment, which is the type of application of the intended uses within the framework of the current renewal, at an overdosed rate when compared to the defended uses (1.94/1.88N with regard to the max. application rate per year), the TRR in grapes was very low (<0.01 mg/kg).

In contrast, the overspray treatment resulted in much higher TRRs in grapes of up to 1.25 mg/kg at 14 days after treatment. The radioactive residues in grapes were identified as glyphosate anion accounting for 77.1% TRR (0.964 mg/kg) and 2.5% TRR (0.031 mg/kg) as AMPA. In the second study (CA 6.2.1/006), glyphosate was also applied as soil treatment, thereby mimicking the type of application of the requested uses in orchards. Although glyphosate was applied at highly overdosed rates, the TRR in grapes was low (<0.01 mg/kg) 7 days after treatment. Some low residue levels were observed in the leaves (0.031 mg TMS cation equiv./kg) and stalks (0.01 mg TMS cation equiv./kg). It can be concluded from this study that 7 days after soil treatment no significant uptake of glyphosate into the grape vines was observed. Due to these low levels, no further investigation took place. Finally, in the third grape study (supportive only), the uptake of ^{14}C-glyphosate or its metabolite AMPA 12 weeks after soil treatment was maximally 0.12% of the applied radioactivity, while after trunk treatment, uptake and translocation was also minimal with maximally 1.57% of the applied activity recovered in vines (leaves and stems), while up to 82.72% of the applied radioactivity was found in treated trunk. Although these percentages in vines are low, which is also true for the calculated TRRs (if they could have been calculated), still these calculated TRRs are in almost all samples >0.01 mg/kg. After hydroponic treatment significant ^{14}C-activity was observed in or on the roots of the grapevines; between 4.7 and 18.7% of the applied ^{14}C-activity (0.8– 4.1 mg/kg) was associated with the roots. Markedly less activity was observed in the aerial portions of the grapevines (<<1% of applied radioactivity). After foliar treatment, the majority of the treatment remained on the treated leaves, but also substantial uptake and translocation has occurred. The majority of the translocated ^{14}C-activity was associated with the stems and leaves (new growth) above the treated leaves and with the roots, while only very little ^{14}C-activity translocation to the fruit was observed whenever fruit was present (which was often not the case). The major residue was glyphosate at different amounts of the TRR: in treated leaves at 70.5–97.1%, new growth above the treatment at 70.4–103.1%, roots and old stock at 87.6–90.2% and grapes (fruit) at 64.6–79.5%. In root and old stock only glyphosate was present, while in treated leaf, new growth and grapes (fruit) the metabolite AMPA was identified as metabolite accounting for 1.5–9.2% TRR, 1.0–2.0% TRR, and <1.0% TRR, respectively.

Conclusion for metabolism in fruit crops
Within all studies investigating the metabolism of ^{14}C-glyphosate in fruits, a similar picture of metabolism was found. In all studies low plant uptake was indicated after soil treatment, which is considered most relevant, since soil application is the type of application for the defended uses on orchard crops. Higher residue uptake was achieved using application scenarios such as hydroponic treatment or foliar treatment which allowed the investigation of the nature of residues. Glyphosate parent compound accounted for the main part of the radioactive residues in the studies where identification of the residues has been conducted (i.e. tree nuts/foliar treatment; apple/foliar treatment; grapes/foliar treatment/2 studies). In some cases, AMPA was identified as minor metabolite. *N*-methyl-AMPA was indicated (not chromatographically separated from AMPA) in one apple study after foliar treatment and only in apple treated leaves, new growth and other new growth (leaves and stem).
It can be concluded that sufficient fruit metabolism studies are available, although many of them are considered supportive only.

Pathway for fruits – non-tolerant plants



N-methyl AMPA Glyphosate AMPA

<u>*Root and tuber vegetables*</u>
Two metabolism studies are available investigating the fate and nature of glyphosate-derived residues in root and tuber crops. An overview on the studies is given in the following table.

Table 2.7.2-2: overview of metabolism studies in root and tuber vegetables

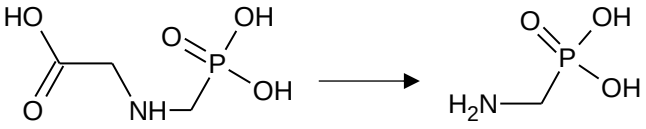
Plant	Application	Application rate	Reference and remark on acceptability
Potato	Soil application	Glyphosate at 23.8 mg per pot or AMPA at 23.4 mg per pot *(application to bare soil)*	CA 6.2.1/008; [redacted] 1975; not acceptable
		Glyphosate at 4.48 kg a.s./ha planting of pre-grown potatoes (BBCH 09) *(weeds treated with glyphosate and incorporated into soil to*	

Plant	Application	Application rate	Reference and remark on acceptability
		simulate ploughing)	
	Foliar application	Glyphosate at 108 µg per plant at pre-bloom stage	
Sugar beets	Soil application	Glyphosate or AMPA at 8.0 mg per pot	CA 6.2.1/009; [redacted] 1976; supportive only
	Foliar application	3.57 µg glyphosate ($^{13}C/^{14}C$ ratio: 13:1) per plant and 0.89 µg glyphosate per leaf	

The nature and magnitude of glyphosate-derived residues after different treatments with glyphosate of potato plants was studied. However, the study is considered not acceptable, and therefore, no further results are shown here, since they are considered not useful or not reliable.
The metabolism study with sugar beets, although considered supportive only, shows minimal uptake of radioactivity into roots or leaves following soil treatment of both glyphosate and AMPA (<0.2%). After foliar treatment with a mixture of ^{13}C- and ^{14}C-glyphosate, the untreated leaves were found to contain 11.9% of the applied radioactivity, while 31.2% of the applied radioactivity had translocated to the roots and 30.2% remained on the treated leaves. The sugar beet root extract from the ^{14}C-glyphosate soil treatments indicated 30% glyphosate, 10% AMPA, and 60% neutral material, while 70% glyphosate and 30% neutral material were found in the extracts of the leaves. The aqueous extracts of the roots and the leaves from the ^{14}C-AMPA soil treatments contained 90% AMPA and 10% neutral material. The major labelled material detected after foliar treatment was glyphosate (85-90% of extracted). The presence of AMPA was not detectable; similarly, no other labelled metabolites were observed.

Conclusion for metabolism in root and tuber vegetables
With only one metabolism study considered as supportive, while the other study is considered as not acceptable, it is more difficult to draw conclusions. As was already observed for the fruit crops, low plant uptake after soil treatment was demonstrated. In addition, parent glyphosate and metabolite AMPA were detected in roots as relevant metabolites. There were also indications of the presence of natural products. No further specific conclusions can be drawn for the root and tuber vegetables.

Pathway for root and tuber crops - non tolerant crops



Glyphosate AMPA

Cereals and grass crops
Several metabolism studies are available investigating the fate and nature of glyphosate-derived residues in cereals and grass crops. An overview of the studies is given in the following table.

Table 2.7.2-3: overview of metabolism studies in cereals and grass crops

Plant	Application	Application rate	Reference and remark on acceptability
Wheat	Foliar application close to harvest	Glyphosate trimesium salt at 5.64 kg/ha for the PMG-label (corresponding to 3.89 kg glyphosate equiv./ha)	CA 6.2.1/010; [redacted] 1989; acceptable
Barley Oats Rice Sorghum	Soil application	Glyphosate at 4.5 kg/ha	CA 6.2.1/011; [redacted] 1974; supportive only
	Hydroponic treatment	Glyphosate at 0.183 mg/mL in hydroponic solution	
Wheat Maize/corn	Soil application	Glyphosate at 4.5 kg/ha	CA 6.2.1/012; [redacted] 1973; supportive only
		AMPA at 1.7 kg/ha	
	Sand culture experiments	Glyphosate at 2.24 kg/ha	

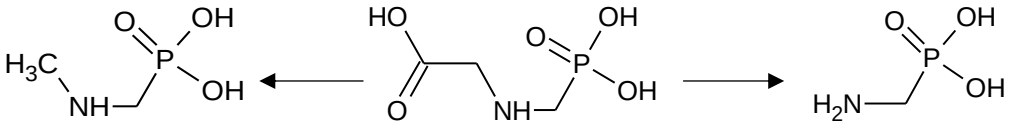
Plant	Application	Application rate	Reference and remark on acceptability
	Hydroponic treatment	Glyphosate 3 mg/24 plants (maize) or 3 mg/72 plants (wheat)	
Pasture (seed mixtures of fescue/alfalfa, bromegrass/red clover and timothy/white clover)	Soil application (pre-emergent)	Glyphosate at 4.48 kg/ha	CA 6.2.1/013; [redacted] 1976; supportive only
Pasture (quackgrass, fescue/alfalfa mixture)	Foliar application to quackgrass followed by incorporation in the soil after 1 week, and after 1 month sowing of fescue/alfalfa mixture	Glyphosate at 1.68 kg/ha	
Pasture (fescue and alfalfa)	Foliar application	$^{13}C/^{14}C$-glyphosate (90:10) at 1.12 kg/ha	
	Pre-harvest application	$^{13}C/^{14}C$-glyphosate (90:10) at 1.12 kg/ha	

In the first wheat study (CA 6.2.1/010), the nature of the residues in plants following the use of the glyphosate trimesium salt was studied in cereals after an application close before harvest. This is the only cereal metabolism study, which is considered fully acceptable. Although the type of application (i.e. foliar application close to harvest) is not compliant with the intended uses as soil application, and the study is also considered overdosed, both deviations would normally lead to higher residues, thereby facilitating investigation of glyphosate metabolism. The main constituent of the TRR in grain, chaff and straw was glyphosate accounting for 90.8, 85.0 and 82.6% of the TRR respectively (corresponding to 2.43, 278 and 103 mg/kg respectively). In addition to glyphosate, AMPA was identified as metabolite in grain, chaff and straw which accounted for 2.8, 3.9 and 3.3% of the TRR, respectively (corresponding to 0.08, 12.8 and 4.1 mg/kg respectively).
In the second study (CA 6.2.1/011), metabolism in several cereals has been investigated: barley, oats, rice and sorghum. Although the study is considered supportive only, it shows that the uptake of glyphosate after soil application is very limited (max. 0.13% of the applied radioactivity was found in the plants). Interestingly, these low percentages of uptake of applied radioactivity still can lead to relevant TRR levels (up to 0.157 mg/kg in rice whole plant), however, also in control plants TRR levels up to 0.23 mg/kg were observed. On the contrary, after hydroponic treatment, more uptake of glyphosate occurred (up to 23% of the applied radioactivity). In the aerial portion (tops) of all crops, glyphosate accounted for the main part of the radioactivity (73.3-76.6% TRR), while 6.5-14.0% of the TRR was identified as AMPA and 1.4 to 5.4% of the TRR was *N*-methyl-AMPA. Barley and sorghum tops, contained the highest percentage of AMPA as well as the highest percentage of *N*-methyl-AMPA. Also in the roots the main part of the radioactivity was identified as glyphosate (19.1-52.6% TRR). The most prominent metabolite was AMPA (2.2 to 7.4% TRR) and 0.4-1.4% TRR was identified as *N*-methyl-AMPA. However, relevant levels of unknown radioactivity (activity remaining at the TLC-origin or indeterminate; up to 0.053 mg/kg in the aerial parts, and up to 0.271 mg/kg in the roots) and several fractions with residual radioactive residues have not been investigated. Furthermore, cereal grains have not been studied. Therefore, it is considered that the described quantitative information on glyphosate metabolism is useful, however, still some relevant information from this study is missing.
The third cereal metabolism study (CA 6.2.1/012) shows both low uptake of glyphosate (max. 0.12% of applied radioactivity), as well as low uptake of AMPA (max. 0.044%), into wheat and maize plants after soil application. As already noted for the previous metabolism study, this low percentage of uptake still can result in relevant quantitative levels up to 0.35 mg/kg. Uptake of glyphosate into plants growing in sand culture after application of an aqueous solution of glyphosate to the sand has also been examined. Only maize gave an uptake of 11.3% of the applied dose into the aerial portion after 18 days. Wheat had an aerial uptake of only 0.03% of the applied ^{14}C-activity after 18 days. Hydroponic treatment was also studied, and resulted in higher uptake levels. These plants were investigated further, and parent glyphosate was an important residue in aerial parts of maize and wheat as well as in their roots. AMPA was found as major metabolite in aerial parts and in roots. *N*-methyl AMPA was also detected as minor metabolite. Separate extractions to investigate the radioactivity in natural products indicated the

incorporation of fragments or $^{14}CO_2$ into natural products (e.g. amino acids and peptides or citric acid cycle intermediates). This third study has been evaluated as supportive only based on similar reasoning as for the second study, i.e. no sampling of the edible part of the plants took place, in several fractions the residual radioactivity should have been further investigated, and the residues >0.01 mg/kg in the soil experiment should have been studied. Still useful qualitative information on glyphosate metabolism can be derived.
In the fourth study (CA 6.2.1/013), the uptake of glyphosate in pasture crops was investigated after soil and foliar treatment. In all experiments with soil application, the uptake was very limited, not exceeding 0.1% of the applied radioactivity. However, as mentioned for the previous studies, although the percentage uptake was low, still relevant levels up to 5.7 mg/kg TRR can be calculated. In directly treated foliage 41.8-69% of the applied radioactivity was recovered after one week. Regrowth after eradication of treated foliage showed residue levels at or below 0.2% of the applied activity. The majority of the radioactive residues extracted from directly treated forage was shown to be glyphosate. Approximately 3% of the radioactivity recovered in extracts of dried fescue forage showed a chromatographic behaviour corresponding to the metabolite AMPA. In conclusion, only qualitative information can be derived from this metabolism study.

Conclusion for metabolism in cereals and grass crops
Although 3 out of the 4 metabolism studies are considered supportive only, the combination of the 4 metabolism studies is considered to provide sufficient acceptable information on glyphosate metabolism in cereals and grass crops. The acceptable metabolism study (CA 6.2.1/010) shows that glyphosate can be considered as the main metabolite in both grain and straw, while AMPA can also be observed. The requested uses within the framework of the renewal of glyphosate concern soil applications, leading to much lower residue levels than can be expected after the foliar application close to harvest, which was studied in this acceptable study. The other three supportive metabolism studies show indeed low glyphosate uptake after soil treatment. In addition, also other metabolites besides glyphosate and AMPA can be observed, such as *N*-methyl AMPA, as well as incorporation into natural products.

Pathway for cereals – non-tolerant crops



N-methyl AMPA Glyphosate AMPA

Pulses and oilseeds
An overview of the metabolism studies with pulses and oilseeds as primary crop is shown in the following table.

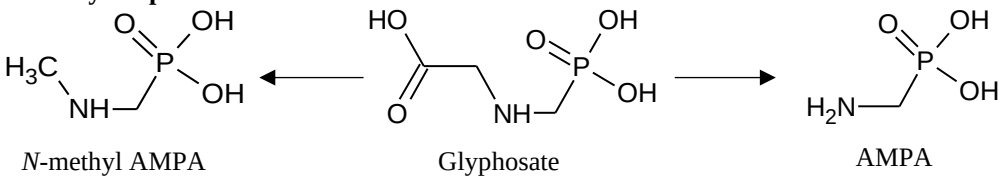
Table 2.7.2-4: overview of metabolism studies in pulses and oilseeds

Plant	Application	Application rate	Reference
Soybean	Soil application (drench)	Glyphosate trimesium salt at 8.40 kg/ha (corresponding to 5.8 kg glyphosate equiv./ha) within two hours after planting the seeds	CA 6.2.1/014; [redacted] 1992; acceptable
Soybean	Soil application	Glyphosate at 4.5 kg/ha or AMPA at 1.7 kg/h	CA 6.2.1/015; [redacted] 1973; supportive only
	Hydroponic sand culture	Glyphosate at 2.24 kg/ha	
	Hydroponic treatment	Glyphosate at 12 mg/24 plants or 50 mg/99 plants or Glyphosate at 12 mg/24 plants (different label) or Glyphosate at 12 mg/24 plants (different label) or Mixture of 13/14C-glyphosate at 50 mg/198 plants or Glyphosate at 12 mg/24 plants for 6 days	
Cotton	Soil application	Glyphosate at 4.5 kg/ha or AMPA at 1.7 kg/ha (corresponding to 2.6 kg glyphosate equiv./ha)	
	Sand culture	Glyphosate at 2.24 kg/ha	
	Hydroponic treatment	Glyphosate at 12 mg/12 plants	

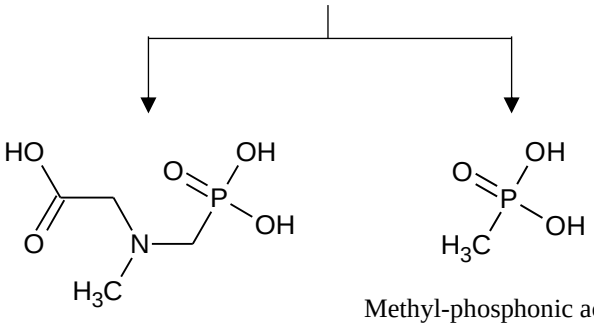
In the acceptable metabolism study with soybeans (CA 6.2.1/014), glyphosate trimesium was applied by soil drench application. The TRRs were 1.76 mg/kg in forage sampled 31 days after the application, 0.859 mg/kg in straw, 0.487 mg/kg in hulls, 0.772 mg/kg in green seeds and 1.31 mg/kg in yellow seeds, respectively, sampled 97 days after application. Within extracts of forage, straw, hulls and yellow seeds glyphosate and its metabolite AMPA were identified accounting for 0.6-4.1 and 1.5-5.7% TRR, respectively. The remaining fractions of the extractable residue (34.1-48.9% TRR) were shown to be radiolabelled natural products mainly consisting of mono- and disaccharides and amino acids and to a lower extent to smaller proteins. The unextractable (bound) residues consisted of natural products, 16.9-25.3% TRR carbohydrates, 1.4-2.9% TRR lignin, 16.0-24.0% TRR protein and 7.6-21.8% TRR crude cellulose. In conclusion, only minor levels of glyphosate or AMPA were found in the various plant parts. Most of the radioactivity was incorporated into natural products like carbohydrates and proteins. This metabolism study has been conducted in line with the method of application of the defended uses, and is considered somewhat overdosed. The study extensively investigated the extractable as well as the unextractable fractions.
In the other metabolism study with oilseeds (CA 6.2.1/015; same study as CA 6.2.1/012), low uptake of glyphosate (max. 0.27% of applied radioactivity) as well as low uptake of AMPA (max. 0.033%) into cotton and soybean was observed after soil application, however, still leading to relevant quantitative levels up to 0.42 mg/kg. Uptake of glyphosate into plants growing in sand culture after application of an aqueous solution of glyphosate to the sand has also been examined. Cotton and soybean had aerial uptakes of only 0.03% and 0.07% of the applied ^{14}C-activity respectively, after 18 days. In the hydroponic experiments the amount of ^{14}C-activity in cotton plants was found up to 3.0% of the applied radioactivity in the aerial part and up to 19.3% of the applied radioactivity in cotton roots, while they were found up to 4.2% and up to 13.9% of the applied radioactivity in soybean aerial parts and roots, respectively. Parent glyphosate was the major residue in aerial parts of cotton and soybean as well as in their roots. AMPA was also found as major metabolite in aerial parts and in roots. Several minor metabolites were also detected, and were indicated as *N*-methyl AMPA in roots, and in soybean forage as AMPA/*N*-methyl-AMPA, methyl phosphonic acid in roots, and *N*-methyl glyphosate in cotton roots. Separate extractions to investigate the radioactivity in natural products indicated the incorporation of fragments or $^{14}CO_2$ into natural products. Within the study the occurrence of minor compounds was also discussed as artefacts from very small impurities in the starting ^{14}C-glyphosate or they may have been formed in the hydroponic solutions via microbial degradation of glyphosate. This metabolism study has been evaluated as supportive only based on the observation that no sampling of the edible part of the plants took place, in several fractions the residual radioactivity should have been further investigated, and the residues >0.01 mg/kg in the soil experiment should have been studied. Still useful qualitative information on glyphosate metabolism can be derived.

Conclusion for metabolism in pulses and oilseeds
The metabolism studies with pulses and oilseeds show that glyphosate and AMPA are important residues. In addition, metabolites such as methyl phosphonic acid and *N*-methyl glyphosate were indicated in soybean and cotton after growing in hydroponic solution. *N*-methyl AMPA was also only indicated in soybean after hydroponic treatment. Furthermore, incorporation of glyphosate into natural products was demonstrated.

Pathway for pulses and oilseeds



N-methyl AMPA Glyphosate AMPA



N-methyl glyphosate

(only identified in one study in cotton roots after hydroponic uptake)

Methyl-phosphonic acid

(only identified in one study in cotton and soybean root after hydroponic uptake)

Miscellaneous crops

Two metabolism studies are available investigating the fate and nature of glyphosate-derived residues in miscellaneous crops (namely coffee and sugar cane). An overview of the studies is given in the following table.

Table 2.7.2-5: overview of metabolism studies in miscellaneous crops

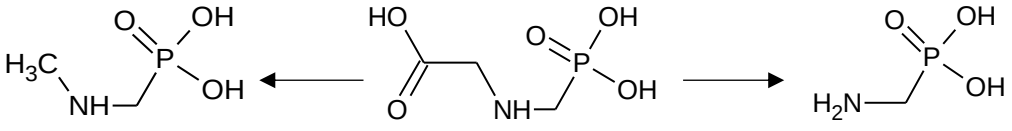
Plant	Application	Application rate	Reference
Coffee	Foliar application	Glyphosate at 0.32 mg/plant, only upper or only lower leaf surface 0.64 mg/plant, upper and lower surface treated 0.608 mg/plant, both surfaces treated, used for further extraction 1.9 mg/plant lower leaf surface on a tree with beans	CA 6.2.1/016; [redacted] 1975; acceptable
	Stem application	Glyphosate at 1.9 mg/plant (coating three of the lower segments of the stems application duration: 5 weeks)	
	Hydroponic treatment	1.1, 3.6 or 11.1 mg/L glyphosate. Treatment duration 3 weeks	
	Soil application	Glyphosate at 4.5 kg/ha, or AMPA at 4.5 kg/ha	
Sugarcane	Foliar application	Glyphosate at 1.96 mg per plant	CA 6.2.1/017; Anonymous 1976; supportive only
	Hydroponic treatment	Glyphosate at 3 mg/plant	

An acceptable metabolism study is available with coffee plants. In coffee plants treated via soil, stem, foliar or hydroponic application the uptake and translocation of radioactivity was minimal. After the soil treatment only 0.052 and 0.061 mg/kg (0.017-0.038% of the applied radioactivity) of ^{14}C-glyphosate and ^{14}C-AMPA, respectively, was found in aerial parts of the tree 8 weeks after the treatment. After the stem treatment, the TRR of the treated stem was 97.41 mg/kg (87.2% of applied radioactivity), whereas the TRR of leaves, untreated stems and roots was 0.050 to 0.687 mg/kg, resulting totally in 2.72% of applied radioactivity. For the foliar uptake of glyphosate, several experiments with ^{13}C- and ^{14}C-glyphosate were used with different formulations and application techniques. In all samples, glyphosate was the major residue present (71.7 to 95.0% TRR). AMPA/N-methyl AMPA accounted for <0.7-<1% TRR. Coffee trees carrying beans were also foliar treated with ^{14}C-glyphosate. In the immature beans 0.02 to 0.05% of the applied radioactivity was found after 4 to 20 weeks after treatment, increasing to 0.94% and to 0.68% of applied radioactivity in green beans and pods as well as in ripe beans, respectively. Glyphosate was the major component of residue in all investigated bean matrices, comprising 91.2 to 98.0% TRR, AMPA/*N*-methyl AMPA amounted to 0.98 to 5.0% TRR. After hydroponic treatment for three weeks most of the applied radioactivity was recovered in roots and in the remaining hydroponic solution. Only 0.1 to 0.2% and 4.3 to 11.7% of applied radioactivity were found in aerial parts and roots, respectively. The significant part of the residue in aerial parts and

roots was identified as the unchanged parent (up to 74.0 and 81.9% TRR, 0.093 and 9.65 mg/kg, respectively). Additionally, a considerable amount of AMPA in aerial parts and roots was found which accounted for up to 14.0 and 8.1% TRR (0.081 and 1.81 mg/kg), respectively.
The study with sugarcane consists of different experiments using either non-labelled glyphosate or ^{14}C-labelled glyphosate. Several of these experiments were not investigating glyphosate metabolism, and in the cases where some metabolism was investigated, several shortcomings were identified. Therefore, the study is considered to be supportive only. It qualitatively provides information that sugarcane roots in hydroponic solution absorb glyphosate, and that AMPA is being formed. In addition, the experiment investigating the foliar absorption of ^{14}C-glyphosate shows that translocation within the sugarcane plant occurs, with glyphosate being the major translocated residue.

Conclusion for metabolism in miscellaneous crops
In sugarcane, glyphosate and AMPA were the only identified compounds. Glyphosate was the major component of residue in all investigated matrices of coffee (treated and untreated leaves, aerial, stem, roots, beans, ripe pods and ripe beans). In the coffee study, AMPA was not chromatographically separated from *N*-methyl AMPA and identified as minor in coffee treated and untreated leaves, aerial, stem, roots, beans, pods and ripe beans.

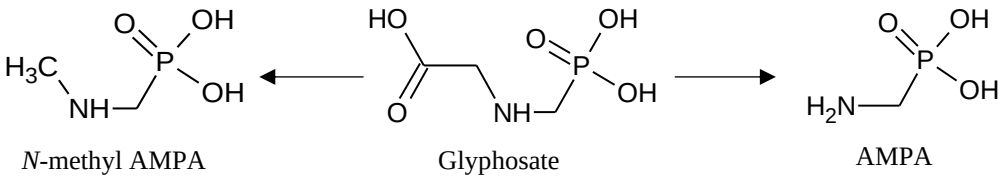
Pathway for miscellaneous crops



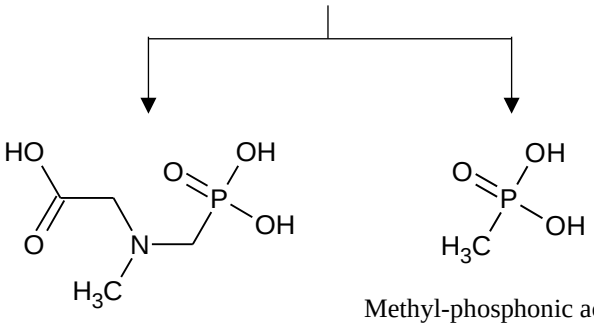
N-methyl AMPA Glyphosate AMPA

Overall conclusion for all non-tolerant crops
Many metabolism studies are available within the renewal dossier for glyphosate, but among these studies several studies have been assessed as supportive only. These supportive studies, however, do provide useful qualitative information on glyphosate metabolism, mostly on the fate of the glyphosate residues within the plants. What is often lacking from these supportive studies, is sufficient information on the identification of the metabolites, and/or the relevant RAC has not been investigated. On the other hand, also several acceptable plant metabolism studies with glyphosate are available: citrus-soil, grape-soil twice, grape-overspray, wheat-foliar, soybean-soil, coffee-foliar/stem/hydroponic/soil.
Altogether, these studies demonstrate a consistent pattern in the metabolism of glyphosate. There is only low uptake of glyphosate after soil treatment, which is considered most relevant, since soil application is the type of application for the defended uses. Importantly, such low percentages of uptake of applied radioactivity still can lead to relevant TRR levels in the plants. Higher residue uptake was achieved using other application scenarios such as hydroponic treatment or foliar treatment, which allowed the investigation of the nature of residues. It is demonstrated that glyphosate is being transported within the plants to some extent.
Glyphosate was the major ^{14}C-component in almost all investigated crops, and AMPA was the major or at least most prominent metabolite. The numerous plant uptake and metabolism studies demonstrate that glyphosate is metabolised in plants to AMPA. *N*-methyl AMPA was also identified in some commodities, namely in soybean forage and roots, and cereal aerial parts and roots. In apple leaves and new growth above the treatment, soybean forage as well as coffee commodities, *N*-methyl AMPA was indicated as a mixture with AMPA.
N-methyl glyphosate and methyl-phosphonic acid were determined in low amounts in one study only when soybean and cotton were grown in hydroponic solution. *N*-methyl glyphosate was only found up to 0.3% TRR in cotton roots, and methyl-phosphonic acid was only found up to 0.3% TRR in soybean roots and up to 2.0% TRR in cotton roots.
The incorporation of glyphosate into natural products (such as mono- and disaccharides, amino acids and to a lower extent to smaller proteins or citric acid cycle intermediates) was shown in some studies. In a few studies, the unextractable residues were further characterised, which also demonstrated incorporation into natural products, such as carbohydrates, lignin, protein or crude cellulose.
In conclusion, it is considered that sufficient plant metabolism studies are available for the assessment of a soil application of glyphosate, which is the relevant type of application within the current renewal dossier. It could be discussed whether foliar applications are also sufficiently covered by the current set of metabolism studies. However, 3 acceptable metabolism studies investigating foliar applied glyphosate are available from different crop groups (grape, wheat and coffee), showing a similar picture. Furthermore, it is not expected that an additional plant metabolism study would lead to any relevant new information. Therefore, it is concluded that sufficient plant metabolism studies are available to cover glyphosate metabolism in conventional crops after both soil as well as foliar application. These conclusions are further confirmed by the rotational crop metabolism studies (see 2.7.7).

Overall pathway considering all non-tolerant crop groups



N-methyl AMPA Glyphosate AMPA



N-methyl glyphosate

(only identified in one study in cotton roots after hydroponic uptake)

Methyl-phosphonic acid

(only identified in one study in soybean and cotton roots after hydroponic uptake)

Genetically modified plants

Genetically modified crops are not within the intended use of the renewal of glyphosate, however, different metabolism studies have been submitted including different genetical modifications such as CP4-EPSPS, CP4-EPSPS and GOX modification, and GAT modification. Based on the genetical modification different enzymes are involved and different metabolites are favoured.

CP4 EPSPS modification and CP4 EPSPS modification/GOX modification

In CP4 EPSPS modified crops the glyphosate tolerance is based on a modified 5-enolpyruvylshikimate-3-phosphate synthase, which is much less susceptible to glyphosate than the enzyme natural occurring in plants.

For the GOX modification, which is often used in combination with CP4 EPSPS, an alternative protein obtained from bacteria – the glyphosate oxidoreductase – is expressed in the plants, causing an accelerated degradation of glyphosate into AMPA.

Several metabolism studies are available investigating the fate and nature of glyphosate-derived residues in these genetically modified plants. An overview on the application scenarios and application rates used is given in the following table:

Table 2.7.2-6: overview of metabolism studies in crops with CP4 EPSPS modification or CP4 EPSPS modification/GOX modification

Plant	Application	Application rate	Reference and remark on acceptability
CP4 EPSPS modification			
Sugar beet	Pre-emergence application	Isotopic mixture of ^{12}C-, ^{13}C- and ^{14}C labelled glyphosate at 0.9 kg/ha	CA 6.2.1/018; [redacted] 2000; acceptable
	Post-emergence application	Isotopic mixture of ^{12}C-, ^{13}C- and ^{14}C labelled glyphosate at 1.08 kg/ha at BBCH 12-14 and at BBCH 19	
CP4 EPSPS modification			
Wheat	Spray application	Isotopic mixture of ^{12}C-, ^{13}C- and ^{14}C labelled glyphosate at 0.84 kg/ha at BBCH 15 and at BBCH 43 to the plant canopy	CA 6.2.1/019; [redacted] 2000; acceptable
CP4 EPSPS and GOX modification			
Maize/corn	Post-emergence application	Isotopic mixture of ^{12}C-, ^{13}C- and ^{14}C labelled glyphosate at 0.93 kg/ha at BBCH 15-16 and 0.84 kg/ha at BBCH 19 with and without soil protection	CA 6.2.1/020; [redacted] 1995; acceptable
CP4 EPSPS and GOX modification			

Rape/canola	Post-emergence applications	Isotopic mixture of ^{12}C-, ^{13}C- and ^{14}C labelled glyphosate at 0.455 kg/ha at BBCH 12-14 (14 days after planting) or at 2x 0.90 kg/ha at BBCH 12-14 and BBCH 16	CA 6.2.1/021; [redacted] 1995; acceptable
CP4 EPSPS modification			
Soybean	Pre-emergence application	Isotopic mixture of ^{12}C-, ^{13}C- and ^{14}C labelled glyphosate at 5.38 kg/ha	CA 6.2.1/022; [redacted] 1994; acceptable
	Early post-emergence application	Isotopic mixture of ^{12}C-, ^{13}C- and ^{14}C labelled glyphosate at 0.84 kg/ha at BBCH 23 (21 days after planting)	
	Sequential post-emergence applications	Isotopic mixture of ^{12}C-, ^{13}C- and ^{14}C labelled glyphosate at 0.84 kg/ha at BBCH 23 (21 days after planting) followed by 1.68 kg/ha at BBCH 51 (43 days after planting)	
CP4 EPSPS modification			
Cotton	Post-emergence applications	Isotopic mixture of ^{12}C-, ^{13}C- and ^{14}C labelled glyphosate at 0.93 kg/ha at BBCH 13-14 and 1.27 kg/ha at BBCH 15-16 with and without soil protection	CA 6.2.1/023; [redacted] 1997; acceptable

The nature of the residues in sugar beet plants (modified to express CP4 EPSPS) was investigated following the use of glyphosate applied either pre-emergent or twice post-emergent. After pre-emergent application, the uptake of glyphosate from soil was very low in sugar beets with TRRs up to 0.006 mg/kg in tops and up to 0.009 mg/kg in roots. After post-emergent treatment, TRRs were much higher (sugar beet tops up to 3.56 mg/kg and roots up to 1.40 mg/kg), showing that a translocation of radioactive residues into the roots occurred. Glyphosate was the major component of the residue in both sugar beet tops and roots treated post-emergent, accounting for 79.7% and 95.3% TRR, respectively. The metabolite AMPA accounted for 1.8% and 3.8% TRR in tops and roots, respectively, and was major in terms of concentration (>0.05 mg/kg in both tops and roots). Glyphosate/AMPA acetylated conjugates accounted for 0.8% TRR in tops and 0.6% TRR in roots. In addition, small amounts of ^{14}C-labelled natural products were indicated (1.4% TRR in tops and 1.2% TRR in roots) after post-emergent treatment.

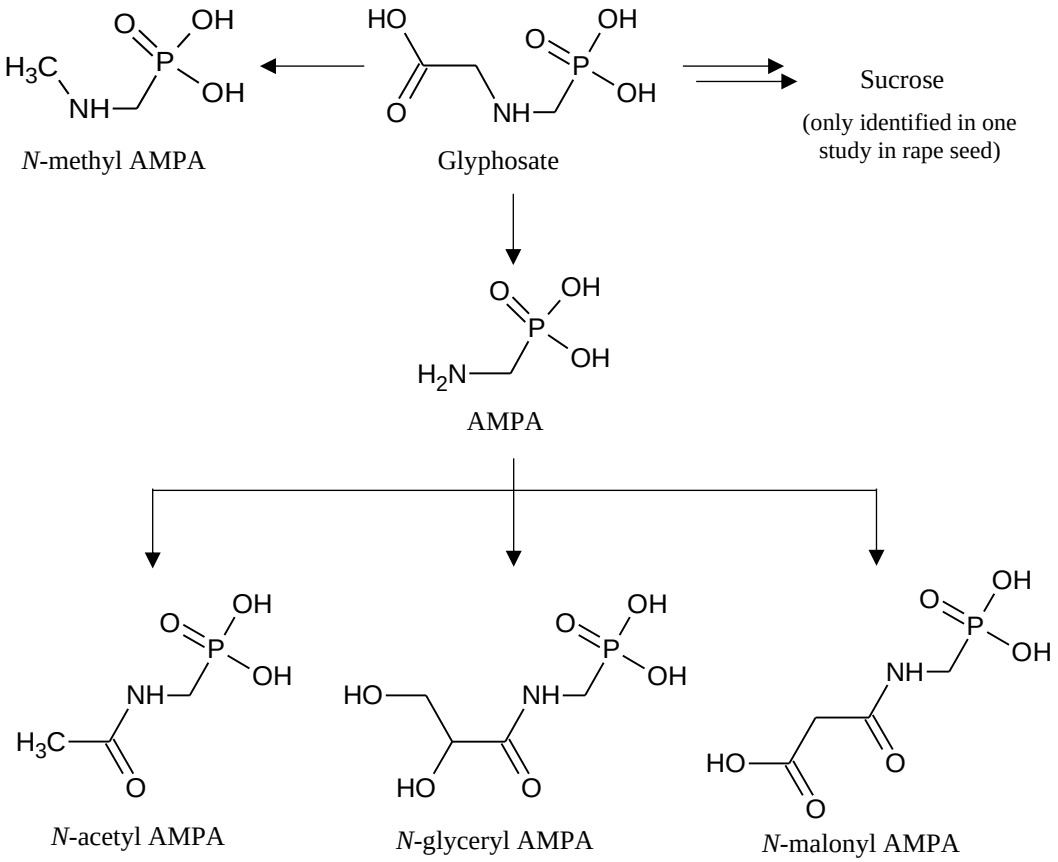
In the first cereals metabolism study, wheat was only genetically modified to express CP4 EPSPS. After two spray applications, the TRR in wheat forage, hay, straw and grain ranged from 12.1 to 34.8 mg/kg with straw containing the highest and grain the lowest level. Glyphosate was the major component of the residue in all wheat matrices (forage, hay, straw and grain accounting for 69.2%-89.4% TRR). AMPA was found to be the major metabolite in wheat grain (10.77 % of the TRR, 1.31 mg/kg). In addition, *N*-glyceryl AMPA was identified as minor metabolite in wheat grain accounting for 0.3% TRR (0.04 mg/kg). Glyphosate/AMPA acetylated conjugates and other AMPA conjugates were characterised in wheat matrices, all accounting for less than 2.4% TRR in any wheat commodity (0.08 – 0.84 mg/kg). The aqueous extracts of wheat matrices contained ^{14}C-labelled natural products (<2% TRR). The radioactive natural products were considered to be derived from the incorporation of $^{14}CO_2$ and other one carbon fragments from ^{14}C-glyphosate degradation into plant constituents.
In the second cereals metabolism study, maize was genetically modified to express CP4 EPSPS and GOX proteins. Two foliar applications were conducted, either when the soil was covered, or when the soil was unprotected. The TRR in ^{14}C-treated maize forage, silage and fodder ranged from 9.1 mg/kg to 14.9 mg/kg for protected treatment, and from 9.6 mg/kg to 19.1 mg/kg for non-protected treatment. Maize grain contained much lower levels of radioactivity; radioactive residues in ^{14}C-treated grain were 0.69 mg/kg and 1.04 mg/kg for soil protected and non-protected treatments, respectively. Glyphosate was observed to be the main radioactive residue in forage, silage and fodder accounting for 67.1 to 83.3% TRR, whereas lower levels of glyphosate were present in grain (2.6 to 7.4% TRR, 0.03 – 0.05 mg/kg). In contrast, AMPA was a major metabolite in all maize commodities found at approximately 4.9% to 15.9% TRR in forage, silage and fodder and 54.1% to 60.3% TRR in grain. Aqueous extracts also contained *N*-glyceryl AMPA accounting for 0.4% to 1.6% TRR in forage, silage and fodder and 6.9% TRR in grain where it was major in terms of concentration (0.05 – 0.07 mg/kg). In addition, low levels (<2% TRR) of glyphosate conjugates and trace levels of other AMPA conjugates are mentioned. Furthermore, aqueous extracts contained ^{14}C-labelled natural products (<3.6% TRR). The radioactivity in oil extracted from grain was shown to be associated with naturally occurring fatty acids. Unextractable residues were less than 5.4% TRR in forage, silage and fodder, while they accounted for up to 25.27% TRR (0.263 mg/kg) in grain. It would have been desirable, if further attempts were made to investigate the RRR, like it has been done for the grain. Acid hydrolysis of extracted grain released almost all of the bound radioactivity (90.2 % from grain). The majority of the acid-released radioactivity was shown to be glucose, derived from the incorporation of $^{14}CO_2$ and other one carbon fragments of glyphosate into maize/corn starch.

Both cereal metabolism studies show similar results on glyphosate metabolism when plants are genetically modified to express CP4 EPSPS.

For the pulses and oilseeds, three metabolism studies are available. In the first one, glyphosate tolerant canola (CP4 EPSPS and GOX modified) was studied. The TRR in canola seed samples taken 87 days after a single early post-emergence application was 0.48 - 0.85 mg/kg. The TRR in canola seed samples taken 79 days after the sequential post-emergence applications were 8.1 - 4.9 mg/kg. AMPA, *N*-glyceryl AMPA, *N*-acetyl AMPA and sucrose were identified in aqueous extracts of seeds, while no glyphosate was detected. In addition, a small percentage of the TRR was characterised as natural products and saponifiable fatty acids. The non-extracted residues amounted to 78.8% TRR (6.38 mg/kg). Several additional extractions were conducted, such as acid, base, enzymatic hydrolysis, simulated gastric fluid followed by simulated intestinal fluid and so on. The enzyme-related results suggest that only a small fraction of the ^{14}C-glyphosate-derived components in the extracted canola meal would be biologically available if ingested by animals. Acidic hydrolysis indicated the presence of radioactive amino acids, organic acids and sugars. The base hydrolysis results suggest that a significant amount of the unextracted residues in meal are due to bound AMPA. Upon hydrolysis, the AMPA is released and partially converted to formate. Thus, results of the numerous experiments to determine the nature of radioactivity in canola meal indicate there are two types of bound radioactivity. One type is the result of incorporation of one carbon ^{14}C fragments of glyphosate into natural products in the seed. The other is postulated to be bound AMPA, which is the primary metabolite of glyphosate in canola.
In the metabolism study with glyphosate-tolerant soybean, expressing CP4 EPSPS proteins, different treatments were investigated. The TRR in soybean forage, hay and seeds after sequential post-emergence treatment amounted to 23.7, 10.4 and 17.5 mg/kg in forage, hay and seeds, respectively. Early post-emergence treatment resulted in TRRs of 0.86, 0.55 and 0.41 mg/kg in forage, hay and seeds respectively. After pre-emergence treatment only 0.24, 0.21 and 0.75 mg/kg were found in forage, hay and seeds respectively. The radioactivity in forage, hay, and seeds treated pre-emergence is characterised as radiolabelled natural plant constituents derived by incorporation of $^{14}CO_2$ from the degradation of ^{14}C-glyphosate in the soil. After post-emergence treatment, glyphosate is slowly metabolised to AMPA, which is the primary plant metabolite. For plants that received the two sequential post-emergence applications, glyphosate accounted for 89.1, 53.6 and 25.2% TRR and AMPA accounted for 6.8, 12.8, and 49.1% TRR in forage, hay, and seeds, respectively. Additional metabolites were identified as *N*-methyl-AMPA, *N*-glyceryl-AMPA, *N*-acetyl-AMPA, and *N*-malonyl-AMPA, all less than 2% TRR, but at relevant quantitative levels. Moreover, 1.0% TRR (0.177 mg/kg) was attributed to AMPA conjugate. Furthermore, the incorporation into natural plant constituents was demonstrated.
Another metabolism study for the CP4 EPSPS modification within the crop group pulses and oilseeds was conducted with glyphosate-tolerant cotton. Foliar applications were made, with and without protecting the soil. The TRR in the forage sample amounted to 15.2 mg/kg without soil protection and 30.4 mg/kg with soil protection. In contrast to the high residues in the forage, the residues in the final harvest stalk, seed and lint samples were all < 0.2 mg/kg in both experiments. Investigation of the nature of residues in both experiments showed comparable results. In forage, 91.5 – 95.7% TRR were present as glyphosate; the most abundant metabolite, AMPA, accounted for less than 2% TRR. A glyphosate-conjugate accounted for up to 0.54% TRR. Natural products accounted for up to 0.83% TRR. In seeds, there was very little AMPA (<1 – 1.38% TRR) relative to glyphosate (12.0 – 23.7% TRR). More than half the radioactive residues in the treated seed samples were either in the oil or remained in the extracted seed. The radioactivity in cotton seeds is characterised as radiolabelled natural plant constituents derived by incorporation of $^{14}CO_2$ from the degradation of ^{14}C-glyphosate in the soil. The largest part of the radioactivity remained unextracted, even after intensive extraction including acidic and basic solvents.
All three metabolism studies within the pulses and oilseeds crop group show a similar metabolic pathway with glyphosate and AMPA being the predominant residues.

Overall conclusion for crops with CP4 EPSPS modification and CP4 EPSPS modification/GOX modification
For all six metabolism studies with CP4 EPSPS or CP4 EPSPS and GOX modification the metabolic pathway was found to be comparable. Glyphosate accounted for the main part in all investigated crops, except for maize grain and soya bean seed, in which AMPA was the major identified residue, while no glyphosate was identified in rape seeds. In all crops the most abundant metabolite was AMPA. Four further metabolites were identified, i.e. *N*-methyl AMPA, *N*-glyceryl AMPA, *N*-acetyl AMPA and *N*-malonyl AMPA. Radioactive residues were also incorporated into natural products (such as cellulose, lignin, starch, sugars, amino acids and fatty acids).

Pathway for crops with CP4 EPSPS as well as CP4 EPSPS and GOX modification



<u>*GAT modification*</u>

In GAT modified plants, glyphosate tolerance is caused by the modified glyphosate *N*-acetyltransferase (*gat*) gene. The new enzyme favours a metabolic pathway normally not observed in plants by acetylation of glyphosate and AMPA into *N*-acetyl glyphosate and *N*-acetyl AMPA, both not showing herbicidal activity. Three metabolism studies are available in which the nature of the residue has been investigated after glyphosate application to GAT modified plants. An overview is provided in the following table:

Table 2.7.2-7: overview of metabolism studies in crops with GAT modification

Plant	Application	Application rate	Reference
GAT modification			
Maize/corn	Pre-emergence application followed by 3 foliar applications	Glyphosate at 4.3 kg/ha and 3 foliar applications each at 1.1 kg/ha	CA 6.2.1/024; [redacted] 2007; acceptable
Rape/canola	Soil pre-emergent application followed by 3 foliar applications	Glyphosate at 4.50 kg/ha (soil) followed by 3 foliar applications at 0.94 to 1.03 kg/ha	CA 6.2.1/025; [redacted] 2010; acceptable
Soybean	Soil pre-emergent application followed by 3 foliar applications	Glyphosate at 3.290 kg/ha (soil) (pre-emergent) followed by 3 foliar applications at 1.410 kg/ha (unifoliolate and seven trifoliolate leaves are fully developed), 2.284 kg/ha (open flower at one of the two uppermost nodes on the main stem with a fully developed leaf) and 0.880 kg/ha (one normal pod on the main stem that has reached its mature pod color)	CA 6.2.1/026; [redacted] 2007; acceptable

GAT modified <u>maize</u> has been investigated following a pre-emergent application and 3 foliar applications with glyphosate. The TRR in forage accounting for 3.476 mg/kg, while at maturity, the majority of the TRR was present in stover (12.24 mg/kg) with 0.69 mg/kg in cobs and 0.28 mg/kg in grain. The major component in forage was glyphosate (58.0% TRR) with *N*-acetyl glyphosate present as major metabolite at 27.0% TRR. AMPA and *N*-acetyl AMPA comprised 4.0% and 1.7% TRR respectively. As was the case for forage, the major component of stover was glyphosate (74.9% TRR) and *N*-acetyl glyphosate was the most abundant metabolite (17.8% TRR). The metabolites

AMPA and *N*-acetyl AMPA were also detected but at much lower levels (3.4% and 1.3% TRR, respectively). The major identified residue in cobs and grain was *N*-acetyl glyphosate, which comprised 63.8% and 51.2% TRR, respectively. *N*-acetyl AMPA was present as minor metabolite at 5.0% and 9.4% TRR respectively. AMPA and glyphosate were detected in grain at lower concentrations, 6.1% and 0.1% TRR, respectively, while not being detected in the cobs.

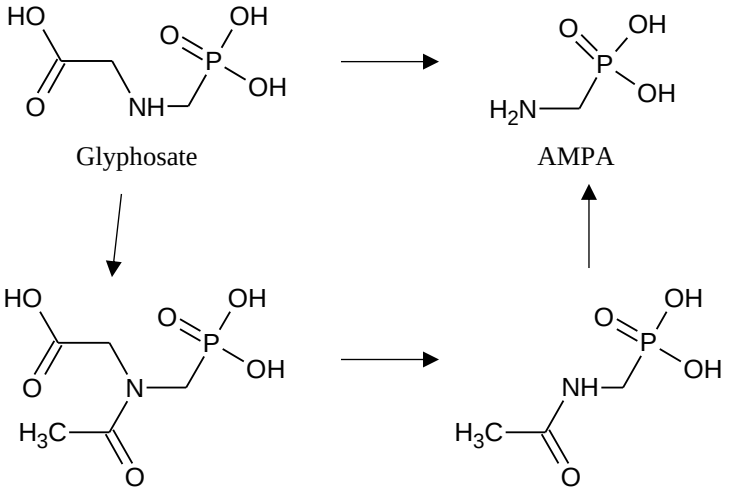
The nature and magnitude of glyphosate residues has also been investigated in GAT modified canola plants. TRRs in foliage were 1.6 – 6.0 mg/kg. *N*-Acetyl glyphosate was the major component accounting for 89.5 – 93.0% TRR. Glyphosate, *N*-acetyl AMPA, and AMPA were also detected in immature foliage at low levels accounting for 3.0% TRR, 3.4% TRR, and 1.4% TRR, respectively. The TRR in immature pods from the pre-harvest sampling immediately prior to the final application was 1.3 mg/kg. *N*-Acetyl glyphosate was the only radioactive component detected accounting for 79.6% TRR. At final harvest, the TRR in mature seed was 2.2 mg/kg. *N*-Acetyl glyphosate was the major radioactive component in the seed accounting for 51.1% TRR. Glyphosate (20.8% TRR; increased compared to earlier sampling events), *N*-acetyl AMPA (14.7% TRR) and AMPA (1.9% TRR) were also detected.

In the third metabolism study GAT modified soybean plants were investigated. The TRR in soybean forage collected 36 days after the pre-emergent soil application contained 0.43 mg/kg. AMPA was the major extractable radioactive component in the forage sample accounting for 39.3% TRR. Glyphosate and *N*-acetyl glyphosate were also detected accounting for 9.1% TRR and 1.9% TRR, respectively. The TRR in hay collected 4 days after the first foliar application contained 13.4 mg/kg. Glyphosate was the major radioactive component detected in the hay sample accounting for 72.5% TRR. *N*-Acetyl glyphosate (19.2% TRR), AMPA (5.3% TRR), and *N*-acetyl AMPA (0.7% TRR) were also detected. In grain, pods and foliage the radioactive residues accounted for 3.14, 17.8 and 22.1 mg/kg after soil followed by three foliar applications respectively. In grain (after soil treatment followed by two foliar applications) and grain, pod and foliage (after soil treatment followed by three foliar applications) *N*-acetyl glyphosate was the predominant metabolite (27.7 to 60.6% TRR, 1.16 to 7.04 mg/kg) while AMPA accounted for 5.3 to 11.2% TRR (0.10 to 2.25 mg/kg). *N*-acetyl AMPA accounted for 23.5% TRR in grain at final harvest (0.74 mg/kg) and ranged between 1.4 and 3.3% TRR (0.26 to 0.57 mg/kg) in foliage (after soil treatment followed by two foliar applications), pod and foliage (after soil treatment followed by three foliar applications).

Overall conclusion for crops with GAT modification

In GAT modified maize, rape and soybean the metabolism of glyphosate was comparable. Parent glyphosate was often retrieved at relevant amounts. In addition, *N*-acetyl glyphosate was the predominant metabolite in almost all matrices (except soybean forage). Furthermore, *N*-acetyl AMPA and AMPA were relevant metabolites present in matrices of all three crops.

Pathway for cereals and oilseeds with GAT modification



Animals

Within the framework of the current renewal of glyphosate, no new animal metabolism studies have been submitted. All existing and previously evaluated metabolism studies have been assessed again with the latest guidelines in Vol. 3, B.7.2.2. Data are summarized here in Vol. 1, 2.7.2, and they are also summarized in Appendix G.

Poultry

There are five metabolism studies available with poultry. One study was conducted using *N*-(phosphono-^{14}C-methyl)glycine, one study was conducted with a 9:1 mixture of *N*-(phosphono-^{13}C/^{14}C-methyl)glycine and amino-^{13}C/^{14}C-methylphosphonic acid, one study was conducted using *N*-(phosphono-^{14}C-methyl)glycine as trimesium salt, and finally one study was conducted using *N*-acetyl-*N*-(phosphono-^{14}C-methyl)glycine.

An overview of the studies is given in the following table.

Table 2.7.2-8: overview of poultry metabolism studies

Animal	**Duration**	**Dose rate**	**Reference and remark on acceptability**
Laying hen	7 days 5 days	Glyphosate at 17.9 mg/kg bw/day or 200 mg/kg feed (expressed in glyphosate equiv.)	CA 6.2.2/001, 1994 Supportive
Laying hen	7 days	Glyphosate and AMPA (9:1 mixture) 9.84 mg/kg bw/day (8.86 mg glyphosate/kg bw/day and 0.98 mg AMPA/kg bw/day) or 120 mg/kg feed (expressed in glyphosate equiv.)	CA 6.2.2/002, 1988 and CA 6.2.2/003, 1988 Acceptable
		Glyphosate and AMPA (9:1 mixture) 8.83 mg/kg bw/day (7.95 mg glyphosate/kg bw/day and 0.88 mg AMPA/kg bw/day) or 120 mg/kg feed (expressed in glyphosate equiv.)	
		Glyphosate and AMPA (9:1 mixture) 29.75 mg/kg bw/day (26.78 mg glyphosate/kg bw/day and 2.98 mg AMPA/kg bw/day) or 400 mg/kg feed (expressed in glyphosate equiv.)	
		Glyphosate and AMPA (9:1 mixture) 8.62 mg/kg bw/day (7.76 mg glyphosate/kg bw/day and 0.86 mg AMPA/kg bw/day) or 120 mg/kg feed (expressed in glyphosate equiv.)	
Laying hen	10 days	Glyphosate trimesium salt at 4.1 mg/kg bw/day or 62.4 mg/kg feed (expressed in glyphosate equiv.)	CA 6.2.2/004 1994 Acceptable
Laying hen	7 days	*N*-acetyl glyphosate 4.4 bw/day or 63.311 mg/kg feed (expressed in *N*-acetyl-glyphosate equiv.)	CA 6.2.2/005, 2007 Acceptable

In the first study (CA 6.2.2/001), the nature of the residues was studied in laying hens after seven or five daily dose application of 17.2-17.9 mg/kg bw/day glyphosate (200 mg/feed). The study is considered as supportive only, however, it shows that glyphosate was not extensively metabolised in laying hens and was rather excreted (63.6-76.45% of applied radioactivity (AR) was recovered in excreta) resulting in low residue levels (less than 0.04% AR) in tissues and eggs, where also unchanged glyphosate was the primary residue.

In the second and third metabolism study, consisting of two reports (CA 6.2.2/002 and CA 6.2.2/003), four groups (three low treatment, including one depuration group and one high treatment) of laying hens were treated with ^{13}C/^{14}C labelled glyphosate and AMPA (9 :1) for seven consecutive days. Elimination of radioactivity via excreta was the primary elimination route, ranging from 81% to 90.5% of AR. Only very low amounts of administrated radioactivity were found in egg yolk (0.01-0.02%AR), egg white (<0.01%AR) and tissues (up to 0.02% AR). The highest TRRs were found in kidney (0.067-7.004 mg/kg), followed by liver (0.079 – 1.914 mg/kg) and egg yolk (0.09 – 0.344 mg/kg). Residues in muscle fat and egg white were lower, not exceeding 0.1 mg/kg. The radioactivity level in tissues in the depuration group were in general lower, with liver having the highest level (0.079 mg/kg). More than 81% of TRR were extractable using chloroform and water and only low amounts of the residues remained unextractable. Glyphosate (28.1%-93.2% TRR; 0.001-6.46 mg/kg) and AMPA (4.2-53.1% TRR; 0.001-0.6 mg/kg) accounted for the majority of the radioactive residues in edible tissues. Only in muscle (thigh and breast) there was some evidence for further metabolization, where a minor unknown metabolite was detected (2.4-16.2% TRR; ≤0.005 mg/kg).

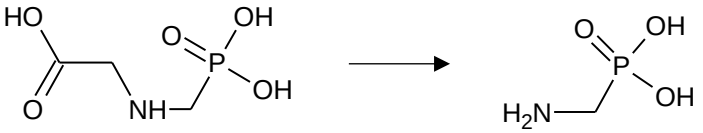
In the fourth study (CA 6.2.2/004) ^{14}C-PMG-labelld glyphosate (glyphosate-anion) was administrated orally for 10 days to laying hens as its trimesium salt. In total 104% of the administrated dose was recovered. The major portion of radioactive residues was recovered in excreta, cage rinse and GI track with contents (103.8%). Radioactive residues associated with edible matrices accounted for 0.13% (0.04% eggs and 0.09% tissues). Highest TRRs were found in kidney (2.17 mg/kg) and liver (0.44 mg/kg). Residues is other edible tissues were between 0.017-0.238

mg/kg. Extractability of residues was high (70-94%) except for breast muscle and egg white where 67.4% and 45.9% of residues were extracted. However in those tissues non-extractable residues were further characterised and accountability was >90%. PMG (34.9-56% TRR; 0.01- 0.23 mg/kg) and AMPA (2.14-18.9% TRR; <0.001-0.08 mg/kg) accounted for the majority of the radioactive residues in all matrices. An unknown compound was present in all matrices, except egg white at very low levels (1.1 – 4.3 % TRR) and it was characterised in two different chromatographic systems.
In egg yolk and fat a major fraction of the ^{14}C-residues was naturally incorporated into lipids. In egg yolk the ^{14}C incorporation was detected in the nonpolar lipid fraction (triglycerides and cholesterol) and in the phospholipid fraction (mainly in phosphatidylcholine). In fat, the ^{14}C-natural incorporation was shown in triglycerides, cholesterol and free fatty acids.

In the last poultry study (CA 6.2.2/005), ^{14}C *N*-acetyl glyphosate was administered to laying hens for 7 consecutive days. Recovery of total administrated dose was 90.18% and administrated compound and its metabolites were further eliminated rapidly by the hens (90.08 % AR). Edible tissues contained ≤0.05% AR. The TRR in edible tissues were in liver 0.5 mg/kg, in muscle 0.029 mg/kg and in fat 0.057 mg/kg. In egg white much less radioactivity was detected (0.01 mg/kg) than in egg yolk (0.229 mg/kg). Extractability of residues ranged in the tissues from 81% to 95%. In both egg white and egg yolk, the most abundant residue identified was *N*-acetyl glyphosate; 41.48 % TRR (0.004 mg/kg) in egg white and 68.4 % TRR (0.157 mg/kg) in egg yolk. Additionally, residues identified in egg white were glyphosate (10.90 % TRR or 0.001 mg/kg) and *N*-acetyl AMPA (4.34 % TRR or <0.001 mg/kg). In egg yolk, glyphosate (5.69 % TRR or 0.010 mg/kg), *N*-acetyl AMPA (1.10 % TRR or 0.002 mg/kg), and AMPA (0.91 % TRR or 0.002 mg/kg) were identified.
The predominant residue found in liver and muscle was *N*-acetyl glyphosate (63.82 and 25.22 % TRR, respectively, or 0.323 mg/kg and 0.009 mg/kg, respectively). Glyphosate was detected in liver up to 16%TRR (0.084 mg/kg) and 7.19 %TRR (0.002 mg/kg) in muscle. Additionally, in muscle AMPA was detected up to 16.69% TRR (0.005 mg/kg) and four minor unknown components which were in total 14.86% TRR, however, with no single metabolite above 10% TRR (or 0.01 mg/kg). In fat glyphosate was the most prominent residue (39.43% TRR; 0.023 mg/kg) followed by *N*-acetyl glyphosate (23.45% TRR; 0.014 mg/kg), AMPA (11.29% TRR; 0.007 mg/kg) and *N*-acetyl-AMPA (10.18% TRR; 0.006 mg/kg).

Overall conclusion on metabolism in poultry
Within all studies investigating the uptake and metabolism of ^{14}C-glyphosate in poultry it was shown that elimination of radioactivity via excreta was the primary elimination route. After administration of glyphosate, a mixture of glyphosate and AMPA or PMG-labelled glyphosate (as trimesium salt) to laying hens, a similar picture on metabolism was found. Glyphosate accounted for the main part of radioactive residues in all studies. Furthermore, AMPA was identified as a major metabolite in egg yolk, kidney, liver and muscle. In one study, the radioactivity was also detected in natural products (e.g. triglycerides, cholesterol phosphatidylcholine and free fatty acids).

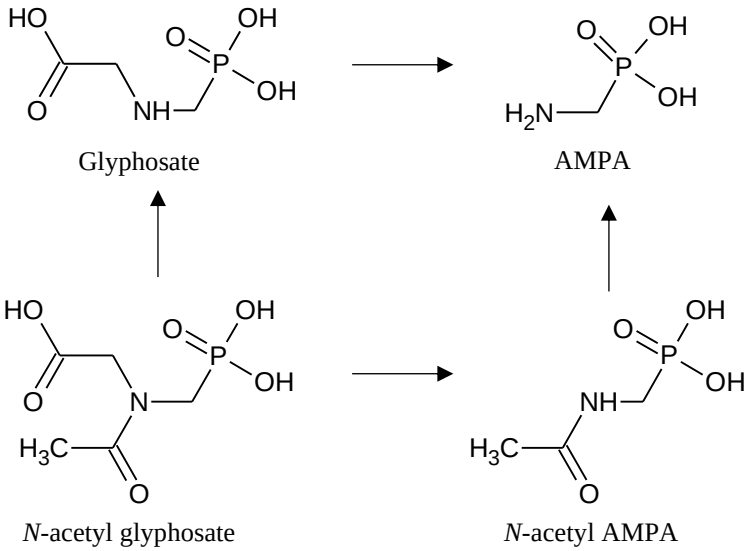
Pathway for livestock (feeding with glyphosate) - poultry



Glyphosate AMPA

After administration of *N*-acetyl glyphosate to laying hens, *N*-acetyl glyphosate accounted for the main part of the radioactive residues, except for fat. In fat, glyphosate was identified as major metabolite. In addition, glyphosate, AMPA and *N*-acetyl-AMPA were detected in egg white, egg yolk, liver, muscle and fat.

Pathway for livestock (feeding with *N*-acetylglyphosate) - poultry



Ruminants

There are five metabolism studies in ruminants (lactating goats) available. One study was conducted using *N*-(phosphono-^{14}C-methyl)glycine, one study was conducted using *N*-(phosphono-^{14}C-methyl)glycine as trimesium salt, one study was conducted with a 9:1 mixture of *N*-(phosphono-^{13}C/^{14}C-methyl)glycine and amino-^{13}C/^{14}C-methylphosphonic acid (AMPA) and finally one study was conducted using *N*-acetyl-*N*-(phosphono-^{14}C-methyl)glycine.

An overview of the studies is given in the following table.

Table 2.7.2-9: overview of ruminant metabolism studies

Ruminant	Duration	**Dose rate**	**Reference and remark on acceptability**
Lactating goat	5 days	Glyphosate at 7.6 mg/kg bw/day or 200 mg/kg feed (expressed in glyphosate equiv.)	CA 6.2.3/001; 1994 Supportive
	3 days	Glyphosate 6.4 mg/kg bw/day or 200 mg/kg feed (expressed in glyphosate equiv.)	
Lactating goat	7 days	Glyphosate as trimesium salt at 2.7 mg/kg bw/day or 63.8 mg/kg feed	CA 6.2.3/002, 1994 Acceptable
Lactating goat	5 days	Glyphosate and AMPA (9:1 mixture) at 2.95 mg/kg bw/day or 120 mg/kg feed (expressed in glyphosate equiv.)	CA 6.2.3/003: 1988 Part I and CA 6.2.3/004: 1988 Part II, Acceptable
	5 days	Glyphosate and AMPA (9:1 mixture) at 2.83 mg/kg bw/day or 120 mg/kg feed (expressed in glyphosate equiv.)	
Lactating goat	5 days	*N*-acetyl-glyphosate at 8.42 mg bw/day or 205.42 mg/kg feed (expressed in *N*-acetyl-glyphosate equiv.)	CA 6.2.3/005; 2007, Acceptable

In the first study *N*-(phosphono-^{14}C-methyl)glycine was administrated to two lactating goats twice daily for five or three days. The study is considered as supportive only, however, it shows that glyphosate was not extensively metabolised and is rapidly excreted resulting in low residue levels in edible tissues and milk (<0.1% AR), where also unchanged parent compound was the primary residue.

In the second study (CA 6.2.3/002) ^{14}C-PMG-labelld glyphosate was administrated orally for 7 days to lactating goats as its trimesium salt. 101% AR was recovered with the main part being excreted up to 100.7%. Radioactivity associated with edible matrices (tissues and milk) was 0.15% of the AR. The highest TRRs were found in kidney (5.58 mg/kg) and liver (0.234 mg/kg). In muscle and fat, 0.026 and 0.018 mg/kg TRR were found, respectively. More than 78% of the TRR was extractable. PMG (glyphosate-anion) (59.4 – 91.3 % TRR or 0.02-4.8 mg/kg) and AMPA

(4.7 – 21.4 % TRR; 0.002-0.42 mg/kg) accounted for the majority of the radioactive residues in liver, kidney, fat and muscle. In milk, PMG (22.3 % TRR or 0.005 mg/kg) and AMPA (2.4 % TRR or 0.001 mg/kg) together represented 25 % TRR. Lactose and triglycerides constituted over 45 % TRR in milk, while material associated with post-extraction milk solids comprised 21 % TRR, which is consistent with natural incorporation into proteins. Presumably other tissues also contained small amounts of radioactivity incorporated into natural components.

In the third and fourth metabolism studies, consisting of two parts (CA 6.2.3/003 and 004) lactating goats were treated with $^{13}C/^{14}C$ labelled glyphosate and AMPA for five consecutive days. Up to 86.7% of the AR was recovered. The main part of the AR was excreted (up to 86.46%) and from <0.01% to 0.17% of the total dose was recovered in tissues and milk. The highest TRRs were detected in kidney (up to 7 mg/kg) and liver (up to 0.49 mg/kg). In muscle and fat, TRRs were up to 0.027 mg/kg and 0.01 mg/kg, respectively. In milk, the radioactive residue ranged between 0.019 and 0.060 mg/kg during the dosing period. In tissue, portions of 73.3 to 97.8 % TRR were extractable. Glyphosate (47.8 – 89.6 % TRR or 0.003 – 6.429 mg/kg) and AMPA (4.9 – 30.7 % TRR or 0.000 – 0.677 mg/kg) accounted for the majority of the radioactive residue in all matrices. Furthermore, an unknown compound was assigned in milk (23.5 – 28.0 % TRR or 0.005 – 0.014 mg/kg). The unknown compound in milk was isolated and further analysed by gel filtration chromatography and acid hydrolysis. The ^{14}C activity appeared to be associated with small molecular weight proteins or glycoproteins.

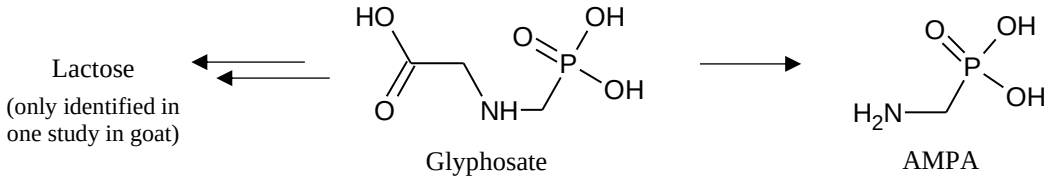
In the last metabolism study, labelled *N*-acetyl-glyphosate was administrated to lactating goats for five consecutive days. Total radioactive recovery was 87.83% AR and *N*-acetyl-glyphosate and its metabolites were rapidly eliminated, primarily in the excreta accounting for 87.74% of applied dose. The TRRs in edible tissues ranged from 0.047 mg/kg (muscle) to 4.67 mg/kg (kidney). In most of the tissues, extractability of residues was high (76-97%) except for muscle and omental fat.

N-acetyl glyphosate was the predominant residue found in all tissues (16.7-77.12% TRR or 0.011-3.7 mg/kg). Glyphosate (14.7% TRR or up to 0.19 mg/kg), *N*-acetyl-AMPA (up to 14.86% TRR or up to 0.021 mg/kg, in fat only) and AMPA (up to 8.45% TRR or up 0.068 mg/kg) were also observed in ruminant tissues.

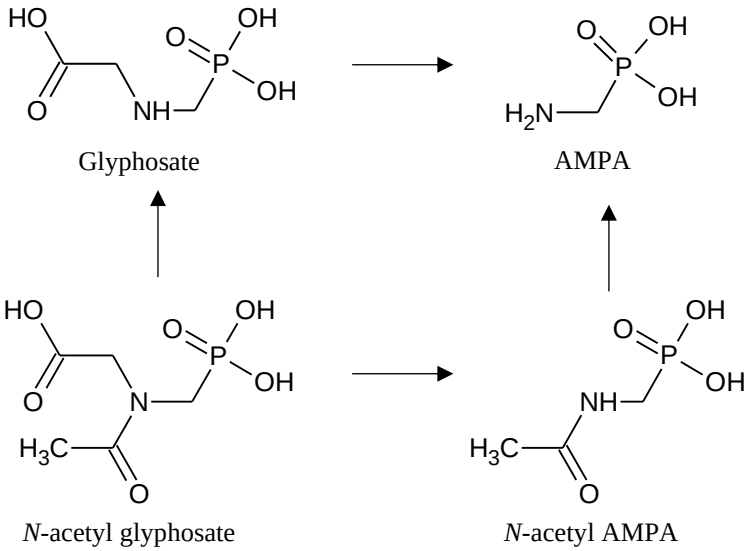
Overall conclusion on metabolism in ruminants

Within all studies investigating the uptake and metabolism of ^{14}C-glyphosate in ruminants, it was shown that elimination of radioactivity via excreta was the primary elimination route. After administration of glyphosate, a mixture of glyphosate and AMPA or PMG-labelled glyphosate (as trimesium salt) to lactating goats, a similar picture on metabolism was found. Glyphosate accounted for the main part of the radioactive residues in in all studies. Furthermore, AMPA was identified as a major metabolite in liver and kidney. In one study, lactose was identified in milk. Furthermore, radioactivity was also detected in natural products (e.g. triglycerides, proteins). After administration of *N*-acetyl glyphosate to one lactating goat, *N*-acetyl glyphosate accounted for the main part of radioactive residues. Additionally, in this study glyphosate was (also) identified as major metabolite in liver and kidney, while AMPA and *N*-acetyl AMPA were major metabolites in liver and fat, respectively.

Pathway for livestock (feeding with glyphosate) - ruminants



Pathway for livestock (feeding with *N*-acetyl glyphosate) - ruminants



Glyphosate AMPA

N-acetyl glyphosate *N*-acetyl AMPA

Fish

According to Commission Regulation (EU) No 283/2013 and Working document SANCO/11187/2013 rev. 3, metabolism studies on fish may be required where a fat-soluble active substance (log Po/w ≥ 3) is used in crops which might be part of fish diet and where residues in feed may occur from the intended applications. Glyphosate and its metabolites AMPA, *N*-acetyl AMPA and *N*-acetyl glyphosate are all no fat-soluble substances:

Log Po/w:

- Glyphosate: -3.2
- AMPA: -2.47
- *N*-acetyl glyphosate: -6.26
- *N*-acetyl AMPA: -2.53

Therefore based on the very low fat solubility, no fish metabolism studies are required.

2.7.3 Definition of the residue

Non-tolerant/conventional plants

Parent glyphosate is clearly the major residue in all investigated crops in the plant metabolism studies, and as such it is considered as a good marker molecule. This has also been visualized in table 2.7.3-1 below, where the identification results of the fully acceptable plant metabolism studies are shown. On the other hand, since all requested uses within the framework of the current renewal of glyphosate concern soil treatments, hardly any residues are being expected, which is confirmed in the supervised residue trials (see 2.7.4). If residues are observed in the magnitude of residues trials, then glyphosate is being detected. Therefore, although often no residues >LOQ are detected, the residue definition for monitoring of conventional crops is proposed as glyphosate. This proposed monitoring residue definition would also cover foliar uses of glyphosate, for which parent is the main residue as well based on the metabolism studies. In addition, results from the confined rotational crop studies (see 2.7.7) can be taken into account for the derivation of the residue definition, and these results confirm the considerations above.

Besides glyphosate, metabolite AMPA is being detected as an important residue in the plant metabolism studies. Based on the available data it has been concluded in the toxicology section that AMPA is of similar toxicity as glyphosate and the reference values of glyphosate can be applied to AMPA. Therefore, exposure to these two analytes should be summed up for consumer risk assessment. Hardly any AMPA is detected in the supervised residue trials, like it is the case for glyphosate (see 2.7.4). To derive a robust residue definition for risk assessment, it is considered appropriate to include both glyphosate and AMPA. However, based on the defended uses within the current framework of renewal, which are only soil treatments, it could be discussed whether inclusion of AMPA into the residue definition is relevant. On the other hand, when also considering foliar uses of glyphosate, it would be more appropriate to include AMPA in the residue definition for risk assessment.

N-methyl AMPA was only detected in coffee ripe beans among the different food commodities investigated, but not chromatographically separated from AMPA in that study. Among the feed items investigated in the plant metabolism studies, *N*-methyl AMPA was only found after hydroponic treatment in barley/oats/rice/sorghum aerial

parts/tops and maize/soybean forage. Since a hydroponic treatment does not reflect a normal use of glyphosate, while *N*-methyl AMPA has not been found in any study with realistic application conditions, the finding of this metabolite is not considered relevant.

N-methyl glyphosate and methyl-phosphonic acid were determined in low amounts in one metabolism study only when soybean and cotton were grown in hydroponic solution. Since it only concerns one study where these two metabolites were observed in cotton and/or soybean roots; the crops were artificially treated by hydroponic treatment; and these roots are not relevant as food or feed item, these metabolites are considered not required for inclusion into the residue definition.

In conclusion, the residue definition for risk assessment of conventional crops is proposed as the 'sum of glyphosate and AMPA, expressed as glyphosate'.

Table 2.7.3-1: Identified components of the fully acceptable plant metabolism studies

Crop and application method	N-rate with regard to the max. application rate per year	Crop part analysed	Glyphosate %TRR (mg/kg[1])	AMPA %TRR (mg/kg[1])	AMPA/ *N*-methyl AMPA %TRR (mg/kg[1])
Citrus, soil (CA 6.2.1/002)	1.35N	Residues in fruit too low for identification	n.a.	n.a.	n.a.
Grape, soil (CA 6.2.1/005)	1.94/1.88N	Residues in fruit too low for identification	n.a.	n.a.	n.a.
Grape, overspray (CA 6.2.1/005)	n.a.	Fruit	77.1 (0.964)	2.5 (0.031)	n.a.
Grape, soil (CA 6.2.1/006)	1.98/1.70N	Residues in fruit too low for identification	n.a.	n.a.	n.a.
Wheat, foliar (CA 6.2.1/010)	n.a.	Grain	90.8 (2.43)	2.8 (0.08)	n.a.
		Straw	82.6 (102.6)	3.3 (4.1)	n.a.
Soybean, soil drench (CA 6.2.1/014)	2.69N (regarding the 'pre-emergence' use as soybeans are not among the intended uses)	Seed	2.6 (0.034)	1.6 (0.021)	n.a.
		Forage	3.3 (0.058)	5.7 (0.1)	n.a.
		Hulls	4.1 (0.02)	1.5 (0.007)	n.a.
Coffee, leaf treatment (CA 6.2.1/016)	n.a.	Bean	91.2 (0.134)	n.a.	4.8 (0.007)
Coffee, soil (CA 6.2.1/016)	2.09N (regarding the 'pre-emergence' use as coffee is not among the intended uses)	Residues too low for identification	n.a.	n.a.	n.a.

[1]mg/kg: mg/kg expressed as glyphosate parent equivalents
n.a.: not applicable

CP4 EPSPS modification and CP4 EPSPS modification/GOX modification

In crops genetically modified to express CP4 EPSPS as well as in CP4 EPSPS and GOX modified crops, both glyphosate and AMPA were the main residues. The metabolic pattern is considered similar to that observed in conventional crops as the CP4 EPSPS modification is not affecting glyphosate metabolism in genetically modified plants. All identified metabolites have been summarized in table 2.7.3-2. In some investigated crop commodities, levels of AMPA were higher than levels of glyphosate, i.e. for maize grain and soybean seed. In rape seed, even no glyphosate could be observed. It seems that AMPA would be a better marker for these commodities. However, it would be helpful for such a discussion if supervised residue trials at relevant application rates were available to assess whether these findings on the presence of glyphosate and AMPA are confirmed. Since genetically modified crops are not within the defended uses for the current renewal of glyphosate, these trials are not available for

discussion in the current framework. To stay in line with what has been proposed in the MRL-review by EFSA, knowing that *N*-acetyl-glyphosate is not expected as metabolite (but is an important metabolite for GAT modified crops; see below), the following residue definition for monitoring for crops with CP4 EPSPS modification with or without additional GOX modification is being proposed: sum of glyphosate, AMPA and *N*-acetyl-glyphosate, expressed as glyphosate. It should be mentioned that if Import Tolerances are requested for these genetically modified crops, then the residue definition should be further discussed by taking into account the results from supervised residue trials with these plants. In addition, based on the 90-day toxicity study it appears that *N*-acetyl glyphosate is not of greater toxicity than glyphosate. However, due to the data gap on genotoxicity no conclusion can be made regarding its reference values (see toxicology section). Therefore, this residue definition is pending further toxicology data.

Regarding the residue definition for risk assessment for crops with the CP4 EPSPS modification or CP4 EPSPS modification/GOX modification, the metabolites *N*-glyceryl AMPA, *N*-acetyl AMPA, *N*-methyl AMPA and *N*-malonyl AMPA should be further considered. Whether or not these four additional metabolites besides metabolite AMPA should be considered relevant for inclusion into the residue definition for risk assessment is difficult to conclude without knowing which GAPs would be applied in practice, and consequently what levels of these metabolites can be expected in the crops. For the time being, it could be an option as a worst-case to include them all in the residue definition, pending further supervised residue data. On the other hand, the contribution of these metabolites to the total exposure from glyphosate and AMPA can be considered very low, based on the metabolism results. Therefore, it would be more appropriate to conclude similar as for the conventional crops: 'sum of glyphosate and AMPA, expressed as glyphosate'. However, genotoxicity should still be addressed for *N*-glyceryl AMPA, *N*-acetyl AMPA, *N*-methyl AMPA and *N*-malonyl AMPA (see toxicology section).

Table 2.7.3-2: Identified components of the plant metabolism studies (CP4 EPSPS modification and CP4 EPSPS modification/GOX modification)

		Glyphosate %TRR (mg/kg[1])	**AMPA %TRR (mg/kg[1])**	***N*-glyceryl AMPA %TRR (mg/kg[1])**	***N*-acetyl AMPA %TRR (mg/kg[1])**	***N*-methyl AMPA %TRR (mg/kg[1])**	***N*-malonyl AMPA %TRR (mg/kg[1])**
Sugar beet (CA 6.2.1/018)	Tops	79.85 (2.74)	1.84 (0.06)	n.a.	n.a.	n.a.	n.a.
	Roots	95.31 (1.33)	3.79 (0.05)	n.a.	n.a.	n.a.	n.a.
Wheat (CA 6.2.1/0019	Forage	89.44 (18.09)	0.76 (0.15)	n.a.	n.a.	n.a.	n.a.
	Hay	83.86 (23.34)	3.45 (0.96)	n.a.	n.a.	n.a.	n.a.
	Straw	69.19 (24.09)	5.08 (1.77)	n.a.	n.a.	n.a.	n.a.
	Grain	72.40 (8.78)	10.77 (1.31)	0.34 (0.04)	n.a.	n.a.	n.a.
Maize (CA 6.2.1/020)	Forage (with soil protection)	80.9 (10.8)	9.4 (1.25)	0.4 (0.05)	n.a.	n.a.	n.a.
	Silage (with soil protection)	77.9 (7.09)	9.0 (0.82)	1.2 (0.11)	n.a.	n.a.	n.a.
	Forage (without soil protection)	71.9 (7.77)	15.9 (1.72)	0.5 (0.06)	n.a.	n.a.	n.a.
	Silage (without soil protection)	67.1 (6.43)	13.1 (1.26)	1.5 (0.14)	n.a.	n.a.	n.a.
	Fodder (with soil protection)	83.3 (12.4)	4.9 (0.73)	1.2 (0.17)	n.a.	n.a.	n.a.
	Grain (with soil protection)	7.4 (0.05)	54.1 (0.37)	6.9 (0.05)	n.a.	n.a.	n.a.
	Fodder (without soil protection)	74.8 (14.27)	11.2 (2.13)	1.6 (0.31)	n.a.	n.a.	n.a.
	Grain (without soil protection)	2.6 (0.03)	60.3 (0.63)	6.9 (0.07)	n.a.	n.a.	n.a.
Rape	Seed (1 application)	n.a.	7.7 (0.037)	3.4 (0.017)	0.9 (0.004)	n.a.	n.a.

(CA 6.2.1/021)	Seed (2 applications)	n.a.	7.1 (0.58)	3.9 (0.31)	0.7 (0.06)	n.a.	n.a.
Soybean (CA 6.2.1/022)	Forage (early post-emergent)	88.5 (0.764)	2.3 (0.020)	n.a.	n.a.	n.a.	n.a.
	Hay (early post-emergent)	64.7 (0.354)	5.3 (0.029)	n.a.	n.a.	0.6 (0.003)	n.a.
	Seed (early post-emergent)	10.1 (0.041)	22.9 (0.093)	1.2 (0.005)	1.0 (0.004)	n.a.	0.9 (0.003)
	Forage (sequential post-emergent)	89.1 (21.078)	6.8 (1.619)	n.a.	n.a.	0.6 (0.140)	n.a.
	Hay (sequential post-emergent)	53.6 (5.582)	12.8 (1.328)	0.8 (0.084)	n.a.	1.3 (0.130)	n.a.
	Seed (sequential post-emergent)	25.2 (4.402)	49.1 (8.579)	1.6 (0.278)	1.4 (0.235)	0.8 (0.131)	1.8 (0.309)
Cotton (CA 6.2.1/023)	Forage (without soil protection)	91.5 (13.9)	1.60 (0.243)	n.a.	n.a.	n.a.	n.a.
	Forage (with soil protection)	95.7 (29.1)	0.66 (0.201)	n.a.	n.a.	n.a.	n.a.
	Seed (without soil protection)	12.0 (0.022)	<1 (<0.002)	n.a.	n.a.	n.a.	n.a.
	Seed (with soil protection)	23.7 (0.025)	1.4 (0.001)	n.a.	n.a.	n.a.	n.a.

[1]mg/kg: mg/kg expressed as glyphosate parent equivalents
n.a.: not applicable

GAT modification

For GAT modified plants a different metabolic pathway can be observed by acetylation of glyphosate and AMPA into *N*-acetyl glyphosate and *N*-acetyl AMPA. This can also be retrieved from the identified metabolites in table 2.7.3-3: besides residues of glyphosate and AMPA, important metabolites for GAT modified crops are *N*-acetyl glyphosate and *N*-acetyl AMPA, of which in particular *N*-acetyl glyphosate was present in large amounts. As it is the case for the crops with the CP4 EPSPS modification or CP4 EPSPS modification/GOX modification, no supervised residue trials are available, since GAT modified crops are not within the defended uses for the current renewal of glyphosate. Therefore, the discussion on the residue definition is more complicated, and needs to be based solely on the available metabolism studies. Since glyphosate could possibly not be a good marker molecule for GAT modified crops, the residue definition for monitoring should rather include *N*-acetyl glyphosate. Therefore, by also combining the discussion on the residue definition for the other genetically modified crops (for which AMPA could particularly be more important; see above), the residue definition for enforcement for GAT modified crops is proposed to be the 'sum of glyphosate, AMPA and *N*-acetyl-glyphosate, expressed as glyphosate'. Similarly as for the CP4 EPSPS modified crops, residue trials are required to confirm this residue definition, and a data gap is set for further toxicology data on *N*-acetyl glyphosate regarding genotoxicity.

As such a combined residue definition for monitoring of all glyphosate tolerant genetically modified plants currently on the market has been derived, rather than separate enforcement definitions for each type of genetical modification, which could make enforcement more complicated. The applicant provided a similar proposal on the residue definition for monitoring of genetically modified crops. And also the approach in the recent MRL-review was the same.

For the consumer risk assessment of GAT modified crops, it seems appropriate to consider the metabolites *N*-acetyl glyphosate and *N*-acetyl AMPA for inclusion. In addition, glyphosate and AMPA could also be present in relevant amounts. Therefore, the following risk assessment residue definition is proposed for GAT modified crops: sum of glyphosate, AMPA, *N*-acetyl glyphosate and *N*-acetyl AMPA, expressed as glyphosate. This residue definition is pending the previously mentioned data gaps on genotoxicity for *N*-acetyl glyphosate and *N*-acetyl AMPA. In addition, *N*-acetyl AMPA is considered not of greater toxicity than glyphosate.

Table 2.7.3-3: Identified components of the plant metabolism studies (GAT modification)

		Glyphosate %TRR (mg/kg[1])	**AMPA %TRR (mg/kg[1])**	***N*-acetyl glyphosate %TRR (mg/kg[1])**	***N*-acetyl AMPA %TRR (mg/kg[1])**
Maize (CA 6.2.1/024)	Forage	58.0 (2.016)	4.0 (0.140)	27.0 (0.937)	1.7 (0.060)

	Stover	74.9 (9.166)	3.4 (0.422)	17.8 (2.188)	1.3 (0.152)
	Cobs	n.a.	n.a.	63.8 (0.435)	5.0 (0.034)
	Grain	0.1 (<0.001)	6.1 (0.016)	51.2 (0.141)	9.4 (0.026)
Rape (CA 6.2.1/025)	Immature foliage	3.0 (0.179)	1.4 (0.084)	89.5 (5.351)	3.4 (0.203)
	Pods (with seeds)	n.a.	n.a.	79.6 (1.013)	n.a.
	Foliage	n.a.	n.a.	93.0 (1.442)	n.a.
	Seeds	20.8 (0.448)	1.9 (0.041)	51.1 (1.101	14.7 (0.316)
Soybean (CA 6.2.1/026)	Forage	9.1 (0.039)	39.3 (0.166)	1.9 (0.009)	n.a.
	Hay	72.5 (9.740)	5.3 (0.704)	19.2 (2.581)	0.7 (0.096)
	Seeds, after 3 applications, of which 2 foliar	22.7 (0.434)	5.3 (0.103)	60.6 (1.156)	n.d.
	Foliage, after 3 applications, of which 2 foliar	43.6 (4.894)	7.4 (0.819)	42.0 (4.699)	2.2 (0.255)
	Seeds, after 4 applications, of which 3 foliar	3.2 (0.102)	11.2 (0.351)	56.9 (1.788)	23.5 (0.738)
	Pod, after 4 applications, of which 3 foliar	56.9 (10.101)	10.2 (1.794)	27.7 (4.906)	3.3 (0.574)
	Foliage, after 4 applications, of which 3 foliar	53.4 (11.791)	10.3 (2.250)	31.9 (7.039)	1.4 (0.308)

[1]mg/kg: mg/kg expressed as glyphosate parent equivalents

In summary for plants, similar residue definitions have been derived as in the EFSA MRL-review from 2019.
Enforcement of conventional crops: glyphosate.
Enforcement of GMO crops: sum of glyphosate, AMPA and *N*-acetyl-glyphosate, expressed as glyphosate.
For risk assessment an overall residue definition for all crops can be proposed, like it has been done in the MRL-review: sum of glyphosate, AMPA, *N*-acetyl glyphosate and *N*-acetyl AMPA, expressed as glyphosate.
The metabolites *N*-acetyl glyphosate and *N*-acetyl AMPA are not relevant for conventional crops and crops with the CP4 EPSPS modification or CP4 EPSPS modification/GOX modification. However, for the crops with the CP4 EPSPS modification or CP4 EPSPS modification/GOX modification, this still needs to be confirmed by supervised residue studies. It is simply a choice of having one general residue definition for risk assessment of all crops, which could be interpreted as being less complicated than two separate residue definitions. Furthermore, the residue definitions are pending data gaps on genotoxicity for *N*-acetyl glyphosate, *N*-glyceryl AMPA, *N*-acetyl AMPA, *N*-methyl AMPA and *N*-malonyl AMPA.

Animals

Poultry and ruminants

The results of the different metabolism studies in livestock are very consistent. After administration of glyphosate or a mixture of glyphosate and AMPA or PMG-labelled glyphosate (as trimesium salt) to laying hens and lactating goats glyphosate is the main residue in all edible matrices. AMPA was additionally identified as a major metabolite in egg yolk, kidney and liver. In all the livestock metabolism studies, it is demonstrated that glyphosate is slowly metabolised to AMPA. Additionally, in one goat study, lactose was also determined in milk and radioactivity was also detected in other natural compounds (e.g. triglycerides, cholesterol and proteins). No other relevant metabolites were identified.
After administration of *N*-acetyl-glyphosate to laying hens and lactating goats *N*-acetyl-glyphosate was the main residue in all edible matrices. Glyphosate was identified as major metabolite in liver, kidney (only ruminants) and fat (only poultry). In addition, AMPA and *N*-acetyl AMPA were major metabolites in liver and fat of lactating goats, while they were only minor metabolites in poultry.

Glyphosate as the parent compound and the compound which was regularly found in animal tissues in metabolism studies is considered as relevant to include in the enforcement residue definition.
When *N*-acetyl-glyphosate has been applied to animals in the study, it was also measured in animal tissues in high concentrations. It is noted that it is a relevant metabolite in genetically modified crops only (see paragraph 2.7.2). However, it should be considered that animals can be exposed not only to glyphosate, but also to N-acetyl-glyphosate by being fed with modified crops, and since it is not possible to distinguish all sources of animal feed, the residue definition should cover all possible scenarios. Therefore, *N*-acetyl-glyphosate is also considered as an important residue marker in animal matrices.

Based on their significant levels in animal tissues as demonstrated in the metabolism studies, glyphosate and *N*-acetyl-glyphosate are both also relevant for risk assessment. *N*-acetyl-glyphosate was detected in the feeding studies, both in poultry and ruminants in all matrices (except milk) at all feeding levels above the LOQ. Glyphosate was also detected above the LOQ in the feeding studies, especially in liver and kidney (ruminants). It should be noted however, that the available feeding studies were highly overdosed compared to the estimated dietary burden for the representative uses (216N for poultry and 80N for ruminants for the lowest dose level). It has been concluded that *N*-acetyl-glyphosate is not of greater toxicity than glyphosate. However, due to the data gap on genotoxicity, no conclusion can be made regarding its reference values.

Metabolite AMPA is a relevant residue for risk assessment in eggs, liver and kidney and muscle (poultry only), as it has been detected in those animal tissues in significant amounts (>10% and/or >0.01 mg/kg). In the feeding studies AMPA has been detected above the LOQ in liver, kidney and eggs, but only at very much higher feeding levels than calculated for the representative uses. Since AMPA can be detected in animal tissues, it could also be considered as a residue marker and it is proposed to include it in the residue definition for enforcement. This would be in line with the proposal made by the applicant and the current residue definition recently established in the framework of the MRL-review (EFSA Journal 2019;17(10):5862)
It should be noted however that, when detected, AMPA is always present at lower levels that glyphosate and N-acetyl glyphosate. Therefore, a need to include this metabolite in the residue definition for enforcement could be actually discussed further.
It is proposed to include AMPA in residue definition for risk assessment in liver, kidney and eggs. It could be also discussed further if AMPA should be included in the risk assessment residue definition for milk and fat, since in those matrices it was not a significant residue in the metabolism study, and it was not observed in any of the feeding studies above the LOQ. However, to facilitate one general residue definition for risk assessment in animal commodities, AMPA has been included.
It has been concluded that AMPA is of similar toxicity as glyphosate and therefore the reference values of the parent compound can be applied.

N-acetyl-AMPA was identified as a (very) minor metabolite in poultry tissues: eggs, liver and muscle. In poultry fat it accounted for 10.2% TRR, however, very low in absolute amounts (0.006 mg/kg). In ruminants, *N*-acetyl-AMPA was only detected in fat (up to 14.8% TRR or 0.02 mg/kg). Based on the toxicological evaluation, it appears that *N*-acetyl-AMPA is not of greater toxicity than glyphosate. However, due to the data gap on genotoxicity no conclusion can be made regarding reference values. It is noted that there is no data available to assess the actual exposure of animals to N-acetyl-AMPA via diet and N-acetyl AMPA has been included in RD-RA for GAT modified plants. To be in line with this last conclusion and the current residue definition recently established in the framework of the MRL-review (EFSA Journal 2019;17(10):5862), it is proposed to include N-acetyl-AMPA in residue definition for risk assessment. However, since it is not expected that N-acetyl-AMPA is detected at significant levels in animal tissues Conversion Factor (CF) from enforcement to risk assessment is proposed as 1 (also in line with Article 12 MRL review).

Proposed residue definitions in animal commodities:

Residue definition for enforcement:
Sum of glyphosate, AMPA and *N*-acetyl glyphosate, expressed as glyphosate.

Residue definition for risk assessment for eggs, liver, muscle, kidney, milk and fat is proposed as:
Sum of glyphosate, AMPA, *N*-acetyl-glyphosate and *N*-acetyl AMPA expressed as glyphosate.

Proposed residue definition for enforcement and risk assessment in animal commodities is in line with current residue definitions established in the framework of Article 12 MRL review of glyphosate.

Expression of the proposed residue definitions is pending further toxicology data for metabolite *N*-acetyl-glyphosate and N-acetyl-AMPA.

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

2.7.4.1 Post-emergence use

A post-emergence use against weeds in orchard crops (citrus, stone and pome fruits, kiwi, tree nuts, banana, and table olives) and vines (table and wine grape, leaves not intended for human consumption) is intended. The uses in table olives and vines will be evaluated separately. The critical GAP in NEU and SEU is identical for all aforementioned crops and is as follows:

2 x 1.44 kg/ha (max. 2.88 kg/ha per year), interval 28 days, PHI 7 days (ground directed, shielded spray, band application).

The following is additionally stated in the GAP: *"Band application in the rows below the trees or as spot treatments. The treated area represents not more than 50 % of the total orchard area. The application rate with reference to the total orchard surface area is not more than 50 % of the stated dose rate."* This restriction, however, is not expected to be of importance for the evaluation of the residues section and is therefore not considered during the assessment.

Furthermore, the GAP states the following: *"Avoid crop contamination during treatment."* This restriction is of importance for the evaluation of the residues section. Based on the available residue trials in olives, it became obvious that residues above the LOQ are expected in case fruits are sampled from the ground. A similar situation is expected in case fruits from orchards or vineyards are sampled from the ground. For the current risk assessment it is assumed that crops will neither be contaminated during treatment, nor between application and harvest, i.e. fruits fallen to the ground after application shall not be harvested. It is important that appropriate risk mitigation measures are taken at national level when granting such authorisations.

The defended use is less critical compared to the critical use evaluated previously which was as follows for both NEU and SEU: 1-3 x 2.88 kg/ha (max. 4.32 kg/ha per year), interval 28 d, PHI n.a. (RAR, 2015).

Detailed study summaries are available in Volume 3, B.7.3.1 and in Appendix G.

Orchard crops (citrus, stone and pome fruits, kiwi, tree nuts, and banana)
For the use of glyphosate on orchard crops, the applicant submitted thirteen studies which investigated the residue levels of glyphosate and AMPA in various crops belonging to the aforementioned crop groups. The tree orchards were treated once with nominal application rates ranging from 2.88 to 3.6 kg/ha, i.e. the trial GAP is not exactly reflecting the intended use. Application rates in the trials, however, reflect at least the critical maximum yearly use rate. This is considered worst-case, however, since residue levels of glyphosate and AMPA were below the LOQ at harvest, this deviation from the intended GAP is accepted. Residue levels were determined at a PHI of 0 days instead of 7 days in multiple trials, however, it is not expected that this has a significant influence on the residue level at harvest considering that metabolism studies showed limited uptake of residues from the soil into the tree, i.e. residue levels in fruits are not expected to significantly increase with longer PHIs.

According to Annex I of Reg. (EC) 396/2005, stone fruits are defined as the whole product after removal of the stem, i.e. including the stone. In the supervised residue trials in stone fruits, however, residue levels were only determined in the fruit without stone (flesh) and not in the stone, i.e. not according to the definition set in Annex I of Reg. (EC) 396/2005. However, residue levels in whole fruits were calculated based on the residue level in flesh and a correction for the weight ratio of flesh and stone; therefore, the results are considered acceptable. An exception are the trials listed under CA 6.3.1/009 where the weight of the stone was not recorded. Since residues were below the LOQ in the flesh, this deviation was accepted.

In all trials, glyphosate was applied as an SL formulation diluted in water. Different glyphosate forms, i.e. the free form, as potassium salt, as isopropylammonium salt, or as trimesium salt, were used, however, it is not expected that this has an influence on the residue level at harvest. A summary of the residue data from the acceptable trials is given in the table below.

Residue data were obtained with acceptable analytical methods (see Volume 1, 2.5) for which acceptable procedural recovery data were generated concurrently with the specimens to-be-analysed. It is noted, however, that additional information regarding the extraction efficiency, and in some trials (2 NEU and 8 SEU) the derivatisation efficiency, is needed for confirmation.

All specimens were also stored in line with the demonstrated periods of storage stability for glyphosate and, based on expert judgement, for AMPA. It is referred to Vol. 3, B.7.3 for more details.

The data in support of the post-emergence use of glyphosate against weeds in tree orchards (citrus, stone and pome fruits, kiwi, tree nuts, and banana) demonstrate that no residues of glyphosate or AMPA above the LOQ (0.05 mg/kg) are expected. It is noted, however, that residues of glyphosate between the LOD and LOQ were determined in several trials across different crops. This indicates that the intended use on orchard crops represents a <LOQ-residue situation rather than a zero-residue situation.

The data are considered acceptable in support of all intended uses in tree orchards based on a risk envelope approach. It is noted that only three acceptable trials from northern Europe are available for evaluation of the orchard use. Considering the <LOQ-residue situation, it would have been desirable to have more data generated in northern Europe in support of the intended use. To support the representative uses in southern Europe, a sufficient number of trials (26) across different orchard crops are available.

For MRL calculation, the NEU and SEU dataset is pooled considering that the GAP in both regions is identical and the datasets are similar. Based on the available data, an MRL of 0.05* mg/kg is calculated for the orchard crops under consideration (citrus, stone and pome fruits, kiwi, tree nuts, and banana), which is covered by the existing MRLs for these crops (0.1*-0.5 mg/kg).

Table 2.7.4.1-1: Summary of residue data in support of the post-emergence use in orchard crops (pending the demonstration of extraction efficiency)

Region	Year	Crop	Number of acceptable trials	Glyphosate (mg/kg)	AMPA (mg/kg)	Reference
Citrus fruit						
SEU	2013	Mandarin	2	2x <0.05	2x <0.05	CA 6.3.1/001[1]
SEU	2017	Orange	2	2x <0.05	2x <0.05	CA 6.3.1/021
Tree nuts						
SEU	2015	Hazelnut	1	<0.05	<0.05	CA 6.3.1/002
SEU	2015	Pistachio	1	<0.05	<0.05	CA 6.3.1/002
Pome fruit						
NEU	2013	Apple	2	2x <0.05	2x <0.05	CA 6.3.1/003[1]
SEU	2013	Apple	2	2x <0.05	2x <0.05	CA 6.3.1/004[1]
NEU	1973-1976	Apple and pear	0	-	-	CA 6.3.1/005
Stone fruit						
SEU	2015	Apricot	4	4x <0.05	4x <0.05	CA 6.3.1/006
SEU	2013	Cherry	2	2x <0.05	2x <0.05	CA 6.3.1/007[1]
SEU	2012	Peaches	1	1x <0.05	1x <0.05	CA 6.3.1/009
NEU	2012	Plum	1	1x <0.05	1x <0.05	CA 6.3.1/009
SEU	2013	Plum	2	2x <0.05	2x <0.05	CA 6.3.1/008[1]
SEU	2017	Plum	4	4x <0.05	4x <0.05	CA 6.3.1/022
Kiwi						
SEU	2015	Kiwi	2	2x <0.05	2x <0.05	CA 6.3.1/019
Banana						
SEU	2015	Banana	3	3x <0.05	3x <0.05	CA 6.3.1/020

[1] Next to extraction efficiency, derivatisation efficiency needs to be demonstrated in these studies/trials.

Vines (table and wine grape, leaves not intended for human consumption)

For the use of glyphosate on vines, the applicant submitted five studies which investigated the residue levels of glyphosate and AMPA. In four studies considered valid by the applicant, vineyards were treated once at nominally 3.6 kg/ha, except for a few trials in which application rates were > 25% deviating from the target application rate. The trial GAP is therefore not exactly reflecting the intended use. Application rates in the trials, however, reflect at least the critical maximum yearly use rate. This is considered worst-case, however, since residue levels of glyphosate and AMPA were below the LOQ at harvest, this deviation from the intended GAP is accepted.

A fifth study was also submitted and shortly described by the applicant, however, the applicant did not consider the study to be reliable due to several deviations from the current OECD guideline (see Volume 3, B.7.3.1.14 for details). The RMS agrees that the study is not reliable for several reasons; consequently no residue values from this study are selected for evaluation.

In all acceptable trials, glyphosate was applied as an SL formulation diluted in water. Different forms of glyphosate, i.e. the free form and as potassium salt, were used, however, it is not expected that this has an influence on the residue level at harvest. A summary of the residue data from the acceptable trials is given in the table below.

Residue data were obtained with acceptable analytical methods (see Volume 1, 2.5) for which acceptable procedural recovery data were generated concurrently with the specimens to-be-analysed. It is noted, however, that additional information regarding the extraction efficiency is needed for confirmation.

Specimens were stored in accordance with the demonstrated period of storage stability for glyphosate and, based on expert judgment, also for AMPA. It is referred to Vol. 3, B.7.3 for more details.

Overall, nine trials conducted in Northern Europe and eight trials conducted in Southern Europe are available; therefore a sufficient number of trials was submitted in support of the representative use in vines in both regions.

For MRL calculation, the NEU and SEU dataset is pooled considering that the GAP in both regions is identical and the datasets are similar (Mann-Whitney U-test). Based on the available data, an MRL of 0.05* mg/kg is calculated for grapes, which is covered by the existing MRLs for table and wine grapes (0.5 mg/kg).

Table 2.7.4.1-2: Summary of residue data in support of the post-emergence use (pending the demonstration of extraction efficiency)

Region	Year	Crop	Number of acceptable trials	Glyphosate (mg/kg)	AMPA (mg/kg)	Reference
Vines						
NEU	2015	Grapes	2	2x <0.05	2x <0.05	CA 6.3.1/010
NEU	2014	Grapes	4	4x <0.05	4x <0.05	CA 6.3.1/011
SEU	2014	Grapes	2	2x <0.05	2x <0.05	CA 6.3.1/011
NEU	2014	Grapes	3	3x <0.05	3x <0.05	CA 6.3.1/012
SEU	2014	Grapes	2	2x <0.05	2x <0.05	CA 6.3.1/012
SEU	2014	Grapes	4	4x <0.05	4x <0.05	CA 6.3.1/013
NEU	1988	Grapes	1	-	-	CA 6.3.1/014[1]

[1] This study is not considered reliable and therefore no residue values were selected for evaluation.

Table olives

For the use of glyphosate on table olives, the applicant submitted four studies which investigated the residue levels of glyphosate and AMPA. Application rates across the different studies ranged from one application at 0.99 to 4.06 kg glyphosate/ha, i.e. trials are partly over- and underdosed compared to the intended GAP. Only trials in which application rates are within 25% of the intended yearly application rate and in which samples were harvested at a PHI of 6-8 days were considered for evaluation. As a consequence, one study is not considered acceptable for evaluation (CA 6.3.1/018).

Residue levels in all trials were only determined in the olive fruits without stone (flesh) and not in the stone itself, i.e. samples were not analysed in accordance with Annex I of Reg. (EC) 396/2005. The weight ratio between the fruit itself (flesh) and the stone was only recorded in one study (CA 6.3.1/015), i.e. the residue levels expressed on a whole fruit basis can be calculated for these samples only. As a worst case approach for risk assessment, however, it is assumed that residue values determined in the other studies are for olive flesh only rather than for the whole fruit and consequently, residue levels would rather be overestimated. For MRL-setting, however, these trials would not be considered acceptable since residues are possibly overestimated and therefore, MRLs would not be calculated according to the ALARA principle.

In all trials, glyphosate was applied as an SL formulation diluted in water. Different forms of glyphosate, i.e. the free form and as trimesium salt, were used, however, it is not expected that this has an influence on the residue level at harvest. A summary of the residue data from the acceptable trials is given in the table below.

The analytical methods used for the determination of glyphosate and AMPA in olive fruits were not fully validated according to the regulatory framework (Vol. 3, B.5). Monsanto method XA001, employed in one study (CA 6.3.1/015) was not fully validated since procedural recoveries were below 70% for glyphosate and AMPA. In the frame of the residue study, concurrent recoveries were determined and these were within the acceptable ranges. Therefore, the analytical method is considered fit for purpose and residue levels are selected for evaluation. It is

noted, however, that additional information regarding the extraction efficiency is needed for confirmation. Method RR92-042B RES, which was used in two studies for the determination of glyphosate, but not AMPA (CA 6.3.1/016 and CA 6.3.1/017), was successfully validated except for the fact that additional information regarding the extraction efficiency is needed for confirmation as well.

In contrast to the above, the analytical method used in the last study with olives (CA 6.3.1/018) was not successfully validated and it is also not fit for purpose. Therefore, the study is not further considered for evaluation.

Specimens were stored in accordance with the demonstrated period of storage stability for glyphosate (18 months in all plant commodities except dry matrices) and, based on expert judgment, also for AMPA. It is referred to Vol. 3, B.7.3 for more details.

In line with the applicant, the RMS does only consider tree-picked olives for evaluation. As stated in the introduction of section 2.7.4.1, appropriate risk mitigation measures shall be established on national level to prevent contamination of olives with residues of glyphosate.

Overall, it is noted that no trials are available for Northern Europe although uses are also intended in this region according to the GAP. Therefore, a data gap is set and additional studies need to be submitted by the applicant (data requirement). In total, seven acceptable trials are available for Southern Europe to address the magnitude of residues of glyphosate in olives. Since table olives are a minor crop in Southern Europe, a sufficient number of trials is available to support this representative use.

Based on the available data, an MRL of 0.05* mg/kg is calculated for table olives, which is covered by the existing MRL for table olives (1 mg/kg).

Table 2.7.4.1-3: Summary of residue data in support of the post-emergence use (pending the demonstration of extraction efficiency)

Region	Year	Crop	Number of acceptable trials	Glyphosate (mg/kg)	AMPA (mg/kg)	Reference
Table olives						
SEU	1995	Table olives	4	4x <0.05	4x <0.05	CA 6.3.1/015
SEU	1995	Table olives	1	<0.05	-	CA 6.3.1/016
SEU	1995	Table olives	2	2x <0.05	-	CA 6.3.1/017
SEU	1988	Table olives	0	-	-	CA 6.3.1/018[1]

[1] No residue values selected for evaluation since the analytical method is not considered acceptable.

2.7.4.2 Post-harvest, pre-sowing, pre-planting, pre-emergence use

A post-harvest, pre-sowing, pre-planting, pre-emergence outdoor use against weeds is intended in the following crops or crop groups: root and tuber vegetables, bulb vegetables, fruiting vegetables, brassica vegetables, leafy vegetables, stem vegetables, and sugar beets. The critical GAP in NEU and SEU is identical and is as follows:

2 x 1.08-1.44 kg/ha (max. 2.16 kg/ha per year), interval 28 days, PHI n.a.

The defended use is less critical compared to the critical NEU and SEU use evaluated in the previous RAR which was a pre-planting, post-planting, and/or pre-emergence use in all seeded or transplanted crops according to the GAP: 1-2 x 1.08-2.16 kg/ha (max. 4.32 kg/ha per year), interval 21 d, PHI n.a.

Detailed study summaries are available in Volume 3, B.7.3.2 and in Appendix G.

For the intended post-harvest, pre-sowing, pre-planting, pre-emergence use of glyphosate on various vegetables, the applicant submitted 15 studies which investigated the residue levels of glyphosate and AMPA in various crops. Ten of these studies are relatively new (all trials conducted across the northern and southern European zone during the growing season 2011) and the other five studies were conducted between 1975 and 1978. Whereas the ten newer studies are considered for evaluation, none of the five older studies are considered reliable for evaluation for various reasons. This is in line with the applicant's conclusion on the reliability of the studies. It is referred to Volume 3, B.7.3.2.10 to B.7.3.2.15 for a detailed evaluation of the older studies, but reasons for considering the studies not reliable include (a combination of) missing information with regard to storage conditions, low procedural recoveries, or the absence of the method validation. Although the studies are not considered for evaluation, it is emphasized

that no data were obtained by the studies that are more critical than data from the ten more recent and reliable studies. The following evaluation is restricted to the ten reliable studies only.

In the ten studies, applications were not exactly performed according to the intended use pattern. One application was performed at a target rate of 2.16 kg/ha instead of two applications at 1.08-1.44 kg/ha, however, the trial GAP reflects the intended maximal yearly use rate. This is considered worst-case, however, since residues were below the LOQ at harvest, this is accepted.

In all trials glyphosate, formulated as isopropylamine salt, was applied as an SL formulation diluted in water. A summary of the residue data from the acceptable trials is given in the table below.

Residue data were obtained with acceptable analytical methods (see Volume 1, 2.5) for which acceptable procedural recovery data were generated concurrently with the specimens to-be-analysed. It is noted, however, that additional information regarding the extraction efficiency is needed for confirmation.

All specimens were also stored in line with the demonstrated periods of storage stability.

In total, 17 trials conducted in northern Europe and 18 trials conducted in southern Europe are considered acceptable for evaluation of the post-harvest, pre-sowing, pre-planting, pre-emergence use. The data are considered acceptable in support of all intended post-harvest, pre-sowing, pre-planting, pre-emergence uses in the different intended crops (root and tuber vegetables, bulb vegetables, fruiting vegetables, brassica, leafy vegetables, stem vegetables, and sugar beets) based on a risk envelope approach. The data demonstrate that no residues of glyphosate or AMPA above the LOQ (0.05 mg/kg) are expected following the intended use pattern. It is noted, however, that residues of glyphosate between the LOD and LOQ were determined in several trials across different crops. This indicates that the intended post-harvest, pre-sowing, pre-planting, pre-emergence use represents a no-residue situation rather than a zero-residue situation.

For MRL calculation, the NEU and SEU dataset is pooled considering that the GAP in both regions is identical and the datasets are similar. Based on the available data, an MRL of 0.05* mg/kg is calculated for the various intended crops or crop groups (root and tuber vegetables, bulb vegetables, fruiting vegetables, brassica, leafy vegetables, stem vegetables, and sugar beets), which is covered be the existing MRLs for these crops (0.1*-15 mg/kg).

Table 2.7.4.2-1: Summary of residue data in support of the post-harvest, pre-sowing, pre-planting, pre-emergence use (pending the demonstration of extraction efficiency)

Region	Year	Crop	Number of acceptable trials	Glyphosate (mg/kg)	AMPA (mg/kg)	Reference
Potato						
NEU	2011	Potato, tuber	2	2x <0.05	2x <0.05	CA 6.3.2/001
SEU	2011	Potato, tuber	2	2x <0.05	2x <0.05	CA 6.3.2/001
Carrot						
NEU	2011	Carrot, root	2	2x <0.05	2x <0.05	CA 6.3.2/002
SEU	2011	Carrot, root	2	2x <0.05	2x <0.05	CA 6.3.2/002
Onion						
NEU	2011	Onion, bulb	2	2x <0.05	2x <0.05	CA 6.3.2/003
SEU	2011	Onion, bulb	2	2x <0.05	2x <0.05	CA 6.3.2/003
Tomato						
NEU	2011	Tomato, fruit	2	2x <0.05	2x <0.05	CA 6.3.2/004
Cucumber						
SEU	2011	Cucumber, fruit	1	<0.05	<0.05	CA 6.3.2/005
Courgette						
NEU	2011	Courgette, fruit	1	<0.05	<0.05	CA 6.3.2/005
SEU	2011	Courgette, fruit	1	<0.05	<0.05	CA 6.3.2/005
Cauliflower						
NEU	2011	Cauliflower, inflorescence	2	2x <0.05	2x <0.05	CA 6.3.2/006
SEU	2011	Cauliflower, inflorescence	2	2x <0.05	2x <0.05	CA 6.3.2/006
Head cabbage						
NEU	2011	Cabbage, head	2	2x <0.05	2x <0.05	CA 6.3.2/007

SEU	2011	Cabbage, head	2	2x <0.05	2x <0.05	CA 6.3.2/007
Lettuce (leaf and head varieties)						
NEU	2011	Lettuce, leaves	2	2x <0.05	2x <0.05	CA 6.3.2/008
SEU	2011	Lettuce, head	2	2x <0.05	2x <0.05	CA 6.3.2/008
Leek						
NEU	2011	Leek, whole plant without roots	2	2x <0.05	2x <0.05	CA 6.3.2/009
SEU	2011	Leek, whole plant without roots	2	2x <0.05	2x <0.05	CA 6.3.2/009
Sugar beet						
SEU	2011	Sugar beet, roots	2	2x <0.05	2x <0.05	CA 6.3.2/010
SEU	2011	Sugar beet, tops/leaves	2	2x <0.05	2x <0.05	CA 6.3.2/010

2.7.4.3 Inter-row use

An outdoor inter-row use against weeds is intended on various vegetables (root and tuber vegetables, bulb vegetables, fruiting vegetables, legume vegetables, and leafy vegetables). The critical GAP in NEU and SEU is identical and is as follows:

1 x 1.08 kg/ha (max. 1.08 kg/ha per year), crop BBCH < 20, PHI 60 days (ground-directed, shielded spray application)

The following is additionally stated in the GAP: *"Applications are performed between the crop rows. The rate refers to the treated area only, which represents not more than 50% of the total area. The application rate with reference to the total surface area is not more than 50% of the stated dose rate."* This restriction, however, is not expected to be of importance for the evaluation of the residues section and is therefore not considered during the assessment.

Furthermore, the GAP states the following: *"Avoid crop contamination during treatment."* This restriction is of importance for the evaluation of the residues section. For the current risk assessment it is assumed that crops will neither be contaminated during treatment, nor between application and harvest. It is important that appropriate risk mitigation measures are taken at national level when granting such authorisations.

In the previous evaluation for renewal of approval of glyphosate, an inter-row use was not part of the defended uses (RAR, 2015).

Detailed study summaries are available in Volume 3, B.7.3.3 and in Appendix G.

For the intended inter-row use of glyphosate on various vegetables (root and tuber vegetables, bulb vegetables, fruiting vegetables, legume vegetables, and leafy vegetables), the applicant submitted nine studies which investigated the residue levels of glyphosate and AMPA in various crops belonging to the aforementioned crop groups. Inter-row applications were mostly performed according to the intended GAP. Some deviations from the GAP were noticed, for instance, applications after BBCH 20 or sampling of commodities at PHIs shorter than 60 days. This is considered worst-case, however, since residues were below the LOQ in those trials, the deviations were accepted.

In all trials, glyphosate was applied as an SL formulation diluted in water. It is noted that glyphosate was formulated as potassium salt, however, this is not expected to have an influence on the residue levels at harvest. A summary of the residue data from the acceptable trials is given in the table below.

Residue data were obtained with acceptable analytical methods (see Volume 1, 2.5) for which acceptable procedural recovery data were generated concurrently with the specimens to-be-analysed. It is noted, however, that additional information regarding the extraction efficiency is needed for confirmation.

All specimens were also stored in line with the demonstrated periods of storage stability.

In total, 13 trials conducted in northern Europe and 28 trials conducted in southern Europe are considered acceptable for evaluation of the inter-row treatment. The data are considered acceptable in support of all intended inter-row uses in vegetables (root and tuber vegetables, bulb vegetables, fruiting vegetables, legume vegetables, and leafy vegetables) based on a risk envelope approach. The data demonstrate that no residues of glyphosate or AMPA above the LOQ (0.05 mg/kg) are expected at the intended PHI of 60 days. It is noted, however, that residues of glyphosate between the LOD and LOQ were determined in one trial on bulb onions. This indicates that the intended inter-row

use on vegetables represents a no-residue situation rather than a zero-residue situation.

For MRL calculation, the NEU and SEU dataset is pooled considering that the GAP in both regions is identical and the datasets are similar. Based on the available data, an MRL of 0.05* mg/kg is calculated for the various intended crop groups (root and tuber vegetables, bulb vegetables, fruiting vegetables, legume vegetables, and leafy vegetables), which is covered be the existing MRLs for these crops (0.1*-3 mg/kg).

Table 2.7.4.3-1: Summary of residue data in support of the inter-row use (pending the demonstration of extraction efficiency)

Region	Year	Crop	Number of acceptable trials	Glyphosate (mg/kg)	AMPA (mg/kg)	Reference
Carrot						
SEU	2015	Carrot, roots	4	4x <0.05	4x <0.05	CA 6.3.3/001
Radish						
SEU	2015	Radish, roots	2	2x <0.05	2x <0.05	CA 6.3.3/002
SEU	2015	Radish, tops/leaves	2	2x <0.05	2x <0.05	CA 6.3.3/002
Bulb onion						
NEU	2015	Onion, bulb	2	2x <0.05	2x <0.05	CA 6.3.3/003
SEU	2015	Onion, bulb	4	4x <0.05	4x <0.05	CA 6.3.3/003
Tomato						
SEU	2015	Tomato, fruit	4	4x <0.05	4x <0.05	CA 6.3.3/004
Cucumber						
NEU	2015	Cucumber, fruit	2	2x <0.05	2x <0.05	CA 6.3.3/005
SEU	2015	Cucumber, fruit	2	2x <0.05	2x <0.05	CA 6.3.3/005
Courgette						
NEU	2015	Courgette, fruit	1	<0.05	<0.05	CA 6.3.3/006
SEU	2015	Courgette, fruit	2	2x <0.05	2x <0.05	CA 6.3.3/006
Head lettuce						
NEU	2015	Lettuce, head	2	2x <0.05	2x <0.05	CA 6.3.3/007
SEU	2015	Lettuce, head	4	4x <0.05	4x <0.05	CA 6.3.3/007
Parsley						
NEU	2015	Parsley, leaves	2	2x <0.05	2x <0.05	CA 6.3.3/008
SEU	2015	Parsley, leaves	2	2x <0.05	2x <0.05	CA 6.3.3/008
Green bean						
NEU	2015	Green beans, whole pods	4	4x <0.05	4x <0.05	CA 6.3.3/009
SEU	2015	Green beans, whole pods	4	4x <0.05	4x <0.05	CA 6.3.3/009

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

Glyphosate is intended to be used on crops that might be fed to livestock and consideration of the occurrence of residues in commodities of animal origin is required.

The supervised residue trials conducted in support of the representative uses showed that residues of glyphosate and AMPA above the LOQ of 0.05 mg/kg are not expected. However, the possible transfer of residues in animal commodities from the proposed uses needs to be considered since residues of glyphosate were between the LOD and LOQ in several trials across the three different representative use patterns, i.e. a complete exclusion of livestock exposure towards residues of glyphosate is not possible.

The exposure of livestock to residues of glyphosate was estimated using the Animal Model 2017. Input values for the model are summarised in Table 2.7.5-1. The LOQ of glyphosate (0.05 mg/kg) was used as input value in case levels of glyphosate and AMPA were both <LOQ in the RACs (and not the sum of LOQs under consideration of a molecular conversion factor as usually required), which is the case for all relevant feed items after primary use of glyphosate. By doing so, the dietary burden is not needlessly overestimated, since levels of AMPA were mostly observed in lower levels than glyphosate in the primary crop metabolism studies on conventional plants. It has also been noted that N-acetyl-glyphosate and N-acetyl-AMPA are not expected above the LOQ in animals tissues, because animals are not expected to be exposed to N-acetyl-glyphosate from the representative uses. Therefore,

those metabolites were not considered in the dietary burden. In case GAT modified crops would become authorised in EU in the future, the dietary burden should be recalculated taking into account actual exposure of animals to these metabolites.

The default processing factors were discarded from the calculation in case all residues were below the LOQ; a processing factor of 1 was therefore considered in the model. Furthermore, it is noted that residues in rotational crops should also be taken into account in the dietary burden (see 2.7.7). Since there is a data requirement for field rotational crop studies, the rotational crop input data should be considered indicative. For glyphosate, input seems to be required from cereal feed items. This input can be derived from an overdosed confined rotational crop study (CA 6.6.1/001), and therefore, proportionality is applied to these residue values of glyphosate. To apply proportionality, the application rate of the rotational crop study is compared with the dose rate, which has been translated from the PECaccumulation level (i.e. 2.3 kg/ha). Only an input for cereal straw is available, while also probably glyphosate residues in rotational cereal forage are relevant. In case of AMPA, no reliable input values can be derived from the available rotational crop data (see 2.7.7), and therefore, AMPA could not be taken into account for the dietary burden calculation. It should therefore be emphasized that the dietary burden probably is an underestimation, pending additional data on AMPA in rotational crops.

Table 2.7.5-1: Input values for the dietary burden calculation[1]

Feed Commodity	Median dietary burden		Maximum dietary burden	
	Input value (mg/kg)	Comment[2]	Input value (mg/kg)	Comment[2]
1 – Forages				
Beet, mangel fodder	0.05	LOQ, use no. 2 & 3	0.05	LOQ, use no. 2 & 3
Beet, sugar tops	0.05	LOQ, use no. 2 & 3	0.05	LOQ, use no. 2 & 3
Cabbage, heads leaves	0.05	LOQ, use no. 2	0.05	LOQ, use no. 2
Cowpea, forage	0.05	LOQ, use no. 3	0.05	LOQ, use no. 3
Cowpea, hay	0.05	LOQ, use no. 3	0.05	LOQ, use no. 3
Kale leaves (forage)	0.05	LOQ, use no. 2	0.05	LOQ, use no. 2
Turnip tops (leaves)	0.05	LOQ, use no. 2 & 3	0.05	LOQ, use no. 2 & 3
Cereal straw[3]	0.14	Rotational crop data from CA 6.6.1/001 for cereal forage[3]; proportionality has been applied (0.4 mg/kg / 2.82)	0.14	Rotational crop data from CA 6.6.1/001 for cereal forage[3]; proportionality has been applied (0.4 mg/kg / 2.82)
2 – Roots & Tubers				
Carrot culls	0.05	LOQ, use no. 2 & 3	0.05	LOQ, use no. 2 & 3
Cassava/tapioca roots	0.05	LOQ, use no. 2 & 3	0.05	LOQ, use no. 2 & 3
Potato culls	0.05	LOQ, use no. 2 & 3	0.05	LOQ, use no. 2 & 3
Swede roots	0.05	LOQ, use no. 2 & 3	0.05	LOQ, use no. 2 & 3
Turnip roots	0.05	LOQ, use no. 2 & 3	0.05	LOQ, use no. 2 & 3
3 – Cereal grains/Crop seeds				
Cowpea seed	0.05	LOQ, use no. 3	0.05	LOQ, use no. 3
Lupin seed	0.05	LOQ, use no. 3	0.05	LOQ, use no. 3
4 – By-products				
Apple pomace, wet	0.05	LOQ, use no. 1	0.05	LOQ, use no. 1
Beet, sugar dried pulp	0.05	LOQ, use no. 2 & 3	0.05	LOQ, use no. 2 & 3
Beet, sugar ensiled pulp	0.05	LOQ, use no. 2 & 3	0.05	LOQ, use no. 2 & 3
Beet, sugar molasses	0.05	LOQ, use no. 2 & 3	0.05	LOQ, use no. 2 & 3
Citrus dried pulp	0.05	LOQ, use no. 1	0.05	LOQ, use no. 1
Potato process waste	0.05	LOQ, use no. 2 & 3	0.05	LOQ, use no. 2 & 3
Potato dried pulp	0.05	LOQ, use no. 2 & 3	0.05	LOQ, use no. 2 & 3

[1] The LOQ of glyphosate was used as input value in case residues of glyphosate and AMPA were both below the LOQ in the RACs, which is the case for all relevant feed items (see body text for details). Besides, the default processing factors were discarded from the model since residues in the RACs were all below the LOQ.
[2] Use no. 1: post-emergence use; use no. 2: post-harvest, pre-sowing, pre-planting, pre-emergence use; use no. 3: inter-row use
[3] In the study report it is described that mature forage has been sampled. However, the mature forage is sampled at the same time as the grain (and chaff) is being collected. Based on this information, it is concluded that the sampled mature forage should be interpreted as cereal straw. Immature forage was also sampled, and could be interpreted as 'real' forage, however, no identification was conducted on these 'real' forage samples.

The results of the dietary burden calculations are shown in Table 2.7.5-2. The calculated dietary burdens for all groups of livestock were found to exceed the trigger value of 0.004 mg/kg bw. The highest dietary burden is calculated for ruminants (0.013 mg/kg bw/d), followed by swine (0.008 mg/kg bw/d) and poultry (0.006 mg/kg bw/d). The available input for rotational crops has no effect on the dietary burden, however, it should be kept in mind that the dietary burden is probably an underestimation, pending additional data on AMPA in rotational crops.

Table 2.7.5-2: Results of the dietary burden calculation

Relevant groups	Dietary burden expressed in				Most critical diet[1]	Most critical commodity[2]		Trigger (0.004 mg/kg bw/day) exceeded (Yes/No)
	mg/kg bw/day		mg/kg DM					
	Median	Max.	Median	Max.				
Cattle (all diets)	0.013	0.013	0.43	0.43	Dairy cattle	Swede	roots	Yes
Cattle (dairy only)	0.013	0.013	0.33	0.33	Dairy cattle	Swede	roots	Yes
Sheep (all diets)	0.013	0.013	0.37	0.37	Lamb	Swede	roots	Yes
Sheep (ewe only)	0.012	0.012	0.37	0.37	Ram/Ewe	Swede	roots	Yes
Swine (all diets)	0.008	0.008	0.34	0.34	Swine (breeding)	Swede	roots	Yes
Poultry (all diets)	0.006	0.006	0.08	0.08	Poultry layer	Swede	roots	Yes
Poultry (layer only)	0.006	0.006	0.08	0.08	Poultry layer	Swede	roots	Yes

[1] When several diets are relevant (e.g. cattle, sheep and poultry "all diets"), the most critical diet is identified from the maximum dietary burdens expressed as "mg/kg bw per day"
[2] The most critical commodity is the major contributor identified from the maximum dietary burden expressed as "mg/kg bw per day".

In table 2.7.5-3, there is an overview presented of all available feeding studies in poultry (three studies), ruminants (three studies) and pigs (one study). For each feeding level there is an N-level calculated compared to the estimated dietary burden for the representative crops.

Table 2.7.5-3 Overview of available livestock feeding studies

Study	Compound administrated	Compounds analysed	Feeding level (expressed as glyphosate or AMPA) [mg/kg bw/d]	N-level compared to estimated dietary burden representative uses
Poultry				
CA 6.4.1/001, 2007 Acceptable	*N*-acetyl glyphosate	*N*-acetyl glyphosate, glyphosate, AMPA, *N*-acetyl-AMPA	1.2	217 N
			4	723 N
			12	2168 N
			40	7229 N
CA 6.4.1/002, 1987 Not acceptable (analytical method not valid)	Glyphosate as its trimesium salt	PMG (glyphosate anion), AMPA	0.025	4.5 N
			0.25	45.2 N
			2.5	451 N
CA 6.4.1/003, 1987 Not acceptable (analytical method not valid)	Glyphosate: AMPA (9:1)	Glyphosate, AMPA	2.4 / 0.25	433 N
			7.1 / 0.76	1283 N
			23.3 / 2.5	4210

Ruminants				
CA 6.4.2/001, 2007 Acceptable	*N*-acetyl glyphosate	*N*-acetyl glyphosate, glyphosate, AMPA, *N*-acetyl-AMPA	1	80 N
			3	240 N
			10	800 N
			30	2340 N
CA 6.4.2/002, 1987 Not acceptable (analytical method not valid)	Glyphosate / AMPA (9:1)	Glyphosate, AMPA	1.4/ 0.16	112 N
			4.1/ 0.46	328 N
			12.7/1.42	1015 N
CA 6.4.2/003, 1987 Not acceptable (analytical method not valid)	Glyphosate as its trimesium salt	PMG (glyphosate anion), AMPA	0.012	1 N
			0.12	9.6 N
			1.25	99.9 N
			7.4	591 N
			25.25	2018 N
Pigs				
CA 6.4.3/001, 1987 Acceptable	Glyphosate/ AMPA (9:1)	Glyphosate, AMPA	1.12 / 0.11	142 N
			3.24 / 0.34	410 N
			11.13 / 1.20	1410 N

Poultry

In all poultry feeding studies, all feeding levels were much higher (4.5-7229N) than the highest estimated dietary burden (0.006 mg/kg bw/d). However, it is noted that the analytical method from the second and third study is considered not acceptable. Therefore, the results of those studies were not used for further risk assessment.

In the first study, four groups of laying hens were dosed for 35 consecutive days with *N*-acetyl glyphosate. In liver, muscle and fat, glyphosate, AMPA and *N*-acetyl-AMPA were not detected above the LOQ in any dose group. *N*-acetyl-glyphosate was found in all edible tissues above the LOQ at all feeding levels. However, since the lowest feeding dose was 217N compared to the dietary burden for the representative uses, no residues of *N*-acetyl-glyphosate are expected above the LOQ in animal tissues at 1N level.

It is noted that *N*-acetyl-glyphosate is not expected in conventional crops above the LOQ and animals are expected to be exposed to glyphosate only (see dietary burden calculation). In the poultry metabolism study, in which was dosed with *N*-acetyl-glyphosate, it has been demonstrated that glyphosate is formed in all poultry tissues (5.6-39% TRR). Taking into account the high overdosed feeding levels, it has been estimated that the available feeding studies with *N*-acetyl-glyphosate, cover possible exposure to glyphosate for all tissues, based on the dietary burden calculation (0.006 mg/kg bw/d). No glyphosate above the LOQ is expected in poultry commodities at the 1N rate, taking into account the requested uses.

Furthermore, since both glyphosate and *N*-acetyl glyphosate were measured in this study and both compounds are part of the residue definition for enforcement and the analytical method used was considered acceptable, data from this feeding study have been used for MRL-setting.

In the second feeding study (not acceptable because the analytical method is not valid), glyphosate trimesium salt was administrated to three groups of hens for 28 consecutive days. Due to lack of reported procedural recoveries of the analytical method at the LOQ level in tissues during sample analysis, results from this study are acceptable for eggs only. Residues of PMG in all egg samples from the 0.5 and 5.0 mg/kg treatment groups were below <0.010 mg/kg. In the 50 mg/kg treatment group, PMG residues were detected at treatment days 7 through 28 with a maximum residue level of 0.015 mg/kg on day 21, returning to below the LOQ at day 28. Residues of AMPA were below the LOQ in all egg samples. Also this feeding study was overdosed compared to the estimated dietary burden, but it confirms the conclusion that no residues of glyphosate and AMPA are expected in eggs.

In the third study (not acceptable because the analytical method is not valid), glyphosate and AMPA (in a 9:1 ratio) were administrated to three group of hens through dietary intake for a period of 28 days. Due to longer sample storage than the demonstrated storage stability, results for eggs and kidney are considered not reliable. Residues of glyphosate and AMPA in all muscle samples in all treatment groups were below the LOQ of 0.05 mg/kg. Residues of glyphosate and AMPA in all fat samples were below the LOQ of 0.05 mg/kg except for one result from day 28 of the highest dose treatment, where 0.056 mg/kg glyphosate was detected. In liver, average glyphosate residues at the lowest feeding level were 0.055 mg/kg and AMPA <0.05 mg/kg. In the second group, the average of glyphosate residues were up to 0.15 mg/kg and 0.076 mg/kg for AMPA. In the highest dose group glyphosate was measured up to 0.6 mg/kg (mean) and AMPA up to 0.29 mg/kg (mean). Again, it is noted that the lowest dose level was more than 400N compared to the estimated dietary burden and no residues of glyphosate and AMPA are expected at the actual feeding level for the representative crops.

Ruminants

In all ruminant feeding studies, almost all feeding levels were much higher than the highest estimated dietary burden

(0.013 mg/kg bw/d (9.6 – 2340N). In one study, in one feeding level, animals were dosed with 1N glyphosate. However, it is noted that the analytical method from the second and third study is considered not acceptable. Therefore, the results of those studies were not used for further risk assessment.

In the first study, four groups of animals were orally dosed with *N*-acetyl glyphosate for 28 consecutive days. Residues of *N*-acetyl glyphosate, glyphosate, AMPA and *N*-acetyl AMPA were measured in edible tissues and milk. In milk, all measured residues were below the LOQ at all feeding levels. In tissues, residue levels were highest in kidney followed generally in decreasing order by liver, fat, and muscle. In each tissue, *N*-acetyl glyphosate was found in higher concentrations than the levels of glyphosate, AMPA, or *N*-acetyl AMPA. In kidney, *N*-acetyl glyphosate was detected above the LOQ in all feeding levels, glyphosate only in the two highest levels and AMPA and *N*-acetyl AMPA only in the highest dose. In liver, fat and muscle glyphosate, AMPA and *N*-acetyl-AMPA were often either detected in higher feeding levels but did not exceed the LOQ, or were not detected at all. Since all feeding levels were overdosed compared to the estimated dietary burden for the representative uses, no *N*-acetyl-glyphosate and its residues are expected above the LOQ in animal tissues at 1N.
It is noted that *N*-acetyl-glyphosate is not expected in conventional crops above the LOQ and animals are expected to be exposed to glyphosate only (see dietary burden calculation). In the ruminant metabolism study, dosed with *N*-acetyl-glyphosate, it has been demonstrated that glyphosate is formed in all ruminants tissues (3.6-14% TRR). Taking into account the high overdosed feeding levels, it has been estimated that the available feeding studies with *N*-acetyl-glyphosate cover possible exposure to glyphosate for all tissues, based on the dietary burden calculation (0.013 mg/kg bw/d). No glyphosate above the LOQ is expected in animal tissues at the 1N rate, taking into account the requested uses.
Furthermore, since both glyphosate and *N*-acetyl glyphosate were measured in this study and both compounds are part of the residue definition for enforcement and the analytical method used was considered acceptable, data from this feeding study have been used for MRL-setting, also for pigs.
In the second study (not acceptable because the analytical method is not valid), glyphosate and AMPA (9 :1) were administrated to three groups of cattle for 28 consecutive days. Residues of glyphosate and AMPA in all milk samples from the highest treatment group were below the LOQ (<0.025 mg/kg); samples from lower dose levels were not analysed. Glyphosate and AMPA residues in fat and muscle samples collected from all animals in all treatment levels were below the LOQ of 0.05 mg/kg. Residues of glyphosate and AMPA at quantifiable levels were found in liver (up to 0.22 mg/kg for glyphosate and 0.18 mg/kg AMPA) and kidney (up to 3.28 mg/kg glyphosate and 0.91 mg/kg AMPA). Also this feeding study was overdosed compared to the estimated dietary burden (112N for the lowest feeding level), but it confirms the conclusion that no residues of glyphosate and AMPA are expected in edible tissues and milk after exposure to glyphosate residues from the representative uses.
In the third study (not acceptable because the analytical method is not valid), glyphosate trimesium salt was administrated to five groups of lactating dairy cows for 28 consecutive days. Due to lack of reported procedural recoveries of the analytical method at the LOQ level in tissues during sample analysis, results from this study are acceptable for milk only. In milk, residues of PMG were above the LOQ (up to 0.04 mg/kg) in the highest dose level only (25 mg/kg bw/d, >2000N). In all other samples no residues above the LOQ were detected. AMPA residues were below the LOQ in all dosage levels evaluated.

Pigs
There is one feeding study available in pigs, where glyphosate and AMPA (9 :1) were administrated to three groups of swine for 28 consecutive days. Residues of glyphosate and AMPA in all fat and muscle samples were below the LOQ, except for the day 28 muscle sample from the highest dose, which contained 0.054 mg/kg of glyphosate. In liver and kidney, residues of glyphosate and AMPA were detected above the LOQ in all feeding levels. However, taking into account that the lowest feeding level was more than 140N compare to the estimated dietary burden for pigs (0.008 mg/kg bw/d), residues in pig tissues are not expected at 1N after exposure to glyphosate from diets containing the representative uses.

Fish
No fish feeding study is available and it is considered not required (see 2.7.2 for the conclusion on the fish metabolism study).

2.7.6 Summary of effects of processing

2.7.6.1 Nature of residues
The nature of residues of glyphosate and its metabolites AMPA and N-acetyl AMPA was investigated in three hydrolysis studies, of which two studies addressing the nature of glyphosate during processing were already available for the previous renewal evaluation of glyphosate (RAR, 2015). It is referred to Volume 3, B.7.5.3.1 for detailed study summaries.

Although minor deviations from the OECD Guideline 507 were noted, these were not considered to have a significant impact on the study outcome. Therefore, all three studies are relied on for evaluation. Based on the available data, glyphosate, AMPA and N-Acetyl AMPA were shown stable during processing conditions simulating pasteurisation, baking/brewing/boiling, and sterilisation.

2.7.6.2 Distribution of the residue in peel and pulp

Results of available and relevant processing studies on the distribution of the residue in peel and pulp are presented in Section 2.7.6.3 below.

2.7.6.3 Magnitude of residues in processed commodities

In the available supervised residue trials, residues of glyphosate and AMPA were always below the LOQ of 0.05 mg/kg, except for trials in ground-picked olives. Since the use on olive is intended for table olive only and appropriate risk mitigation measures shall be established on national level to prevent fruits from being contaminated with residues, processing studies for olive oil are not required. Studies with olives were nonetheless provided by the applicant and these are described below. Besides, studies were submitted that address the magnitude of residues during processing in citrus fruits and potatoes, although such studies are not required. An evaluation of these studies is also provided below.

Detailed study summaries are available in Volume 3, B.7.5.3 and in Appendix G. An overview of the available processing factors is also given in Table 2.7.6.3-1.

Citrus fruits

One study was submitted to address the magnitude of residues during processing of citrus fruits (orange, lemon, grapefruit). Due to several deviations, however, the RMS considers the study to be not reliable for the calculation of processing factors. It is referred to Volume 3, B.7.5.3.1 for a detailed evaluation of the study and its shortcomings.

Although the study is not used to calculate processing factors, it is worthwhile to mention that, although residues levels of glyphosate were mostly below the LOQ in whole fruits, residue levels seemed to concentrate in certain processed fractions. This indicates that low levels of residues of glyphosate may indeed be present in the RACs. It is therefore unlikely that the ground-directed use in orchard crops indeed represents a zero-residue situation, as suggested by the applicant. The RMS, however, recognises that the trials were all overdosed compared to the representative GAP (3 x 4.48 or 3 x 8.97 kg/ha in the trials versus 2 x 1.44 kg/ha (max. 2.8 kg/ha per year) in the defended GAP).

Potatoes

One study was submitted to address the magnitude of residues of glyphosate and AMPA in potato tubers and the processed fractions chips, wet peel (from chips processing), flakes, wet peel (from flakes processing), dry peel (from flakes processing) and granules. No residues above the LOQ of 0.05 mg/kg were found for glyphosate in potato tubers (RAC) after treatments of either 4.2, 8.4, 21, or 42 kg/ha and PHIs of 97-104 days. Therefore, no processing factors could be derived for glyphosate. Only in samples of wet peel (flakes) and dry peel (flakes), residues of glyphosate of 0.06 and 0.08 mg/kg, respectively, were found after treatment at 42 kg/ha. Similar to citrus fruits, these results indicate that glyphosate was present in the RAC following exaggerated application rates, thus supporting the conclusion that a zero-residue situation is not given after a soil-directed treatment.

In contrast to glyphosate, residues of AMPA were determined in three trials/plots conducted at dose rates of 1 x 21 kg/ha or 1 x 42 kg/ha. It is noted that the trials/plots were not considered independent and therefore, only one mean processing factor can be derived. Furthermore, it is noticeable that residues of AMPA were higher than glyphosate, whereas in the primary crop metabolism studies and the supervised residue trials, levels of AMPA were not exceeding the levels of glyphosate.

The study, however, is not considered acceptable for evaluation since the analytical method is not successfully validated (Vol. 3, B.5). Since no processing studies are required according to the regulatory framework, this has no impact on the overall risk assessment.

Olives

Three studies were submitted to address the magnitude of residues of glyphosate and AMPA in ground-picked olive fruits and the processed fraction raw and/or refined olive oil. Glyphosate was applied, depending on the study, at a target rate of 0.36-2.16 kg/ha to the soil under the olive trees and samples were taken at a PHI of 0-41 days.

No residues above the LOQ were determined for AMPA in any of the trials, therefore no processing factors could be derived. It is noted, however, that specimens were not stored in accordance with the demonstrated period of

storage stability for AMPA since AMPA was shown to be stable in soybean seeds only and data are not sufficient to allow an extrapolation to all high oil content matrices or to all plant commodities. Therefore, it is not possible to ascertain whether residues of AMPA were indeed below the LOQ at harvest, or whether residues declined during storage. The results with regard to AMPA are therefore pending the submission of an additional storage stability study for AMPA. Since processing studies are not required for the intended uses, no data requirement needs to be set. However, if processing studies would be required in the future, then storage stability of AMPA needs be addressed to validate these olive processing trials. In contrast to AMPA, residues of glyphosate were determined in multiple, but not all, trials and processing factors could be calculated.

For raw olive oil, the following processing factors were derived for glyphosate: <0.03, <0.04, <0.05, <0.05, <0.10, <0.11, <0.12, <0.24, <0.29, <0.39, and <0.45. The corresponding median processing factor is <0.11. For refined olive oil, the following processing factors were derived for glyphosate: <0.05, <0.24, <0.39, and <0.45. The corresponding median processing factor is <0.32.

The analytical method used for the determination of glyphosate and AMPA in olive fruits and oil were not fully validated according to the regulatory framework (Vol. 3, B.5). Monsanto method XA001, employed in the three studies with olives (CA 6.5.3/004, CA 6.5.3/005, and CA 6.5.3/005) was not fully validated since procedural recoveries were below 70% for glyphosate and AMPA. In the frame of the processing study, concurrent recoveries were determined and these were within the acceptable ranges. Therefore, the analytical method is considered fit for purpose and residue levels are selected for deriving processing factors. It is noted, however, that additional information regarding the extraction efficiency is needed for confirmation.

Table 2.7.6.3-1: Overview of the acceptable processing factors.

RAC/Processed commodity	Individual processing factors	Median processing factor	Conversion factor for RA
Glyphosate			
Olive/Raw oil	<0.03, <0.04, <0.05, <0.05, <0.10, <0.11, <0.12, <0.24, <0.29, <0.39, <0.45	<0.11	1[1]
Olive/Refined oil	<0.05, <0.24, <0.39, <0.45	<0.32	1[1]

[1] Conversion factor determined to be 1 since no residues of AMPA were determined in the RAC or processed commodities.

2.7.7 Summary of residues in rotational crops

Confined rotational crop metabolism

No new rotational crop metabolism studies have been submitted within the current renewal of glyphosate. All existing and previously evaluated metabolism studies have been assessed again with the latest guidelines in Vol. 3, B.7.6.1. Data are summarized here in Vol. 1, 2.7.7, and they are also summarized in Appendix G. In total, six confined rotational crop studies are available (four using *N*-(phosphono-^{14}C-methyl)glycine and two using *N*-(phosphono-^{14}C-methyl)glycine as trimesium salt), in which the fate and nature of glyphosate-derived residues has been investigated. An overview is provided in the following table.

Plant	Application	Application rate	Reference and remark on acceptability
Rotational crops: lettuce, wheat and radish	Soil application (soil was aged 30, 120 and 365 days until planting of rotational crops)	Glyphosate at 6.5 kg/ha	CA 6.6.1/001; [redacted] 1998; supportive only
Rotational crops: lettuce, wheat and radish As a primary crop seeds of soybean were planted immediately prior to application; soybeans were harvested before planting the rotational crops	Soil application (soil was aged 35, 63 and 308 days until planting of rotational crops)	Glyphosate trimesium salt at 5.617 kg/ha (3.87 kg/ha expressed as glyphosate equivalents) or 9.51 kg/ha (split in three monthly applications, 6.56 kg/ha expressed as glyphosate equivalents)	CA 6.6.1/002; [redacted] 1993; acceptable
Rotational crops: lettuce, barley and carrot	Application on planted rye grass, (soil was aged 30,	Glyphosate at 4.16 kg/ha	CA 6.6.1/003; [redacted] 1990; acceptable

Plant	Application	Application rate	Reference and remark on acceptability
A crop of soybeans was planted 7 days after application on the planted rye grass; soybeans were harvested before planting the rotational crops	119 and 364 days until planting of rotational crops)		
Rotational crops: wheat and turnip	Soil application (soil was aged 35, 95 and 370 days until planting of rotational crops)	Glyphosate trimesium salt at 6 kg/ha (4.12 kg/ha expressed as glyphosate equivalents)	CA 6.6.1/004; [redacted] 1989; supportive only
Rotational crops: beet, cabbage and wheat Primary crops (soybean, cabbage, wheat and beet) were planted 3 days after application	Soil application (soil was aged 30, 120 and 365 days until planting of rotational crops)	Glyphosate at 4.48 kg/ha	CA 6.6.1/005; [redacted] 1978; not acceptable
Rotational crops: pea, bean, carrot, cabbage, sweet corn Primary crops (pea, string bean, carrot, cabbage) were sampled 4-11 weeks after treatment	Foliar application (rotational crops were planted at PBIs of 29 – 79/101 days; within a 1-23 day interval after harvest of the primary crops)	Glyphosate at 4.48 kg/ha (application at the maximum plant growth of primary crops)	CA 6.6.1/006; [redacted] 1976; supportive only

The first confined rotational crop study (CA 6.6.1/001) was considered as supportive only. The TRR detected in the various crops, in both mature and immature growth stages, was generally highest in the early planting and sampling intervals and lower in later intervals. The rotational crops from the 30 days PBI contained 0.24 - 1.6 mg/kg TRR in edible matrices and up to 4.8 mg/kg TRR in inedible matrices. Crops from the 120 days PBI contained TRRs of 0.15 – 0.7 mg/kg and 0.17 – 1.4 mg/kg for edible and inedible matrices, respectively. Crops from the 360 days PBI contained TRRs of 0.02 – 0.16 mg/kg and 0.01 – 0.19 mg/kg for edible and inedible matrices, respectively. Hence, there was a more significant decrease in TRR between the 120 and 365 day planting interval than between the 30 day and 120 day planting interval. Even though TRRs were relatively high, parent glyphosate was only detected at concentrations <0.05 mg/kg in mature, edible samples (lettuce leaves, wheat grain and radish root) of all three rotations. In mature samples of wheat forage and chaff, glyphosate accounted for <0.05 mg/kg, 0.3 – 0.4 mg/kg and <0.05 – 0.06 mg/kg for the first, second and third rotation, respectively. AMPA residues were only seen at concentrations above the limit of quantification (0.05 mg/kg) in mature 30 and 120 day wheat forage, chaff, and seed, accounting for 0.1 – 0.4 mg/kg. This study only provides information on the TRR in several crop parts after rotation, while no investigation took place on extractability, and on the subsequent identification/characterization of other metabolites besides glyphosate and AMPA. Therefore, the majority of the residues has not been investigated, and as such no information on the metabolism can be abstracted from the study. These were the major deficits based on which the study is considered to only provide supportive information.

In the second rotational crop metabolism study (CA 6.6.1/002), the same rotational crops were investigated as in the first study. After removal of the primary crop, the rotational crops were planted into the subplots at 35, 63, and 308 days after treatment. The TRR levels in matrices obtained from rotational crops were relatively low, not exceeding 0.1 mg/kg, except for lettuce (0.127 mg/kg). The rotational crops from the 35 and 63 PBI contained TRRs of 0.020 – 0.076 mg/kg and 0.021 – 0.127 mg/kg, respectively. Crops from the 308 PBI contained TRRs of 0.010 – 0.038 mg/kg. However, although no TRR has been determined in the primary crops, it could be that the TRRs in the rotational crops are underestimated, since part of the radioactive residues has already be trapped by the soybean as primary crop. This is further confirmed by the findings in the third confined rotational crop, where residues up to 0.43 mg/kg were measured in the primary crop (see next paragraph), which are as such no longer available for uptake by the rotational crops. AMPA was found as major metabolite at levels of 8.7 - 34% TRR. Glyphosate was also detected in most samples, however its levels were <2.3% TRR. Residues after extraction with water and chloroform were further investigated; they were identified as being carbohydrates as glucose, fructose and malic acid and characterised as being starch, lignins, amino acids and cellulose.

In the third confined rotational crop study (CA 6.6.1/003), the primary crop was harvested and the plots rototilled before planting rotational crops at 30, 119, and 364 days after glyphosate treatment. The rotational crops from the 30 days PBI contained 0.037 – 0.188 mg/kg of glyphosate equivalent residues. Crops from the 119 days PBI contained residues of 0.017 – 0.078 mg/kg. Carrots, barley, and lettuce from the 364 days PBI contained residues of 0.0096 to 0.061 mg/kg. However, since the primary crop already contained TRRs ranging between 0.08 and 0.43 mg/kg, it could be that the TRRs in the rotational crops are underestimated in this study, as these residues are no longer available for uptake by the rotational crop. Analysis of rotational crop samples revealed two residue components, AMPA and a polar metabolite (called Metabolite 1 within the report) characterised as being a mixture of sugars, primarily glucose and fructose. Glyphosate was present only in lettuce, barley straw and grain of the first rotation 1.0 – 9.8% TRR and in lettuce of the second rotation 1.6% TRR. AMPA ranged from 3.7 – 17.9, 1.1 – 14.2 and 7.7 – 20.0% TRR in the matrices of the crops of the first, second and third rotation, respectively. Metabolite 1 amounted to 7.7 – 40.8, 6.3 – 24.9, 6.6 – 31.9% TRR in the matrices of the crops of the first, second and third rotation, respectively. Residues after further extraction steps were identified as being starch, lignin and cellulose, as well as biopolymers of glucose.

With regard to the fourth rotational crop metabolism study (CA 6.6.1/004), rotational crops wheat and turnip were planted at PBIs of 35, 95 and 370 days after treatment. Total glyphosate equivalent residues in wheat seeds, chaff and stalks/leaves were 0.25, 0.29 and 0.46 mg/kg grown on soil aged for 35 days, 0.28, 0.25 and 0.51 mg/kg (on soil aged for 95 days) and 0.06, 0.1 and 0.11 mg/kg (on soil aged for 370 days). In turnip leaves and bulbs the radioactive residues amounted to 0.02 mg/kg for both commodities of turnips grown on soil aged for 35 days, to 0.09 and 0.03 mg/kg (on soil aged for 95 days) and were detected at 0.03 and 0.02 mg/kg (on soil aged for 370 days). The radioactive residues in the plant matrices were not extracted, so no characterisation or identification of residues was performed. This is considered as the main deficit, since in particular in wheat commodities the TRR was sufficiently high. The study is, therefore, only considered as supportive.

The fifth confined rotational crop study (CA 6.6.1/005) was considered not acceptable. Concerning radioactive residues, there was a reduction in the amount of uptake of ^{14}C-activity with time. The rotational crops from the 30 days PBI scenario contained 0.002 – 0.018 mg/kg of glyphosate and 0.003 – 0.041 mg/kg AMPA, only for wheat residues were higher (0.046 mg/kg for glyphosate and 0.128 mg/kg for AMPA). Residues from respective plant materials for the 120 days PBI decreased to <0.001 – 0.014 mg/kg for glyphosate and 0.001 – 0.010 mg/kg for AMPA. Residues from respective plant materials for the 365 days PBI further decreased to <0.001 – 0.004 mg/kg for glyphosate and <0.001 – 0.004 mg/kg for AMPA. The study suffered from many shortcomings. Like for some other metabolism studies in the current dossier, no attempts have been made to investigate the unextracted residues, while their levels were above the trigger for further characterization. However, since it is even for some crops unclear what part of the crop has been sampled, extractability was in many cases low, crop growth was poor, and results on identification are considered not very reliable, the study is considered not acceptable

The final confined rotational crop study (CA 6.6.1/006) was considered as supportive only, in which also 2 different soils have been investigated. In primary crops, the residues were higher for plants grown on silty loam soils (up to 1.07 mg/kg) than for plants grown on sandy loam soil (up to 0.22 mg/kg). The radioactive residues detected in rotational crops grown on the two different soils were comparable (all between about 0.040 and 0.280 mg/kg). For crops grown on sandy loam soil, glyphosate was the major component detected in the plant extracts of primary and rotational crops. Glyphosate was detected at up to 0.137 mg/kg in primary crops and up to 0.128 mg/kg in rotational crops. AMPA was less abundant in these extracts and was detected between 0.002 and 0.044 mg/kg. Components that were characterised upon their elution behaviour and are designated as ‘neutrals’, ‘others’ or ‘indeterminates’, were detected at up to 0.037 mg/kg in the extracts of primary and rotational crops from this sandy loam soil. No further investigations were conducted to identify those other ^{14}C-products. In plant extracts from primary crops grown on silt loam soil, the amounts of AMPA (found at up to 0.041 mg/kg), were generally about twice as high as the concentrations of glyphosate. Glyphosate was not present in the extracts of rotational crops and AMPA was found at only low amounts (up to 0.004 mg/kg). The major part of radioactive residues were neutrals and/or indeterminates, representing up to 0.140 mg/kg. No further investigations were conducted to identify those other ^{14}C-products. In particular, the observation that the residual radioactive residues were often not further investigated, while the levels were higher than 0.01 mg/kg or 0.05 mg/kg, is considered an important deviation. Similarly, relevant levels of extractable residues should have been further investigated.

Overall conclusion on rotational crop metabolism

Glyphosate and AMPA were almost the only identified residues in the different crops after rotation (see table 2.7.7-1). It can be concluded that the metabolism in rotational crops is similar to the metabolism in primary crops, however, AMPA was identified as the major metabolite in the rotated crops, due to its formation in soil. In addition, several natural products were identified/characterized. Of course, it is as expected that primary crop and rotational crop metabolism is similar, since for both types it concerns a soil application of glyphosate after which crops are planted. Therefore, the results of both sets of metabolism studies can be considered together when deriving residue definitions (see 2.7.3).

In particular the two fully acceptable confined rotational crop studies have been used to draw overall conclusions on the relevant residues. Since in both these acceptable studies, crops from 3 different crop categories have been

studied, this is considered sufficient to address rotational crop metabolism. Based on the results from these studies, it could be discussed whether AMPA would be a better marker than glyphosate to monitor residues in rotational crops. On the other hand, both glyphosate and AMPA remain below 0.05 mg/kg in the acceptable confined studies (see table 2.7.7-1). From the rotational crop metabolism studies that are considered as supportive only, still relevant information on the levels of glyphosate and AMPA in crops can be derived, which shows that both compounds were >0.05 mg/kg in cereal commodities (CA 6.6.1/001, CA 6.6.1/005) and pea leaves and pods (CA 6.6.1/006). As such, from study CA 6.6.1/001, input for the dietary burden has been derived (see 2.7.5).

Rotational crop data are relevant for the 'post-harvest, pre-sowing, pre-planting, pre-emergence outdoor use' (see 2.7.4.2) with a maximum application of 2.16 kg/ha per year, and the inter-row use (see 2.7.4.3) with a maximum application of 1.08 kg/ha per year. Based on the worst-case application rate of 2.16 kg/ha per year, all confined rotational crop studies can be considered at least 1.8N overdosed with regard to the glyphosate application rate. The DT90 for both parent and metabolite AMPA exceeds 100 days. Therefore, PECaccumulation values have been calculated for both compounds in the fate section (Vol. 3, CP, B.8.2.1, table 8.2.1-21). Since no or less tillage is expected due to the use of glyphosate, the 'PECsoil, accumulation over 5 cm soil' has been used as a worst-case (although also PECaccumulation values over 20 cm soil have been made available). As such, the relevant PECsoils to be considered for the assessment on rotational crops are 3.1 mg/kg for glyphosate and 4.1 mg/kg for AMPA. These PECvalues have been translated into dose rates to assess whether the rotational crop studies adequately address the potential accumulation in soil: 2.3 kg/ha for glyphosate and 3.1 kg/ha for AMPA. Regarding glyphosate, all confined studies can be considered overdosed (up to 2.8N), and are consequently dosed at sufficiently high application rates. With regard to AMPA, obviously no application rates are available from the confined rotational crop studies to compare the PECsoils with.
In some of the confined studies (CA 6.6.1/001, CA 6.6.1/004) TRRs in soil are available (directly after application and at every PBI), but no individual levels of glyphosate and AMPA. In the second and third confined study (CA 6.6.1/002, CA 6.6.1/003), also individual soil levels of glyphosate and AMPA are available (directly after application and at several PBIs). The soil values from these studies can be compared with the PECaccumulation levels to evaluate whether these levels are sufficiently high to draw reliable conclusions whether or not residues of glyphosate and/or AMPA >LOQ can be expected in rotational crops.

As already concluded in the previous paragraph, to estimate possible glyphosate residues in rotational crops, the studies have been dosed at sufficiently high dose rates. Although the soil levels of glyphosate in none of the confined studies reach the calculated PECaccumulation (CA 6.6.1/002: max. 2.11 mg/kg glyphosate / 59.5 %TRR at day 0 in 0-10 cm soil; and CA 6.6.1/003: max. 0.64 mg/kg glyphosate / 90.5 %TRR at day 0 in 0-15 cm soil), this is considered acceptable, since the applied dose rates have been assessed as being overdosed and the measured soil levels have been determined over a higher soil depth than the 5 cm soil over which the relevant PEC has been calculated (additionally, it seems that the extraction method in CA 6.6.1/002 was not very efficient with only 59.5 %TRR glyphosate at day 0).
For AMPA, the assessment can only be based on the measured AMPA levels in soil. In CA 6.6.1/002, AMPA was max. 0.84 mg/kg in 0-10 cm soil, 34 days after treatment of 3.87 kg/ha (expressed as glyphosate equivalents), while in CA 6.6.1/003, AMPA was max. 0.30 mg/kg in 0-15 cm soil, 125 days after treatment of 4.16 kg/ha glyphosate. Therefore, the soil concentrations of AMPA in the confined studies are considered to be significantly too low to cover possible soil levels of AMPA after multiannual applications (with and without tillage).

For cereals, it is already clear that residues of glyphosate and/or AMPA >0.05 mg/kg can be expected in both food as well as feed items, based on the confined rotational crop studies. In addition, glyphosate residues >0.05 mg/kg were observed in one sample of pea pods and one sample of pea leaves in an overdosed study. In none of the other investigated rotational leafy crops (lettuce, radish tops, cabbage, beet foliage, carrot leaves, string bean leaves) residues of glyphosate >0.05 mg/kg were observed. Therefore, it is considered acceptable to conclude that no glyphosate residues >0.05 mg/kg are to be expected in rotational leafy crops. Also for rotational root crops no glyphosate residues >0.05 mg/kg are expected. However, these findings need to be confirmed with field rotational crop studies, in particular for cereal commodities (data requirement). In addition, field rotational crop studies are also required to evaluate the magnitude of the levels of AMPA in all relevant rotational crops. The applicant already announced that a limited field rotational crop study will be conducted in the near future to cover the maximum yearly application rate of 2.16 kg as/ha and repeated uses every year. The planned study design involves treatment of soil with a mixture of parent glyphosate and AMPA.

Based on the available rotational crop data, it can be concluded that input values for the dietary burden calculation and for the consumer risk assessment need to take into account possible residues in rotational crops. From the confined studies, glyphosate input values need to be derived only from cereal feed items, since glyphosate in cereal grain and other crops is expected to remain <0.05 mg/kg (and as such no MRLs are required for rotational crops). This glyphosate input is considered relatively reliable, since the studies have been conducted at the appropriate dose

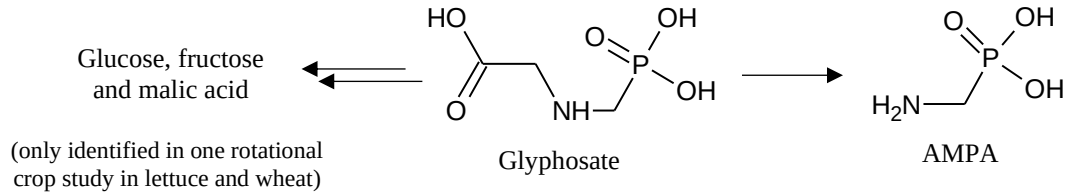
rate to investigate glyphosate residues. However, as already described, these values need to be confirmed by field studies. For AMPA, it is considered not possible to derive reliable input values, since no field studies are available; the confined studies are too much underdosed (also with regard to the PECaccumulation value over 20 cm soil, when tillage would be taken into account) to apply proportionality; and if a factor would need to be derived based on proportionality, it would be complicated to decide on a factor, because AMPA in soil has only been measured in two studies, where it strongly fluctuates (as expected) in time. Therefore, AMPA derived from rotational crops has not been taken into account for the dietary burden calculation and the consumer risk assessment. As such, both these exposure assessments probably reflect an underestimation.

Table 2.7.7-1: Identified components of the fully acceptable confined rotational crop studies

		Glyphosate %TRR (mg/kg[1])	**AMPA %TRR (mg/kg[1])**	**Glyphosate/AMPA %TRR (mg/kg[1])**
CA 6.6.1/002 (the identification of glucose, fructose and malic acid is not separately mentioned in this table)				
Lettuce	PBI 35 days	0.7 (0.001)	20.4 (0.015)	4.4 (0.003)
Wheat grain		n.d. (<0.001)	34.0 (0.026)	3.7 (0.003)
Wheat straw		0.4 (0.0002)	11.7 (0.006)	-
Wheat forage		0.5 (0.0001)	20.5 (0.005)	-
Radish roots		1.8 (0.0004)	8.7 (0.002)	-
Radish tops		0.9 (0.0002)	12.3 (0.002)	-
Lettuce	PBI 63 days	0.9 (0.001)	18.5 (0.024)	4.9 (0.006)
Wheat grain		2.3 (0.002)	25.8 (0.024)	2.3 (0.003)
Wheat straw		0.3 (0.0002)	12.7 (0.008)	-
Wheat forage		n.d. (<0.001)	20.5 (0.007)	-
Radish roots		1.7 (0.0004)	11.0 (0.002)	-
Radish tops		1.1 (0.0002)	9.5 (0.002)	-
CA 6.6.1/003				
Lettuce 70 DALT	PBI 30 days	3.8 (0.0041)	14.6 (0.0158)	-
Lettuce 90 DALT		n.d.	8.1 (0.0039)	-
Lettuce 105 DALT		2.9 (0.0028)	14.1 (0.0137)	-
Barley straw		1.0 (0.0018)	3.7 (0.0065)	-
Barley grain		9.8 (0.0184)	17.9 (0.0336)	-
Carrot tops		n.d.	1.4 (0.0007)	-
Carrot roots		n.d.	11.1 (0.0041)	-
Lettuce 147 DALT	PBI 119 days	n.d.	4.6 (0.0027)	-
Lettuce 167 DALT		1.6 (0.0009)	9.1 (0.0050)	-
Lettuce 181 DALT		n.d.	12.4 (0.0046)	-
Barley straw		n.d.	9.6 (0.0054)	-
Barley grain		n.d.	14.2 (0.0111)	-
Carrot tops		n.d.	1.1 (0.0003)	-
Carrot roots		n.d.	8.2 (0.0014)	-
Lettuce 399 DALT	PBI 364 days	n.d.	13.3 (0.0076)	-
Lettuce 425 DALT		n.d.	10.5 (0.0045)	-
Lettuce 455 DALT		n.d.	20.0 (0.0056)	-
Barley straw		n.d.	7.7 (0.0047)	-
Barley grain		n.d.	15.7 (0.0074)	-
Barley forage		n.d.	16.6 (0.0093)	-
Carrot roots and tops		n.a.		

[1]mg/kg: mg/kg expressed as glyphosate parent equivalents

Pathway for rotational crops



2.7.8 Summary of other studies

2.7.8.1 Effect on the residue level in pollen and bee products

Based on the decision-making scheme presented in the Technical guidelines for determining the magnitude of pesticide residues in honey and setting Maximum Residue Levels in honey (SANTE/11956/2016 rev. 9), the effect of the defended uses on the residue level in honey needs to be addressed since applications on non-target plants (in-field weeds and adjacent plants) are intended which might take place during the flowering period from April to September. The applicant submitted a tunnel residue trial, EU-monitoring data, as well as six Category A publications (see 2.7.8.2) considered relevant for risk assessment. The respective studies and publications are summarised in detail in Volume 3, B.7.7.1.

Four tunnel residue trials were performed in Germany in 2019 to investigate the residue levels of glyphosate and AMPA in honey collected by honey bees from *Phacelia tanacetifolia* (known by the common names lacy phacelia, blue tansy or purple tansy). Three of the four trials were considered to be performed in accordance with the technical guidelines (SANTE/11956/2016 rev. 9) for tunnel residue trials, although some minor deviations were noted. One trial, however, was not considered acceptable due to insufficient sampling quantities.

Residue data were obtained with an acceptable analytical method (see Volume 1, 2.5) for which acceptable procedural recovery data were generated concurrently with the specimens to-be-analysed. It is noted, however, that additional information regarding the extraction efficiency of the analytical method is needed for confirmation.

All samples were stored in accordance with the demonstrated periods of storage stability.

Residue levels of glyphosate and AMPA determined in the acceptable trials are shown in Table 2.7.8.1-1. According to the technical guidelines (SANTE/11956/2016 rev. 9), at least four trials are required to propose an MRL, i.e. one additional trial needs to be submitted (data requirement). It is furthermore noted that only data generated in northern Europe are available. In the technical guideline (SANTE/11956/2016 rev. 9), it is not explicitly stated that data from northern and southern Europe are required, however, the RMS is of the opinion that it would have been desirable to also have some data generated in southern Europe considering that residue levels on melliferous crops may be different in southern Europe.

Although no MRL can be proposed based on the available data, it is obvious that the existing MRL of 0.05* mg/kg needs to be raised to accommodate the intended uses. For the sake of completeness, it is noted that an MRL of 20 mg/kg is calculated based on the three acceptable trials and using the OECD MRL calculator.

Table 2.7.8.1-1: Summary of residue data in honey (pending the demonstration of extraction efficiency)

Region	Year	Crop	Number of acceptable trials	Glyphosate (mg/kg)	AMPA (mg/kg)	Reference
Honey						
NEU	2019	Honey	3	0.87, 3.2, 6.9	2x <0.025, 0.028	CA 6.10.1/001

Next to the tunnel residue trial, the applicant provided EU-monitoring data based on results obtained in 2016 and 2017. A total of 406 unique samples were analysed for parent glyphosate. Out of these, 212 samples were also analysed for AMPA. The LOQs were variable and ranged between 0.01 and 0.05 mg/kg for parent glyphosate (except for one sample for which the reported LOQ was 0.14 mg/kg) and between 0.01 and 0.03 mg/kg for AMPA. Measurable residues of glyphosate above the LOQs were found in 42 samples and these residues ranged between 0.01 mg/kg and 0.61 mg/kg. The residues of AMPA were always < LOQ.

The technical guidelines (SANTE/11956/2016 rev. 9) allow the setting of temporary MRLs in honey based on

monitoring data according to two different methods:

According to the FAO spice method, the MRL is set at the upper 95th confidence limit for the 95th percentile, considering the samples with measurable residues only (i.e. ≥ LOQ). A minimum of 58-59 samples with measurable residues are required. Since only 42 results ≥ LOQ are available, this method is not applicable. It is nevertheless noted that, in case the FAO spice method would be used on the reduced dataset, the 95th percentile would be 0.563 mg/kg, i.e. an MRL of 0.6 mg/kg would be proposed.

According to the FAO extraneous MRL (EMRL) approach, the MRL is estimated based on the 99th or 99.5th percentile of the entire dataset (including results < LOQ). Based on the EU monitoring data for 2016-2017, the 99th and 99.5th percentile residue levels for glyphosate in honey are 0.310 and 0.584 mg/kg, respectively. Therefore, based on the available data, it seems appropriate to set the MRL for glyphosate in honey at 0.6 mg/kg.

Obviously, the MRL proposal based on the available tunnel residue trial is considerably higher than the proposed MRL based on EU-monitoring data (20 mg/kg (based on the limited dataset of three trials) cf. 0.6 mg/kg). In case both field trials and monitoring data are available, the data from field trials should prevail for the setting of a permanent MRL in contrast to a temporary MRL based on monitoring data. This principle is in line with the proposal made by the European Commission (see SCPAFF residues, 15-16 June 2020).

Next to the tunnel residue trial and the monitoring data obtained by official EU monitoring laboratories, the applicant provided six publications belong to the Category A, i.e. studies considered reliable and relevant for risk assessment. The RMS considered the studies indeed reliable or reliable with restrictions, however, the studies are considered less relevant for evaluation. The main reasons for this is the fact that data were not obtained by official monitoring laboratories and the fact that the MRL of glyphosate for honey is not proposed based on monitoring data (see above). Nevertheless, the RMS notes that the data obtained in the six publications are all well in line with the monitoring data obtained in 2016 and 2017 by the EU-monitoring laboratories and that the results demonstrate that the existing MRL of glyphosate in honey needs to be raised.

2.7.8.2 Public literature

A literature search for glyphosate and its metabolites has been conducted. Data are summarized in Vol. 3, B.7.8. The search strategy is considered acceptable. For the residues section, 11 articles have been included as Category A studies, and have been presented in the accompanying dossier sections in Vol. 3, B.7. Since there are many publications which contain some monitoring results; or which contain analytical method development data, probably also containing some monitoring results; and the results from such publications are not directly expected to have impact on the risk assessment parameters; while such publications can be considered 'socially relevant', the RMS has made a selection for inclusion of such publications into the renewal dossier. In addition, the RMS proposes to include all publications related to the use of glyphosate as desiccant (i.e. pre-harvest application). Furthermore, the RMS has requested to include all Category C studies, which all relate to possible microbe-related effects. These study summaries have been requested, and these have been provided by the applicant. None of them are considered to have further impact on the existing risk assessment parameters. There is one data requirement: to provide a summary of the article from Krüger et al. (2014), including an assessment.
The applicant also provided a white paper (CA 6.10.1/002), in which they have reviewed available assays for the analysis of glyphosate in food, water and beverages, urine and other substances; reviewed reports in which glyphosate has been monitored in food or urine and other consumer items, thereby converting these findings into exposure estimates; and finally compared these estimates with health-based guidance values.
In conclusion, there is many public literature which contain some kind of analytical/monitoring results of glyphosate in food. Although the quantitative levels of glyphosate in such papers are not always reliable, the presence of glyphosate in food is not unexpected, and not of concern either as they are almost always below the MRL, and more importantly, exposure calculations are always below the toxicological reference values. However, for the current renewal of glyphosate, a new consumer exposure calculation will be conducted (see 2.7.9).

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

The dietary exposure for consumers has been calculated using the toxicological endpoints presented in 2.6.10.1 and 2.6.10.2. The ADI (0.1 mg/kg bw/d) and the ARfD (1.5 mg/kg bw) for glyphosate, which are being proposed in the current renewal framework, have been used. Furthermore, the STMRs and HRs for the representative uses, and EFSA PRIMo rev 3.1 have been used to calculate the consumer exposure. The input values for the consumer risk assessment can be found in table 2.7.9-1. Probably additional input values are required from glyphosate and AMPA residues in rotational crops. Glyphosate residues are not expected above the LOQ of 0.05 mg/kg in rotational crops, but this needs to be confirmed by field studies. To quantify AMPA residues in rotational crops, also field studies are needed. Since these data are not yet available, the consumer risk assessment is considered provisional, and probably an

underestimation.
The supervised residue trials conducted in support of the representative uses showed that residues of glyphosate and AMPA above the LOQ of 0.05 mg/kg are not expected (see 2.7.4). Therefore, the LOQ of glyphosate (0.05 mg/kg) was used as input value in case levels of glyphosate and AMPA were both <LOQ in the RACs (and not the sum of LOQs under consideration of a molecular conversion factor as usually required). By applying this approach, the consumer risk assessment is considered not too much overestimated, but still sufficiently worst-case. For animal commodities, where no residues above the LOQ are expected, values of a combined LOQ according to the residue definition for risk assessment were used. In the previous EFSA peer review and in the MRL-review of glyphosate, a similar approach was taken.

Table 2.7.9-1: Input values for the consumer risk assessment[1]

Commodity	Chronic risk assessment		Acute risk assessment	
	Input value (mg/kg)	Comment	Input value (mg/kg)	Comment
Citrus fruit	0.05*	LOQ glyphosate, see 2.7.4.1	0.05*	LOQ glyphosate, see 2.7.4.1
Tree nuts	0.05*	LOQ glyphosate, see 2.7.4.1	0.05*	LOQ glyphosate, see 2.7.4.1
Pome fruit	0.05*	LOQ glyphosate, see 2.7.4.1	0.05*	LOQ glyphosate, see 2.7.4.1
Stone fruit	0.05*	LOQ glyphosate, see 2.7.4.1	0.05*	LOQ glyphosate, see 2.7.4.1
Table and wine grapes	0.05*	LOQ glyphosate, see 2.7.4.1	0.05*	LOQ glyphosate, see 2.7.4.1
Table olives	0.05*	LOQ glyphosate, see 2.7.4.1	0.05*	LOQ glyphosate, see 2.7.4.1
Kiwi	0.05*	LOQ glyphosate, see 2.7.4.1	0.05*	LOQ glyphosate, see 2.7.4.1
Banana	0.05*	LOQ glyphosate, see 2.7.4.1	0.05*	LOQ glyphosate, see 2.7.4.1
Root and tuber vegetables	0.05*	LOQ glyphosate, see 2.7.4.2	0.05*	LOQ glyphosate, see 2.7.4.2
Bulb vegetables	0.05*	LOQ glyphosate, see 2.7.4.2	0.05*	LOQ glyphosate, see 2.7.4.2
Fruiting vegetables	0.05*	LOQ glyphosate, see 2.7.4.2	0.05*	LOQ glyphosate, see 2.7.4.2
Brassica vegetables	0.05*	LOQ glyphosate, see 2.7.4.2	0.05*	LOQ glyphosate, see 2.7.4.2
Leafy vegetables, herbs and edible flowers	0.05*	LOQ glyphosate, see 2.7.4.2	0.05*	LOQ glyphosate, see 2.7.4.2
Legume vegetables	0.05*	LOQ glyphosate, see 2.7.4.3	0.05*	LOQ glyphosate, see 2.7.4.3
Stem vegetables	0.05*	LOQ glyphosate, see 2.7.4.2	0.05*	LOQ glyphosate, see 2.7.4.2
Sugar beet roots	0.05*	LOQ glyphosate, see 2.7.4.2	0.05*	LOQ glyphosate, see 2.7.4.2
Animal meat, milk and egg	0.1*	Combined LOQ according to the residue definition for monitoring	0.1*	Combined LOQ according to the residue definition for monitoring
Animal liver, kidney, fat, edible offals, and other products	0.2*	Combined LOQ according to the residue definition for risk assessment	0.2*	Combined LOQ according to the residue definition for risk assessment
Honey	3.2	STMR, taking into account both glyphosate and AMPA, see table 2.7.8.1-1, pending the submission of one additional trial	6.9	HR, taking into account both glyphosate and AMPA, see table 2.7.8.1-1, pending the submission of one additional trial

[1] The LOQ of glyphosate was used as input value in case residues of glyphosate and AMPA were both below the LOQ in the RACs.

The TMDI is maximally 9% of the ADI (NL toddlers). It is concluded that no chronic risk has to be expected for any of the European consumer groups (see table 2.7.9-2). Also a calculation of the IESTI has been performed. The IESTI for honey is maximally 2% of the ARfD (NL toddlers), while for the other representative crops and animal commodities the IESTI is lower, and therefore, no acute consumer risk has to be expected (see table 2.7.9-3).

It is concluded that no chronic or acute risk has to be expected for European consumers resulting from treatment of crops with glyphosate according to the GAP of the representative use for the current renewal of glyphosate.

It should be kept in mind that the consumer risk assessment is indicative, since there are some data requirements. Data gaps on genotoxicity for certain metabolites (see 2.7.3) are considered not relevant for the proposed uses on conventional crops. In particular, additional input is required from glyphosate and AMPA residues in rotational crops, and full acceptability of the residue data needs to be confirmed by additional information on extraction efficiency in all supervised residue trials (for further details see the section on analytical methods). Glyphosate residues are not expected above the LOQ of 0.05 mg/kg in rotational crops, but this needs to be confirmed by field studies. To quantify AMPA residues in rotational crops, field studies are needed. Therefore, the indicative consumer risk assessment should probably be considered an underestimation. However, since orchard crops are considered permanent crops, no rotational crop studies are required for this defended use. As such, the consumer risk assessment for the orchards is considered indicative, only with regard to the confirmation of extraction efficiency of the analytical method.

Table 2.7.9-2: Report of the chronic dietary consumer intake assessment to glyphosate for the uses supported for the renewal

European Food Safety Authority

EFSA PRIMo revision 3.1 2019/03/19

Glyphosate

LOQs (mg/kg) range from: to:

Toxicological reference values

ADI (mg/kg bw/day):	0,1	ARfD (mg/kg bw):	1,5
Source of ADI:		Source of ARfD:	
Year of evaluation:		Year of evaluation:	

Input values

Details - chronic risk assessment | Supplementary results - chronic risk assessment

Details - acute risk assessment/children | Details - acute risk assessment/adults

Comments:

Normal mode

Chronic risk assessment JMPR methodology (IEDI/TMDI)

No of diets exceeding the ADI : ---

TMDI/NEDI/IEDI calculation (based on average food consumption)	Calculated exposure (% of ADI)	MS Diet	Expsoure (µg/kg bw per day)	Highest contributor to MS diet (in % of ADI)	Commodity / group of commodities	2nd contributor to MS diet (in % of ADI)	Commodity / group of commodities	3rd contributor to MS diet (in % of ADI)	Commodity / group of commodities	Exposure resulting from MRLs set at the LOQ (in % of ADI)	Exposure resulting from commodties not under assessment (in % of ADI)
	9%	NL toddler	8,63	6%	Milk: Cattle	0,5%	Apples	0,3%	Bananas		
	5%	UK infant	5,03	4%	Milk: Cattle	0,2%	Potatoes	0,2%	Honey and other apiculture produc		
	4%	NL child	4,32	2%	Milk: Cattle	0,4%	Sugar beet roots	0,3%	Apples		
	4%	FR toddler 2 3 yr	4,26	3%	Milk: Cattle	0,2%	Apples	0,1%	Sugar beet roots		
	4%	DE child	4,09	2%	Milk: Cattle	0,6%	Apples	0,3%	Honey and other apiculture produc		
	4%	FR child 3 15 yr	3,82	2%	Milk: Cattle	0,2%	Sugar beet roots	0,2%	Oranges		
	3%	UK toddler	3,20	2%	Milk: Cattle	0,2%	Potatoes	0,2%	Sugar beet roots		
	3%	SE general	2,62	1%	Milk: Cattle	0,4%	Bovine: Muscle/meat	0,2%	Potatoes		
	2%	DK child	2,40	1%	Milk: Cattle	0,2%	Swine: Muscle/meat	0,1%	Bovine: Muscle/meat		
	2%	ES child	2,38	1%	Milk: Cattle	0,1%	Bovine: Muscle/meat	0,1%	Poultry: Muscle/meat		
	2%	DE women 14-50 yr	2,35	1%	Milk: Cattle	0,2%	Sugar beet roots	0,1%	Apples		
	2%	DE general	2,33	1%	Milk: Cattle	0,2%	Sugar beet roots	0,1%	Honey and other apiculture produc		
	2%	FR infant	2,32	2%	Milk: Cattle	0,1%	Potatoes	0,1%	Apples		
	2%	RO general	2,31	1%	Milk: Cattle	0,2%	Potatoes	0,1%	Swine: Muscle/meat		
	2%	GEMS/Food G11	1,87	0,8%	Milk: Cattle	0,2%	Potatoes	0,1%	Swine: Muscle/meat		
	2%	GEMS/Food G15	1,84	0,7%	Milk: Cattle	0,2%	Potatoes	0,1%	Swine: Muscle/meat		
	2%	GEMS/Food G07	1,79	0,6%	Milk: Cattle	0,2%	Potatoes	0,1%	Poultry: Muscle/meat		
	2%	NL general	1,78	0,8%	Milk: Cattle	0,1%	Sugar beet roots	0,1%	Potatoes		
	2%	IE adult	1,68	0,4%	Milk: Cattle	0,2%	Sweet potatoes	0,1%	Potatoes		
	2%	GEMS/Food G08	1,67	0,6%	Milk: Cattle	0,2%	Swine: Muscle/meat	0,2%	Potatoes		
	2%	GEMS/Food G10	1,59	0,5%	Milk: Cattle	0,1%	Potatoes	0,1%	Poultry: Muscle/meat		
	1%	GEMS/Food G06	1,31	0,2%	Milk: Cattle	0,2%	Tomatoes	0,1%	Potatoes		
	1%	ES adult	1,25	0,5%	Milk: Cattle	0,1%	Bovine: Muscle/meat	0,1%	Swine: Muscle/meat		
	1%	FR adult	1,21	0,4%	Milk: Cattle	0,1%	Wine grapes	0,1%	Swine: Muscle/meat		
	1%	DK adult	1,14	0,5%	Milk: Cattle	0,1%	Swine: Muscle/meat	0,1%	Potatoes		
	1,0%	LT adult	0,98	0,4%	Milk: Cattle	0,2%	Potatoes	0,1%	Swine: Muscle/meat		
	0,8%	UK adult	0,81	0,3%	Milk: Cattle	0,1%	Potatoes	0,1%	Bovine: Muscle/meat		
	0,7%	UK vegetarian	0,74	0,3%	Milk: Cattle	0,1%	Potatoes	0,0%	Oranges		
	0,7%	PT general	0,71	0,3%	Potatoes	0,1%	Wine grapes	0,1%	Apples		
	0,6%	FI 3 yr	0,64	0,2%	Potatoes	0,1%	Bananas	0,1%	Cucumbers		
	0,6%	IE child	0,57	0,4%	Milk: Cattle	0,0%	Potatoes	0,0%	Swine: Fat tissue		
	0,5%	FI 6 yr	0,49	0,2%	Potatoes	0,0%	Bananas	0,0%	Cucumbers		
	0,5%	PL general	0,47	0,2%	Potatoes	0,1%	Apples	0,0%	Tomatoes		
	0,4%	IT toddler	0,38	0,1%	Tomatoes	0,0%	Potatoes	0,0%	Apples		
	0,3%	IT adult	0,34	0,1%	Tomatoes	0,0%	Apples	0,0%	Potatoes		
	0,3%	FI adult	0,26	0,1%	Potatoes	0,0%	Apples	0,0%	Tomatoes		

Conclusion:
The estimated long-term dietary intake (TMDI/NEDI/IEDI) was below the ADI.
The long-term intake of residues of Glyphosate is unlikely to present a public health concern.

Table 2.7.9-3: Report of the acute dietary consumer intake assessment to glyphosate for the uses supported for the renewal

Acute risk assessment /children	Acute risk assessment / adults / general population
Details - acute risk assessment /children	Details - acute risk assessment/adults

The acute risk assessment is based on the ARfD.
The calculation is based on the large portion of the most critical consumer group.

Show results for all crops

Results for children
No. of commodities for which ARfD/ADI is exceeded (IESTI) ---

IESTI

Highest % of ARfD/ADI	Commodities	MRL / input for RA (mg/kg)	Exposure (µg/kg bw)
2%	Honey and other	0 / 6,9	25
0,8%	Milk Cattle	0 / 0,1	12
0,5%	Potatoes	0 / 0,05	7,7
0,5%	Melons	0 / 0,05	7,6
0,5%	Pears	0 / 0,05	6,9
0,4%	Oranges	0 / 0,05	6,6
0,4%	Watermelons	0 / 0,05	6,1
0,4%	Apples	0 / 0,05	5,4
0,3%	Bananas	0 / 0,05	4,9
0,3%	Peaches	0 / 0,05	4,8
0,3%	Grapefruits	0 / 0,05	3,9
0,2%	Table grapes	0 / 0,05	3,6
0,2%	Cucumbers	0 / 0,05	3,3
0,2%	Carrots	0 / 0,05	3,2
0,2%	Kiwi fruits (green, red,	0 / 0,05	3,1

Expand/collapse list

Results for adults
No. of commodities for which ARfD/ADI is exceeded (IESTI) ---

IESTI

Highest % of ARfD/ADI	Commodities	MRL / input for RA (mg/kg)	Exposure (µg/kg bw)
0,6%	Honey and other	0 / 6,9	9,5
0,3%	Milk Cattle	0 / 0,1	3,9
0,1%	Head cabbages	0 / 0,05	2,1
0,1%	Watermelons	0 / 0,05	2,0
0,1%	Melons	0 / 0,05	2,0
0,1%	Milk Goat	0 / 0,1	1,8
0,1%	Swedes/rutabagas	0 / 0,05	1,7
0,1%	Table grapes	0 / 0,05	1,7
0,1%	Oranges	0 / 0,05	1,5
0,1%	Pears	0 / 0,05	1,5
0,1%	Milk Sheep	0 / 0,1	1,5
0,10%	Potatoes	0 / 0,05	1,5
0,09%	Yams	0 / 0,05	1,4
0,09%	Apples	0 / 0,05	1,4
0,09%	Cucumbers	0 / 0,05	1,4

Total number of commodities exceeding the ARfD/ADI in children and adult diets (IESTI calculation)

Results for children
No of processed commodities for which ARfD/ADI is exceeded (IESTI) ---

IESTI

Highest % of ARfD/ADI	Processed commodities	MRL / input for RA (mg/kg)	Exposure (µg/kg bw)
0,4%	Sugar beets (root) / sugar	0 / 0,6	5,5
0,3%	Potatoes / fried	0 / 0,05	4,7
0,3%	Pumpkins / boiled	0 / 0,05	4,4
0,3%	Witloofs / boiled	0 / 0,05	4,4
0,3%	Broccoli / boiled	0 / 0,05	3,9
0,2%	Cauliflowers / boiled	0 / 0,05	3,5
0,2%	Escaroles/broad-leaved er	0 / 0,05	3,3
0,2%	Potatoes / dried (flakes)	0 / 0,23	3,0
0,2%	Leeks / boiled	0 / 0,05	2,9
0,2%	Apples / juice	0 / 0,05	2,7
0,2%	Oranges / juice	0 / 0,05	2,6
0,2%	Turnips / boiled	0 / 0,05	2,5
0,2%	Parsnips / boiled	0 / 0,05	2,5
0,2%	Sweet potatoes / boiled	0 / 0,05	2,5
0,2%	Florence fennels / boiled	0 / 0,05	2,3

Expand/collapse list

Results for adults
No of processed commodities for which ARfD/ADI is exceeded (IESTI) ---

IESTI

Highest % of ARfD/ADI	Processed commodities	MRL / input for RA (mg/kg)	Exposure (µg/kg bw)
0,2%	Pumpkins / boiled	0 / 0,05	2,8
0,1%	Sugar beets (root) / sugar	0 / 0,6	2,2
0,1%	Cauliflowers / boiled	0 / 0,05	2,1
0,1%	Beetroots / boiled	0 / 0,05	1,9
0,1%	Celeries / boiled	0 / 0,05	1,7
0,1%	Apples / juice	0 / 0,05	1,7
0,08%	Broccoli / boiled	0 / 0,05	1,2
0,08%	Courgettes / boiled	0 / 0,05	1,1
0,07%	Parsnips / boiled	0 / 0,05	1,1
0,07%	Kohlrabies / boiled	0 / 0,05	1,1
0,07%	Wine grapes / juice	0 / 0,05	1,0
0,07%	Escaroles/broad-leaved	0 / 0,05	1,0
0,06%	Florence fennels / boiled	0 / 0,05	0,97
0,06%	Turnips / boiled	0 / 0,05	0,95
0,06%	Cassava roots / boiled	0 / 0,05	0,95

Conclusion:
No exceedance of the toxicological reference value was identified for any unprocessed commodity.
A short term intake of residues of Glyphosate is unlikely to present a public health risk.
For processed commodities, no exceedance of the ARfD/ADI was identified.

2.7.10 Proposed MRLs and compliance with existing MRLs

Table 2.7.10-1: Overview of the proposed MRLs and compliance with existing MRLs for glyphosate

Commodity	**Results from supervised residue trials (mg/kg)** Mo: Glyphosate RA: Sum of glyphosate and AMPA, expressed as glyphosate	**STMR**	**HR**	**Proposed MRL (mg/kg)**	**Existing MRL (mg/kg)**	**Remarks**
Orchard crops (citrus, stone and pome fruits, kiwi, tree nuts, and banana)	NEU Mo: 3x <0.05 RA: 3x <0.05 SEU Mo: 26x <0.05 RA: 26x <0.05	Mo: 0.05 RA: 0.05	Mo: 0.05 RA: 0.05	0.05*	0.1*-0.5	Post-emergence use. Appropriate risk mitigation measures shall be established on national level to prevent crop contamination. Combined NEU dataset on apple (2) and plum (1). Combined SEU dataset on mandarin (2), orange (2), hazelnut (1), pistachio (1), apple (2), apricot (4), cherry (2), peach (1), plum (6), kiwi (2), and banana (3). NEU and SEU datasets are pooled and data can be extrapolated to all orchard crops based on a risk envelope approach. Since residues of glyphosate and AMPA were both <0.05 mg/kg, only the LOQ of glyphosate was considered for the calculation of residues according to the RD-RA. Existing MRL is sufficiently high in support of the intended use. It is noted, however, that additional information regarding the extraction efficiency, and in some trials (2 NEU and 8 SEU) the derivatisation efficiency, of the analytical method is needed for confirmation.
Vines (table grapes and wine grapes)	NEU Mo: 9x <0.05 RA: 9x <0.05 SEU Mo: 8x <0.05 RA: 8x <0.05	Mo: 0.05 RA: 0.05	Mo: 0.05 RA: 0.05	0.05*	0.5	Post-emergence use. Appropriate risk mitigation measures shall be established on national level to prevent crop contamination. NEU and SEU datasets are pooled for deriving the MRL and risk assessment values. Since residues of glyphosate and AMPA were both <0.05 mg/kg, only the LOQ of glyphosate was considered for the calculation of residues according to the RD-RA. Existing MRL is sufficiently high in support of the intended use. It is noted, however, that additional information regarding the extraction efficiency of the analytical method is needed for confirmation.

Table olives	NEU Mo: - RA: -	Mo: - RA: -	Mo: - RA: -	-	1	Post-emergence use. Appropriate risk mitigation measures shall be established on national level to prevent crop contamination. No data available for NEU. Since residues of glyphosate and AMPA were both <0.05 mg/kg, only the LOQ of glyphosate was considered for the calculation of residues according to the RD-RA. It is noted, however, that AMPA was determined in 4 trials only. Existing MRL is sufficiently high in support of the intended use. It is noted, however, that additional information regarding the extraction efficiency of the analytical method is needed for confirmation.
	SEU Mo: 7x <0.05 RA: 4x <0.05	Mo: 0.05 RA: 0.05	Mo: 0.05 RA: 0.05	0.05*	1	
Root and tuber vegetables, bulb vegetables, fruiting vegetables, brassica, leafy vegetables, stem vegetables, and sugar beets	NEU Mo: 17x <0.05 RA: 17x <0.05	Mo: 0.05 RA: 0.05	Mo: 0.05 RA: 0.05	0.05*	0.1*-15	Post-harvest, pre-sowing, pre-planting, pre-emergence use. Combined NEU dataset on potato (2), carrot (2), onion (2), tomato (2), courgette (1), cauliflower (2), head cabbage (2), leaf lettuce (2), and leek (2). Combined SEU dataset on potato (2), carrot (2), onion (2), cucumber (1), courgette (1), cauliflower (2), head cabbage (2), head lettuce (2), leek (2), and sugar beet (2). NEU and SEU datasets are pooled and data can be extrapolated to all root and tuber vegetables, bulb vegetables, fruiting vegetables, brassica, leafy vegetables, stem vegetables, and sugar beets based on a risk envelope approach. Since residues of glyphosate and AMPA were both <0.05 mg/kg, only the LOQ of glyphosate was considered for the calculation of residues according to the RD-RA. Existing MRL is sufficiently high in support of the intended use. It is noted, however, that additional information regarding the extraction efficiency of the analytical method is needed for confirmation.
	SEU Mo: 18x <0.05 RA: 18x <0.05					

Root and tuber vegetables, bulb vegetables, fruiting vegetables, legume vegetables, and leafy vegetables	NEU Mo: 13x <0.05 RA: 13x <0.05	Mo: 0.05 RA: 0.05	Mo: 0.05 RA: 0.05	0.05*	0.1*-3	Interrow use. Appropriate risk mitigation measures shall be established on national level to prevent crop contamination. Combined NEU dataset on onion (2), cucumber (2), courgette (1), head lettuce (2), parsley (2), and green beans (4). Combined SEU dataset on carrot (4), radish (2), onion (4), tomato (4), cucumber (2), courgette (2), head lettuce (4), parsley (2), and green beans (4). NEU and SEU datasets are pooled and data can be extrapolated to all root and tuber vegetables, bulb vegetables, fruiting vegetables, legume vegetables, and leafy vegetables based on a risk envelope approach. Since residues of glyphosate and AMPA were both <0.05 mg/kg, only the LOQ of glyphosate was considered for the calculation of residues according to the RD-RA. Existing MRL is sufficiently high in support of the intended use. It is noted, however, that additional information regarding the extraction efficiency of the analytical method is needed for confirmation.
	SEU Mo: 28x <0.05 RA: 28x <0.05					
Honey	NEU Mo: 0.87, 3.2, 6.9 RA: 0.91, 3.2, 6.9	Mo: 3.2[#] RA: 3.2[#]	Mo: 6.9[#] RA: 6.9[#]	-	0.05*	No MRL proposed due to insufficient data (data requirement). It is furthermore noted that additional information regarding the extraction efficiency of the analytical method is needed for confirmation. [#] Calculation of risk assessment values based on a limited dataset only.
	SEU Mo: - RA: -	Mo: - RA: -	Mo: - RA: -	-	1	
Animals meat, eggs, milk	No residues above the LOQ are expected in animal commodities: milk, eggs and meat when exposed to diet including the (by-products) of intended uses.	-	-	0.1*	0.05*	
Animals liver, kidney, fat	No residues above the LOQ are expected in animal commodities: liver, kidney and fat when exposed to diet including the (by-products) of intended uses.	-	-	0.2*	0.05*-2	

2.7.11 Proposed import tolerances and compliance with existing import tolerances

Not applicable.

2.8 FATE AND BEHAVIOUR IN THE ENVIRONMENT

2.8.1 Summary of fate and behaviour in soil

Route of degradation in soil

Under laboratory aerobic conditions, reliable information on the route of degradation of glyphosate are available from 11 soils. The extent of mineralisation was high with a maximum amount of 70.6 % AR after 121 days (mean of replicates). The formation of non-extractable residues reached a maximum amount of 21.6 % AR (mean of replicates) after 90 days. The major degradation product observed in soil under aerobic conditions is aminomethylphosphonic acid (AMPA). AMPA was found with a maximum occurrence of 42.4 % AR in laboratory studies. Additional information from terrestrial field dissipation studies indicate that AMPA reached a maximum occurrence of 46.9 % in field.

Under anaerobic laboratory conditions, the degradation of glyphosate slowed down while the degradation pathway remained identical to that under aerobic conditions. The only major degradation product observed was AMPA with a maximum occurrence of 29.7 % AR after 84 days of anaerobic incubation. Mineralisation was negligible under anaerobic conditions and non-extractable residues increased by a maximum of 10 % AR during the anaerobic incubation phase.

A single laboratory soil photolysis study is considered as reliable and shows that degradation of glyphosate in soil is slightly enhanced by irradiation. Mineralisation reached 14.6% AR after 30 days under irradiated conditions against 5.4% AR in the dark control. The extent of non-extractable residues was similar in irradiated and dark control, with maximum of 19.4% AR after 14 days and 17.4% AR after 21 days, respectively. The major degradation product observed was AMPA with a maximum occurrence of 8.2 % AR after 7 days in irradiated samples (6.1 % AR after 3 days in dark control samples). No further photolytic degradation products were observed at levels above 5 % AR.

The proposed degradation pathway of glyphosate in soil is available below.

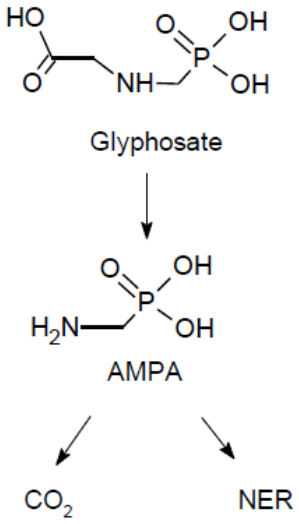


Figure 2.8.1-1: Proposed degradation pathway of glyphosate in soil

Rate of degradation in soil

Laboratory data
The rates of degradation of glyphosate and its metabolite AMPA were evaluated following the recommendations of the FOCUS Kinetic guidance.

The rate of degradation of glyphosate in standard dark aerobic laboratory studies has been determined in 10 different soils at 20/25°C. The degradation of glyphosate is mostly better described by biphasic kinetics.
The trigger DT_{50} and DT_{90} values of glyphosate range from 0.7 to 78.9 days and from 14.9 to 1660 days, respectively.
Modelling DT_{50} values for glyphosate for modelling in parent-only fits range from 2.2 to 161.1 days (pooling SFO kinetics, FOMC DT_{90}/3.32 and slow phase DFOP DT_{50} as recommended in FOCUS kinetics guidance). Corresponding modelling DT_{90} (used for assessment of pH dependence) range from 7.2 to 378.4 days.
Modelling DT_{50} values for glyphosate for modelling in a pathway fit were based on DFOP kinetics, also when <10% parent remains, because FOMC kinetics (leading to DT_{50}=DT_{90}/3.32) cannot be applied in a linked model run with a metabolite. In the pathway fit, normalized modelling DT_{50} ranged between 0.1 and 10 days for fast-phase and between 2.4 and 161.1 days for slow-phase. Normalised modelling DT_{90} values (used for the assessment of pH dependence) range between 6.4 and 378.4 days.
Based on the available modelling values, pH dependence cannot be excluded, with higher persistence with decrease of soil pH (see detailed evaluation in Vol 3 CA B 8).

The rates of degradation of metabolite AMPA are mostly issued from parent-applied studies and were also investigated in three soils under dark aerobic laboratory conditions in AMPA-applied studies. Degradation of AMPA followed single-first-order degradation. The trigger DT_{50} and DT_{90} values of AMPA range from 28.6 to 1040 days and from 95 to 3450 days, respectively. Modelling DT_{50} were in the range of 13-1040 days, with formation fraction of 0.196-0.480 (mean 0.29) from glyphosate. Based on the available values, pH dependence cannot be excluded, with higher persistence with decrease of soil pH (see detailed evaluation in Vol 3 CA B 8).

Under anaerobic laboratory conditions glyphosate does not degrade significantly.

Field data
The rates of degradation of glyphosate and its metabolite AMPA were evaluated following the recommendations of the FOCUS Kinetic guidance and EFSA $DegT_{50}$ guidance.

Information on the dissipation of glyphosate in soil under field conditions was investigated in several dissipation trials, conducted in Europe, USA and Canada. An Ecoregion Crosswalk exercise was performed to evaluate the representativeness of sites from outside EU for European conditions. A data gap has been set for the applicant to provide a comparison of actual field sites properties instead of default root ecoregions.

Several data gaps were also identified regarding the kinetic analysis of the field data. Based on the currently available data, reliable endpoints could be obtained for a limited number of sites.

For glyphosate, reliable field $DissT_{50}$ (trigger endpoints) were obtained from a total of six sites. Degradation is biphasic. $DissT_{50}$ and $DissT_{90}$ range between 1.1-13.7 days and 54.4-201 days, respectively. RMS notes that no field dissipation study was performed in Southern Europe. However one site in California is considered as representative of Southern Europe conditions (from the ecocrosswalk region comparison with ENASGIPS).

Reliable modelling $DegT_{50}$ were obtained from 2 sites only (32.6-46 days). pH dependence cannot be assessed due to the limited dataset. With only two field modelling DT_{50} considered reliable at this time of the assessment, normalized field data are pooled with laboratory values, following the EFSA $DegT_{50}$ guidance (2014).

Metabolite AMPA was analysed in the available field dissipation studies and occurred at a maximum occurrence of 46.9 %. No reliable trigger endpoints could be derived at this time (data gap on the kinetic fittings are identified on two soils). RMS highlights that for AMPA the two field DT_{50} would only cover a pH of 7.8. Since AMPA was shown to be more persistent in laboratory under acidic conditions, this range of pH investigated in field would not be sufficient. In any case, a data gap for additional field data is identified.

All of the field studies are “legacy studies” as qualified by the EFSA $DegT_{50}$ guidance. No modelling DT_{50} could be derived for AMPA since it occurred at more than 5% before 10 mm rain.

Assessment in relation to the P-criteria
The assessment is done according to the DG SANCO Working Document on "Evidence Needed to Identify POP, PBT and vPvB Properties for Pesticides" (2012, rev. 3).

The criteria for persistence (P) in soil, as stated in Regulation (EC) 1107/2009, are DT_{50} >120 days for PBT and >180 days for POP and vPvB.

When considering laboratory degradation rates, best-fit (including SFO, FOMC, DFOP kinetics) DT_{50} values at 20°C for glyphosate are < 120 days in the 10 available soils. When considering DT_{90}/3.32 when degradation was

best described by biphasic model, estimated DT_{50} values at 20°C for glyphosate are > 120 days in 1 soil over 10 (18 Acres, DFOP DT_{90}/3.32=177 d) and > 180 days in 2 soils over 10 (18 Acres, DFOP DT_{90}/3.32=177 d and Arrow, FOMC DT_{90}/3.32=500 d).
When considering field data, the 6 available best-fit field $DissT_{50}$ (not normalised, FOMC and DFOP kinetics) are < 120 days. When considering DT_{50} estimated from biphasic DT_{90}/3.32, all 6 estimated field $DissT_{50}$ are again below 120 days. The 2 available field modelling $DegT_{50}$ (normalised) are also < 120 days.

Based on all available data, it is therefore concluded that the P-criteria in soil is not fulfilled for glyphosate.

Adsorption

The adsorption of glyphosate was investigated in 11 batch adsorption studies. Reliable results were obtained on 10 soils. The calculated adsorption coefficients normalised to organic carbon content, $K_{F,OC(ads)}$, range from 1031 to 9615 mL/g (geometric mean: 4348 mL/g). The Freundlich exponents expressed as 1/n are in the range of 0.546 to 0.777 (arithmetic mean: 0.682). Glyphosate is considered as low mobile to immobile in soil according to McCall classification. Adsorption of glyphosate was found to be not dependent on pH of soil.

The adsorption of AMPA was investigated in 6 batch adsorption studies. Reliable results were obtained on 8 soils. The calculated adsorption coefficients $K_{F, OC(ads)}$ (normalised to organic carbon content) range from 1160 to 5650 mL/g (geometric mean: 2541 mL/g). The Freundlich exponent 1/n is in the range of 0.707 to 0.875 (arithmetic mean: 0.767). AMPA is considered as low mobile to immobile in soil according to McCall classification. Adsorption of AMPA was found to be not dependent on soil pH.

Mobility in soil

Since reliable adsorption coefficients for glyphosate and AMPA were obtained in adsorption/desorption studies, additional mobility studies are not strictly required. However several column and aged column leaching studies were provided.
Only one aged column leaching study provided reliable results. Under the conditions of the study peformed on a sandy soil, glyphosate and AMPA were found to be immobile, with no residues found deeper than 12 cm. The radioactivity in the leachates did not exceed 0.1% AR. These results support the results from the batch adsorption studies.
The mobility of glyphosate was investigated in several literature studies (column leaching, lysimeter and field leaching). Although results from these studies cannot be used to derive reliable endpoints for regulatory risk assessment, the studies bring supportive information regarding the mobility of glyphosate. In particular, preferential flow was demonstrated in some of the studies, mainly in clay soils, as expected for many herbicides applied on bare soil.

2.8.2 Summary of fate and behaviour in water and sediment [equivalent to section 11.1 of the CLH report template]

All the studies considered reliable to characterize both biotic and abiotic degradation of the active substance in water and sediment are used for CLP purposes. They are reported in the following table.

Table 68: Summary of relevant information on rapid degradability (biotic and abiotic degradation)

Method	Results	Key or Supportive study	Remarks	Reference
Ready biodegradability OECD 301 F	Biodegradation after 28 days was 26%. Glyphosate is not readily biodegradable under the conditions of the test.	Key study	-	[redacted] 2009 (see Vol. 3 B.8.2.2.1 (AS) for details)
Inherent biodegradability OECD 302 B	Biodegradation after 28 days was 2%. Glyphosate is not inherently biodegradable under the conditions of the test.	Key study	-	[redacted] 1991 (see Vol. 3 B.8.2.2.1 (AS) for details)

Method	Results	Key or Supportive study	Remarks	Reference
Inherent biodegradability OECD 302 B	Biodegradation after 28 days was 0%. Glyphosate is not inherently biodegradable under the conditions of the test.	Key study	-	[redacted] 1990 (see Vol. 3 B.8.2.2.1 (AS) for details)
Hydrolysis BBA-Merkblatt No. 55, part I and II (October 1980)	Glyphosate is stable to hydrolysis at 5, 7 and 9 (more than 90% of the applied active substance remain at the end of the study).	Key study	-	[redacted] 1993 (See Vol. 3 B.8.2.1.1)
Hydrolysis OECD 111	Glyphosate is stable to hydrolysis at 4, 7 and 9 (more than 90% of the applied active substance remain at the end of the study).	Key study	-	[redacted] 1992 (See Vol. 3 B.8.2.1.1)
Hydrolysis US EPA 540/9-85-013: section 161-1	Glyphosate is stable to hydrolysis at 5, 7 and 9 (more than 90% of the applied active substance remain at the end of the study)	Key study	-	[redacted] 1990 (See Vol. 3 B.8.2.1.1)
Photolysis Japan MAFF 12-Nousan-No. 8147, Part 2-6-2	Artificial light (Xenon arc lamp, with cut-off for wavelengths < 290 nm) – Continuous irradiation Direct photolysis: no degradation of glyphosate Indirect photolysis: 21.5% AR remains as glyphosate after 12 days. Estimated DT50 are 33.9-34.4 days (solar days, Tokyo)	Key study	-	[redacted] 2005 (See Vol. 3 B.8.2.1.2)
Photolysis U.S. EPA 540/9-82-021 Section 161-2	Artificial light (Xenon arc lamp, with cut-off for wavelengths < 290 nm) – Continuous irradiation Direct photolysis: degradation of glyphosate is slightly enhanced under irradiated conditions Estimated DT50 are 33, 69 and 77 d at pH 5.1, 7.3 and 9.2, respectively.	Key study	-	[redacted] 1992 (See Vol. 3 B.8.2.1.2)
Photolysis U.S. EPA 540/9-82-021 Section 161-2	Natural sunlight. Mean light intensity between 7684 and 16789 µW/cm². Direct photolysis: no degradation of glyphosate at pH 5, 7 and 9.	Key study		[redacted] 1990 (See Vol. 3 B.8.2.1.2)
Aerobic mineralisation OECD 309	DT50 for glyphosate are 12.3 and 21.8 days at low and high dose, respectively. Mineralisation is 23.1-26.5% AR after 62 days.	Key study	-	[redacted] 2020 (See Vol. 3 B.8.2.2.2)
Degradation in water/sediment systems BBA Guideline Part IV, 5-1 SETAC 1995	After 100 days, glyphosate amounts to 0.8-5.1% AR in the water phase, 3.7-58.2% AR in the sediment phase and 4.5-63.3% AR in total system. Maximum amount in sediment:	Key study	-	[redacted] 1999 / Kinetic analysis in [redacted] 2020 (See Vol. 3 B.8.2.2.3)

Method	Results	Key or Supportive study	Remarks	Reference
	15.9-58.2% AR after 3-100 d. Mineralization: 5.9-48.0% AR after 100 days. In total system, DT50 ranged between 8.4-196 days (DT90: 45.6-902 days). In water compartment, DissT50 ranged between 5.0-7.9 days (DissT90: 22.7-78.2 days). In sediment, DissT50 is 33.9 days (DT90: 112.6 days).			
Degradation in water/sediment systems BBA Guideline Part IV, 5-1	After 100 days, glyphosate amounts to 0.3-2.4% AR in the water phase, 29.2-44.2% AR in the sediment phase and 29.5-46.6% AR in total system. Maximum amount in sediment: 53.1-61.4% AR after 7 d. Mineralization: 17.8-23.5% AR after 100 days. In total system, DT50 ranged between 15.8-121.6 days (DT90: 329->1000 days). In water compartment, DissT50 ranged between 1.1-2.0 days (DissT90: 22.2-28.7 days). In sediment, DissT50 is 158.7 days (DT90: 965.3 days).	Key study	-	[redacted] 1993 / Kinetic analysis in [redacted] 2020 (See Vol. 3 B.8.2.2.3)

Results from these studies are summarized below. A detailed assessment can be found in Volume 3 B.8 (AS).

2.8.2.1 Rapid degradability of organic substances

2.8.2.1.1 Ready biodegradability

One study relative to the glyphosate ready degradability was provided. Biodegradation after 28 days was 26%. Based on this study, glyphosate is considered not readily biodegradable under the conditions of the test.

2.8.2.1.2 BOD5/COD

No data available.

2.8.2.2 Other convincing scientific evidence

2.8.2.2.1 Aquatic simulation tests

In the mineralization study, glyphosate was found to be well degraded in natural surface water under aerobic conditions at 20°C in the dark with half-lives of 12.3 and 21.8 days, for low and high dose, respectively. Maximum mineralisation of glyphosate was 26.5 and 23.1 % AR, while non extractable radioactivity accounted for 14.0 and 8.8 % AR at the end of the study, in the low and high dose, respectively. AMPA was the only major metabolite identified and was almost exclusively detected in the water phase. The maximum amounts of AMPA, detected in the water phase, were 42.7 and 39.8 % AR, in the low and high dose, respectively.

Analysis by a secondary chromatographic method showed the presence of an unidentified peak with >5 % AR. Several attempts to identify this peak were not successful. Analyses by a tertiary chromatographic method showed that this peak was comprised of three individual peaks. Further attempts to characterize this radioactivity are currently made and will be reported in an amendment to this study report. A data gap is identified for the notifier to provide the amended report when available.

In water/sediment systems, glyphosate degraded in the water phase and also partitioned to the sediment where it was further degraded. Mineralisation reached a maximum amount of 48 % AR after 100 days. The formation of non-extractable residues reached a maximum amount of 22.0 % AR after 100 days. The major degradation products observed in water/sediment systems were AMPA and hydroxymethylphosphonic acid (HMPA). AMPA was determined in water, sediment and total system with maximum occurrences of 15.7 % AR after 14 days, 18.7 % AR after 58 days and 27.1 % AR after 30 days, respectively. HMPA was not observed in sediment extracts but in the water phase with a maximum occurrence of 10.0 % AR after 61 days.

The proposed degradation pathway for glyphosate in water/sediment system is presented below.

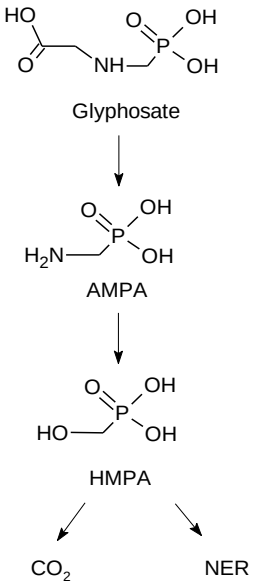


Figure 2.8.2- 1 : Proposed degradation pathway of glyphosate in water/sediment systems

In addition, 4 water/sediment studies with the metabolite AMPA applied provide further information on the behaviour of AMPA in aquatic systems. Reliable results were obtained on 7 water/sediment systems. The results of these studies showed rapid dissipation of AMPA from the water phase by adsorption to the sediment (maximum 63.8% AR after 30 days) followed by microbial degradation to CO_2. The results demonstrated the degradation of AMPA to carbon dioxide and non-extractable residues. Mineralisation reached a maximum of 40.1 % AR after 104 days. The formation of non-extractable residues reached a maximum amount of 40.7 % AR after 29 days. In addition, formation of 1-oxo-AMPA was observed. It should be considered in more details whether this metabolite 1-oxo-AMPA exceeds the trigger for further assessment. A data gap is identified for the applicant to further address this metabolite, quantitatively or qualitatively.

The reliable results for glyphosate, AMPA and HMPA were evaluated according to the current FOCUS kinetic guidance.

The degradation/dissipation of glyphosate in water / sediment systems was mainly described by biphasic kinetics. The persistence DT_{50} and DT_{90} of glyphosate for the total system range from 8.4 to 196 days and from 45.6 to >1000 days, respectively. In addition, the persistence $DissT_{50}$ and $DissT_{90}$ for the water phase range from 1.1 to 7.9 days and from 22.2 to 78.2 days, respectively. The persistence $DissT_{50}$ and $DissT_{90}$ for the sediment phase range from 33.9 to 158.7 days and from 112.6 to 965.3 days, respectively. For modelling purpose the geometric mean $DegT_{50}$ in the total system is 143.3 days (n = 4).

The degradation/dissipation of AMPA in water / sediment systems is described by both single-first-order and biphasic kinetics. The persistence DT_{50} and DT_{90} of AMPA for the total system ranged from 2.4 to 172.8 days and from 29.2 to >1000 days, respectively. In addition, the persistence $DissT_{50}$ and $DissT_{90}$ for the water phase range from 0.6 to 172.8 days and from 5.1 to 573.9 days, respectively. The persistence $DissT_{50}$ and $DissT_{90}$ for the sediment phase could be derived from one system only and are 168.1 days and 558.3 days, respectively. For modelling purpose the geometric mean $DegT_{50}$ in the total system, derived from evaluation at Level P-I and Level M-I dissipation is 98.7 days (n = 7).
The trigger and modelling DT50 and DT90 of HMPA for the total system ranged from 10 to 128.8 days and from 33.4 to 427.8 days, respectively.

Assessment in relation to the P-criteria
The assessment is done according to the DG SANCO Working Document on "Evidence Needed to Identify POP, PBT and vPvB Properties for Pesticides" (2012, rev. 3).

The criteria for persistence (P) in water, as stated in Regulation (EC) 1107/2009, are DT_{50} >40 days for PBT and >60 days for POP and vPvB. The criteria for persistence (P) in sediment, as stated in Regulation (EC) 1107/2009, are DT_{50} >120 days for PBT and >180 days for POP and vPvB.

Based on the available study on aerobic mineralisation in water, the DT_{50} in water (SFO) at 20°C are < 40 days (please refer to the LoEP for detailed values).

According to the DG SANCO Working Document, the total system DT_{50} from water/sediment systems should be compared with the most relevant compartment. Due to strong adsorption, sediment is considered as the most relevant compartment for glyphosate. Based on information from water/sediment total systems, best-fit DT_{50} values at 20°C for glyphosate are > 120 days in 2 systems over 4 and > 180 days in 1 system over 4 when considering the best fit DT_{50} derived from biphasic models in the 4 systems (please refer to the LoEP for detailed values). When considering total system DT50 estimated from biphasic DT_{90}/3.32, DT_{50} values at 20°C for glyphosate are 13.7, 99.2, 271.8 and >300 d; therefore they are > 120 days and > 180 days in 2 systems over 4.

Based on the available data, it is considered that the P-criteria in water is not fulfilled and the P-criteria in sediment is fulfilled.

Impact of water treatment processes
It is considered that the degradation pathway linked with water treatment processes has been sufficiently investigated and there are no indications that harmful disinfection by-products would be formed.

2.8.2.2.2 Field investigations and monitoring data (if relevant for C&L)

No monitoring data available which are considered relevant for CLH.

2.8.2.2.3 Inherent and enhanced ready biodegradability tests

Two studies relative to the inherent biodegradability of glyphosate were provided. Based on these studies, glyphosate is not inherently biodegradable under the conditions of the tests.

2.8.2.2.4 Soil and sediment degradation data

Please refer to 2.8.1 for soil degradation and to 2.8.2.2.1 for sediment degradation (water/sediment systems).

2.8.2.2.5 Hydrolysis

Glyphosate was found to be hydrolytically stable in sterile buffers of pH 4, 5, 7 and 9.

2.8.2.2.6 Photochemical degradation

Direct photolysis of glyphosate is not expected to be an important process since the substance does not absorb light in the right wavelength spectrum.
Glyphosate was stable in experiments on direct photolysis in sterile distilled water under artificial sunlight and in buffer solutions at pH 5, 7 and 9 under natural sunlight. In another study, degradation of glyphosate was slightly enhanced under artificial irradiated conditions compared to dark conditions. AMPA was found at levels above 10 %

at pH 7.3 and 5.1 with maximum amounts of 11.6 and 16.0 %, respectively. A data gap is identified to update the kinetic adjustments provided to determine a photolysis DT_{50} for glyphosate.
Glyphosate was significantly degraded by indirect photolysis. Besides the natural compound methanediol (up to 52 %AR after 12 days) and the known metabolite AMPA (up to 19.6% AR after 12 days), no degradation products were observed above 10 %.

2.8.2.2.7 Other / Weight of evidence

No additional data available.

2.8.2.3 Conclusion on rapid degradability

Glyphosate is considered not readily biodegradable under the conditions of the available test. Glyphosate was also shown to be not inherently biodegradable under the conditions of 2 tests. Results from hydrolysis and water/sediment studies show that glyphosate is not degraded in the aquatic environment to a level > 70 % within a 28-day period. As a consequence, glyphosate is considered not rapidly degradable.

2.8.3 Summary of fate and behaviour in air

The vapour pressure of glyphosate is 1.31×10^{-5} Pa (25 °C). Based on EVA 3.2, this is equivalent to a vapour pressure of 6.81×10^{-6} Pa at 20°C. According to FOCUS Air criteria, glyphosate can be classified as not volatile from soil and plants.
No significant volatilisation of glyphosate from plants and soil was observed after the application of glyphosate in laboratory experiments.

Glyphosate degrades very rapidly in air with an estimated half-life of 0.135 days (1.625 hours), indicating that long-range transport is not expected.

Due to no significant UV-absorption, direct photolysis in air is not relevant. In case reaching the atmosphere, glyphosate will rapidly be removed by photochemical oxidative degradation.

Based on glyphosate properties, the active substance is not considered volatile and has no potential for long range transport according to FOCUS guidance Air (2008). However, it should be noted that glyphosate is quantified in a national exploratory pesticide campaign in air in France. Please refer to section B.8.5 for more details.

2.8.3.1 Hazardous to the ozone layer

2.8.3.1.1 Short summary and overall relevance of the provided information on hazards to the ozone layer

Based on the available data presented under 2.8.3, there is no evidence that glyphosate may present a danger to the structure and/or the functioning of the stratospheric ozone layer.

2.8.3.1.2 Comparison with the CLP criteria

Based on the available data presented under 2.8.3, there is no evidence that glyphosate may present a danger to the structure and/or the functioning of the stratospheric ozone layer.

2.8.3.1.3 Conclusion on classification and labelling for hazardous to the ozone layer

Based on the available data presented under 2.8.3, there is no evidence that glyphosate may present a danger to the structure and/or the functioning of the stratospheric ozone layer.

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

An extensive review of existing monitoring data have been submitted, including collection of public monitoring data (raw data and aggregated data from national authorities and any regional/national agencies - aggregated data refers to information provided in publicly available reports, *e.g.* from environmental agencies or research institutes.) and review of open literature. For the current approval renewal, there are 10 new applicant studies, 7 existing

applicant studies and several published peer-reviewed papers (considered reliable or reliable with restrictions) covering the monitoring of glyphosate and its principal metabolite AMPA in soil, groundwater, surface water, transitional water, sediment, drinking water and air.

The studies and publications assessed cover a number of different spatial extents ranging from pan-EU and country, to regional/provincial, and even specific locations/fields. Similarly, they cover a range of temporal scales ranging from a single sampling occasion to multi-monthly and annual sampling schemes.

The data from public monitoring have been collated and analysed by applicant with regard to compliance of regulatory triggers, considering that the whole EU data set was large enough to capture a range of agronomic, geographical, pedoclimatic and hydrogeological situations, as well as providing a good temporal coverage allowing assessment of the state of a compartment in different seasons and hydrological regimes.

However, if the collected data indeed covers a wide variety of situations (as the public monitoring programs are aimed at), RMS highlights that further information and analysis are precisely missing to get a clear picture of what the overall data set really captures, notably in terms of relation to use pattern of the active substance and temporal percentile.

Reasoning on the whole data set to get a rate of compliance to regulatory triggers or to calculate any 90th, 95th or 99th percentile concentration as proposed by applicant is *de facto* biased; these should be taken with caution as they do not cover consistent situations, and cannot be compared to those considered in risk assessment.

The overall rates of compliance of the collated data with different RACs and thresholds as well as maximum reported concentrations in each compartment are provided in the table below**Table**. RMS higlights that only maximum values from EU public monitoring data set and from literature data that could be assimilated to EU public monitoring data (notably in terms of scale, compartment of interest for EU approval) are included in the following table. Literature data relying on specific experiment (specific conditions, flux concentration rather than environmental compartment), although may be considered reliable are not considered in the table below.

Table 2.8.4-1: Summary of minimum reported rates of quantification, of compliance with regulatory acceptable concentrations (RAC) or relevant thresholds and reported maximum concentrations for glyphosate (GLY) and AMPA in each environmental compartment

		GLY				AMPA			
	Dataset Size	Quantif (% samples	RAC[1]/ Threshold (µg/L)	Compliance (%)	Max Conc. (µg/L unless stated)	Quantif (% samples)	RAC[1]/ Threshold (µg/L)	Compliance (%)	Max Conc. (µg/L unless stated)
Soil	Small	~21%	94.6* mg/kg	100	2.05 mg/kg	~42%	26.4 mg/kg	100	1.92 mg/kg
Ground water	Very Large	~2%	0.1	99.38	1005 39.21[4]	~2.9%	10.0[2]	99.99	19.0
Surface Water	Very Large	~40%	100	99.99	3400[¤]	~64%	1200	99.99	3369[¤]
Tidal Water	Very Small	~7%	100	100	1.2	~33.1%	1200	100	0.9
Drinking Water	Small/ Medium	-[$]	0.1	99.84	0.92	-[$]	0.1[3] 10.0[2]	99.78 100	3.0
Sed	Small/ Medium	-	NA	-	2.84 mg/kg <4.0	-	NA	-	9.56 mg/kg <4.0
Air	Very Small	~7% to ~56%	NA	-	1.225 ng/m3	~1.3%	NA	-	-

NA – Not available
1 - Regulatory acceptable concentration
2 - Threshold for non-relevant metabolite
3 - Threshold value chosen to allow statistical comparisons only
4 – Maximum excluding outliers
* The value of 94.6 mg/kg is a RAC derived for soil macroorganisms, and correspond to the NOEC divided with a safety factor of 5. For microorganisms, no significant effect is observed for a tested NOEC (highest tested concentration) of 33.1 mg/kg.
[$] Frequency of quantification not available for a EU combined data, data from individual MS only
[¤] Maximum concentration to be confirmed once additional data are provided by applicant on outlier exclusion procedure.

In conclusion, it is an extended data set for most compartments that have been collected by applicant, although not always equally spatially distributed throughout EU (see summary for each compartment below). RMS emphasizes very few exceedance of the regulatory triggers are detected for each compartment but it often remains many

uncertainties to set into context these results.

Particular attention should be paid to the results in surface water and air.
The number of detection above LOQ (respectively ~40% and ~64% samples EU-wide for GLY and AMPA) tend to indicate that the active substance is widely and regularly found in surface water. This indeed reflects the spread and diversity of use of glyphosate containing products, but it still cannot be evaluated on which extend actual peak concentration and exceedance of the RAC in relation to pesticide use of glyphosate is caught by these monitoring programs. These levels of quantification highlight the necessity of implementing better-reasoned practices for glyphosate containing products, in order to limit environmental contamination.
The few available data in air also shows a high frequency of quantification of glyphosate in air, despite its intrinsic properties which indicate no significant potential of volatilization. Further data would be necessary to confirm these observations.

Summary of soil monitoring data

Regarding the collection of public monitoring data for soil compartment, applicant reported that there were hardly any official programs in place targeting monitoring of glyphosate or its metabolites residues in soil. Raw data for glyphosate and AMPA were available for the German federal state of Brandenburg. Aggregated monitoring data at the EU level for soil were obtained in the form of a research article.

Data from the German federal state of Brandenburg: The small number of raw data (57 samples from 29 sites, covering 9 years period) of GLY and AMPA analyses from agricultural soils were assessed against the soil regulatory acceptable concentration (RAC) of 94.6 mg/kg for GLY and 26.38 mg/kg for AMPA. Analysis indicates that GLY is quantified in ~30% of samples (total 43 samples) and AMPA is quantified ~86% of the samples (total 14 samples) No analyses exceeded the RAC or came close to doing so with the maximum measured concentration being 0.25 mg/kg for GLY and 0.975 mg/kg for AMPA. However, these samples cannot be related to land use (not reported) although study authors suggested it was largely agricultural land (visual assessment of locations in GIS), neither to a specific soil layer depth, since sampling method is not described.

In aggregated monitoring data report, 300 of the samples have been collected as part of the LUCAS topsoil project Results from these data suggest GLY is quantified in ~21% of 317 soil samples, AMPA is quantified in ~42% of 317 soil samples. None exceeds the RAC with the maximum concentration being 2.05 mg/kg for GLY and 1.92 mg/kg for AMPA, associated with permanent crops (vineyards) in central Portugal. However, the measured concentration should be regarded with caution since the exact sampling depth is unknown (15/20cm), and in any case higher than the one that would be considered for risk assessment in permanent crops (5cm). Also, the glyphosate application amounts, as well as the time passed since last application are not known.

Within the open literature review, there are five published peer-reviewed papers submitted for soil monitoring data. These papers report concentrations that are partly not directly comparable with the soil compartment that is typically assessed as part of the approval process, e.g. concentrations in soil pore water, or unconsistent soil layer depth sampling compared to what is considered in risk assessment. They were identified in the formal literature search conducted for the current submission and cover a wide range of use settings, predominantly agricultural, including rotational and permanent crops.

All reported soil concentrations are well below the RACs of 94.6 mg/kg for glyphosate (GLY) and 26.4 mg/kg for AMPA. Concentrations are also well below the NOEC of 33.1 mg/kg (highest tested concentration) for soil microorganisms. However, it should be kept in mind that the measured concentration from these monitoring programs or literature articles are only valid for the time and place they represent, and are not equivalent to the PECsoil calculated for risk assessment purpose.

Summary of ground water monitoring data

Regarding the collection of public monitoring data, an extended monitoring data set was collected throughout 14 EU countries ([redacted], 2020).

The whole combined EU monitoring data set for surface water represents >251 000 samples collected from >37 800 sampling sites for glyphosate and >230 000 samples collected from >34 400 sampling sites for AMPA. It is dominated by French data (~79.1%/82.4% of the samples for GLY/AMPA) with smaller contributions from Denmark (~5.8%/6.4% for GLY/AMPA), Germany (~5.7%/5.2%) and Austria (~3.8% for GLY).

Detection of GLY above the limit of quantification (>LOQ) in GW samples of EU combined data set was ~2% ranging from as low as 0.2% in AT to as high as 10.3% in ES. Compliance of the combined EU data set with the 0.1 µg/L threshold was 99.4% of samples from 97% of sites, indicating few exceedances (~0.6% of samples from ~3.0% of sites). The assessment of outliers identified 10 outliers in the dataset and if these are excluded the maximum concentration is reduced to 39.2 µg/L which is well below the SW RAC (for groundwater fed ecosystems). Case studies exploring elevated rates of groundwater detection in ES and the UK, suggest these findings are most likely a function of direct contamination, like spray drift into open wells.

Detection of AMPA above the limit of quantification (>LOQ) in GW samples of EU combined data set was ~2.9, ranging from as low as 0.4% in ES to as high as 19.5% in BE. Compliance with the regulatory threshold of 0.1 µg/L was very high (99.3% of samples) with few exceedances (~0.7% of samples) indicated. Compliance with the 10 µg/L regulatory threshold for a non-relevant metabolite was 99.998% of samples from 99.994% of sites, indicating rare exceedances (~0.002% of samples from ~0.006% sites). The maximum concentration is reported to be 19 µg/L. Although it exceeds the regulatory threshold for a non-relevant metabolite of 10 µg/L, a consumer risk assement based on this maximum value of 19 µg/L indicates that the exposure via drinking water for the most vulnerable consumers (infants) was estimated representing less than 1% of the ADI. In addition, this maximum concentration is below the SW RAC (for groundwater fed ecosystems).

However, it should be kept in mind that these conclusions based on % of compliance toward regulatory triggers cannot be related to a consistent groundwater type, or to any clear temporal or spatial percentile, either to any actual use pattern of the active substance. This because key information on description of monitoring locations are often missing in such public monitoring data and were thus not included in the applicant's analysis. It is neither possible to evaluate the vulnerability to leaching of the sampling sites. Frequency and regularity of sampling have also not been included as criteria in the data analysis, although the sampling effort can clearly be very different from a site to another. This was shown in a submitted study specific to French public monitoring data.

Regarding the exceedance of the 0.1µg/L trigger for glyphosate, there are 1496 samples >0.1 µg/L distributed on 1128 sites, with maximum number of samples >threshold at single site being 13. For AMPA they are 1511 samples >0.1 µg/L distributed on 994 sites, with maximum samples >threshold at single site being 37. However, too little information are given on this analysis to confirm applicant conclusions that the exceedances were considered as non-systematic given that very small proportion were consecutive sampling. Additional assessment could be performed on this point to confirm the exceedance are not related to long-term contamination in some locations. This is a data gap identified for applicant. On the contrary, it cannot be evaluated on which extent the high percentile of compliance with regulatory triggers indicated by study authors (0.1 µg/L triggers represents the 98.976th percentile concentration for glyphosate) is influenced by a total absence of use of the active substance in the catchment areas of the sampling locations. However RMS notes that this influence might be limited in the case of glyphosate, considering the wide spread and diversity of uses of glyphosate containing products (including agricultural and non-agricultural uses, professional and non professional uses).

Specific elucidation was nevertheless performed in some cases where MS showed lower compliance rates with the 0.1 µg/L threshold.

[redacted] (2016) also provides a review of glyphosate and AMPA monitoring for groundwater across EU. It is an update of previous review from [redacted] (2012) that was included in the RAR 2015. The raw data collected in this report overlaps the data from [redacted], 2020. However, in some cases it gave further information on some findings above 0.1 µg/L. Overall, as indicated previously by RMS of the RAR 2015, it remains often unclear if findings above the authorisation limit originate from a technically correct and regulation compliant use of the respective plant protection products in agricultural areas, or misuses or if construction defects on the groundwater abstraction points are reasonable for the limit exceedances etc.

Within the open literature review, groundwater monitoring data were obtained from eighteen published peer-reviewed papers. Detailed results from these are reported in volume 3CA_B8 in the dedicated section for monitoring data.

Summary of surface water monitoring data

Regarding the collection of public monitoring data for surface water, an extended monitoring data set was collected throughout 8 EU countries and 2 large transboundary catchments relating to the Rhine and Danube river basins.

The whole combined EU monitoring data set for surface water represents >291 000 samples collected from >13 800 sampling sites for glyphosate and >269 000 samples collected from >12 400 sampling sites for AMPA. It is dominated by French data (~65%/68% for GLY/AMPA) with smaller contributions from Belgium (9% for both

GLY and AMPA), Germany (~8.5%/9% for GLY/AMPA), the Netherlands (~5.6%/5.0 for GLY/AMPA) and Spain (~4.9% for GLY).

Detection of GLY above the limit of quantification (>LOQ) in SW samples was ~40%. Detection of AMPA >LOQ in SW samples was ~64%.

Glyphosate quantification

Compliance of the concentration results with the GLY RAC was 99.994% of samples; 99.90% of sites and the exceedances (0.006% of samples; 0.10% of sites) were on separate non-consecutive occasions (0.003% of samples being consecutive). Note that this analysis was performed based on a RAC of 400 µg/L initially proposed by applicant, while RMS final proposed RAC is 100 µg/L. However, this does not significantly impact the compliance rates (see below).

A small number (58) of high maximum concentrations in the dataset were considered to be outliers by study authors and once excluded a maximum concentration of 57 µg/L was retained, and compliance of 100% with any of the RAC values. However, very few justification on the value considered outliers was provided in the report. Some maximum values over the RAC (up to 558 µg/L) considered outliers should be further justified. This is identified as a data gap for the applicant, to clarify the method used for outlier removal, and the detail of the exluded values. Although the influence of removing the data considered outliers by applicant been proved to have no significant impact on the overall conclusions on rate of compliance with the RAC, it has to be ckecked that no reliable higher maximum concentration could be retained from this data set.

Since detailed results of maximum concentrations are not available, the analysis and the % compliance cannot be updated by RMS based on the RAC of 100 µg/L. However, considering that about 58 samples considered "outliers" by applicant are above 57 µg/L (applicant indicated the maximum concentration is 57 µg/L when excluding outliers), the overall compliance with the lower RAC of 100 µg/L would not be significantly different than the one presented by applicant.

Regarding Environmental Quality Standard (EQS), no EU-wide EQS values, annual average (AA) or maximum allowable concentration (MAC), were available for assessment as broader ecosystem endpoints. Consideration of the MS GLY surface water data against MS EQS values indicates that the presence of GLY is not expected to have any adverse impacts on ecosystems with a near total compliance (99.987%) across the large EQS-MAC dataset (~228 000 samples from ~9 000 sites) with very few exceedances (0.013% of samples; 0.22% of sites) identified. Similarly, 100% compliance for the large EQS-AA dataset (~11 000 years from ~1 600 sites) is indicated with no exceedances identified.

Regarding the threshold of 0.1 µg/L, detection for glyphosate above the threshold of 0.1 µg/L was ~23% of samples (~54.0% of sites), ranging from 3.4% in AT to 57.5% in BE. Note that this comparison is reported for information and is only relevant for locations where the surface water is actually intended to supply drinking water production. The proportion of sampling locations potentially intended to supply drinking water is unknown.

AMPA quantifications

Compliance of the concentrations with the AMPA RAC of 1200 µg/L was very high (99.999% of samples; 99.976% of sites) with infrequent exceedances (0.001% of samples from 0.024% of sites) occurring on 3 separate non-consecutive occasions.

Regarding Environmental Quality Standard (EQS), no EU-wide EQS values, AA or MAC, were available for assessment as broader ecosystem endpoints. Consideration of the MS AMPA surface water data against MS EQS values indicates that no sites showed average annual concentration >EQS-AA in the MS where such trigger is defined.

Assessment against the threshold of 0.1 µg/L was also undertaken; detection above the threshold of 0.1 µg/L was ~47.5% of samples (~67.6% of sites), ranging from 16.3% in AT to 77.7% of samples in BE. Note that this comparison is reported for information and is only relevant for locations where the surface water is actually intended to supply drinking water production. The proportion of sampling locations potentially intended to supply drinking water is unknown.

The conclusions of the study authors is that GLY and AMPA residues are frequently detected in surface water, but they do not pose risk to the environment. If indeed the results show very few exceedance of the RAC and other Environmental Quality Standard, any straightforward risk assessment conclusions based on these findings should be regarded with caution, as key information are lacking to get a clear picture of what these data capture in terms of use pressure and temporal percentile. The number of detections above LOQ (respectively ~40% and ~64% samples

EU-wide for GLY and AMPA) tends to indicate that the active substance is widely and regularly found in surface water. This indeed reflects the spread and diversity of use of glyphosate containing products, but it still cannot be evaluated on which extend actual peak concentration and exceedance of the RAC in relation to pesticide use of glyphosate is caught by these monitoring programs..

Frequency and regularity of sampling have not been included as criteria in the data analysis, although the sampling effort can clearly be very different from a site to another. Regarding use of active substance, the surface water sampling sites cannot be related to any use pattern of the active substance. No sufficient information is available to evaluate the proportion of sampling sites that are really located down gradient of area where the active substance is used, or even just likely to be used. However RMS notes that this might have less importance in the case of glyphosate, considering the wide spread and diversity of uses of glyphosate containing products (including agricultural and non-agricultural uses, professional and non professional uses).

Within the open literature review, surface water monitoring data were obtained from forty seven published peer-reviewed papers. Detailed results from these are reported in volume 3CA_B8 in the dedicated section for monitoring data.

Summary of transitional/tidal water monitoring data

Regarding the collection of public monitoring data, concentrations of glyphosate (GLY), AMPA and HMPA in transitional water arising from public monitoring datasets have been collected from regional/national environment agencies. Since tidal water is usually not accounted for in regulatory assessment for active substance approval, these data are considered as supportive. Only few data were collated.

Raw data from monitoring in tidal water are reported for a limited number of sites (~800 samples from 22 sites) from DE and UK. These include a variety of tidal water bodies including estuaries, lagoons and near shore brackish areas. The bulk of the data (~46% for GLY and 100% for AMPA) came from the DE dataset which comprises 15 sites located along the Baltic Sea coastline of Germany in the Bundesland of Mecklenburg-Vorpommern. This dataset covered 9 years spanning the period 2009 – 2018.

Within the 260 samples, GLY was quantified in 6.9% of samples, and AMPA was quantified in 33.1%. The maximum measured concentrations were 0.18 µg/L for GLY, and 0.9 µg/L for AMPA, which are below the RAC and EQS thresholds.

The dataset from the UK comprised 8 sites distributed unevenly along the east coast of England. It covered 9 years spanning the period 2000 to 2009. Within the 303 samples, GLY was quantified in 8.9% of samples. The maximum measured concentrations was 1.2 µg/L for GLY which is below the RAC and EQS thresholds.

Within the open literature review, there is only a single published peer-reviewed paper submitted for transitional/tidal water monitoring data. Detailed results are reported in volume 3CA_B8 in the dedicated section for monitoring data.

Summary of drinking water monitoring data

Regarding the collection of public monitoring data, concentrations from public monitoring datasets of glyphosate (GLY), AMPA and HMPA in drinking water have been collected from regional/national environment agencies as well as published peer reviewed publications from literature searches.

It should be preliminary noted that the data collated are very limited and outdated for many countries. The process of determining the reliability of the data is not clearly described so as the definition of drinking water taken into account, which is not always clear. Results from different water supplies (groundwater, surface water and "other sources") are gathered for each country and it is sometimes unclear whether the data reported in the summary tables and the statement indicated in the conclusions refer to raw or treated water. There is no precise indication on the origin of raw data for drinking water (*i.e.* ground water, surface water..) although this was part of the information to be collected in the described methodology; RMS aknowledges that few information is usually publicly available on the sampling location and the origin of raw water cannot be further indicated when collecting raw data. A data gap is however set for the applicant to clarify the definition of "drinking water" considered in this review of monitoring data.

Very little un-aggregated drinking water data was available for analysis (~8000 samples for GLY, ~7000 for AMPA). Bulk of the data (~86% for GLY and 99% for AMPA) came from Sweden (SE). Only data for GLY were available in Ireland (IE) (14% of the data). A small dataset from Germany (DE) is limited to the federal state of Schleswig-Holstein. The SE dataset comprises records from 1998 to 2014, the DE data set covers 2012 to 2018 while that from IE are from 2017 only.

For DE, results indicated 3 exceedance of 0.1 µg/L for GLY and 1 for AMPA, likely being isolated cases although the overall number of analysis is very limited. Aggregated data provides information on a wider set of data. Exceedances were very marginal representing less than 0.2% of samples.

For SE, raw data collected indicated 5 sampling >0.1 µg/L for GLY and 6 for AMPA, with maximum concentration of 0.17 µg/L for GLY and 0.680 µg/L for AMPA. All exceedances are indicated to be old (≤2007) and significant strides have been made in SE since the introduction of the water protection regulations in 2004 through delineation of water protection zones. This is consistent with data from [redacted], 2015 that does not further report any detection above 0.1 µg/L for the period 2008-2015.

For FR, data were collected comes from different sources with variable degree of detail and are limited to the periods 2001-2003 and 2010-2012. There is very few details on the samples exceeding the trigger. As in previous review of [redacted] 2008, the report indicates that further investigations failed to establish any coherent relationships between these detections and factors, such as seasonal occurrence, raw water quality, type of aquifer, analysis and water treatment. In fact, several of the samples with glyphosate were found in chlorinated waters; although it has been shown that chlorine effectively remove glyphosate. Overall, the evidence points to isolated detections, most likely due to contamination at the sampling stage or problems with analyses, rather than any indication of a persistent presence in drinking water.
RMS supplements this overview with published data at FR level on glyphosate and AMPA measurements in drinking water[26]: Through the period 2007-2016, for glyphosate the annual number of analyses for drinking water were between 4 293 and 15 003, and the proportion of yearly observed exceedance of 0.1 µg/L were between 0.09% and 0.30%. For AMPA in the same period the number of annual analyses was between 4138 and 14422, and the observed yearly exceedance of 0.1 µg/L were between 0.08% and 0.27%.

In BE, detection above >0.1 µg/L are reported from aggregated data for year 2016 (2 for GLY, 1 for AMPA) representing less than 0.2 % of total samples. Data collated from Flanders in 2013, showed no excedence for 17 samples collected.

In DK, single detection above >0.1 µg/L for GLY is reported from aggregated data for period 2014-2016, representing 0.07% of total samples. No detection above >0.1 µg/L have been reported in a data set for the period 2011-2013.

For NL, a maximum concentration for glyphosate of 3.0 µg/L from aggregated report is reported but no further details are given (number of sample, time of sampling). In another study, results are not clear and are mixing results from raw water intakes and treated water.

In SP, the sampling which exceeded 0.1 µg/L seem to be isolated cases (2 in 2013, 1 in 2012, 4 in 2011). However, the report indicates that there is no detail on the samplings such as actual concentrations found, whether they occurred at one or more sampling points. Data indicates that glyphosate was monitored in a relatively small proportion of water supply zones; the number of sites and sampling frequency is not known, as only the total number of analyses per year has been reported.

In the UK, there is no further details on the sampling found above 0.1 µg/L. They are isolated cases, representing 0.030 % of the analyses performed during the 2008-014 period

Glyphosate has not been found at concentrations at or above 0.1 µg/L in Austria, the Czech Republic and Switzerland.

There were no reported exceedances for AMPA in most countries, with exceptions in France (13 samples) and Germany (one sample).

[26] Glyphosate. Phytopharmacovigilance : Synthèse des données de surveillance. Appui scientifique et technique n°2017-04. ANSES. Octobre 2018

Within the open literature review, two publications were presented. Detailed results are reported in volume 3CA_B8 in the dedicated section for monitoring data.

Summary of sediment monitoring data

Regarding the collection of public monitoring data for sediment compartment, a small number (~2 700 analyses from ~550 sampling sites) of GLY and AMPA analyses from riverine sediment were collected and analysed. These were from two MS, FR and SE.

The bulk of the data (~91% for GLY and ~99% for AMPA) comes from the FR dataset which comprises ~541 sites, primarily in the north of France from a subset of departments. This dataset covers 13 years spanning the period 2005 – 2017. Monthly sampling effort for both GLY and AMPA is limited to the months of May through December and appears to be unimodal with lower sampling intensities in the early/latter months

The dataset from SE comprises ~12 sites distributed around the country targeting research catchments and locations. The GLY dataset covers 10 years spanning the period 2003 to 2012 while the AMPA data is restricted to 2006. Monthly sampling effort appears to be inconsistent and targets predominantly September.

The maximum measured concentrations were 2.84 mg/kg (FR) and 0.9 mg/kg (SE) for GLY, 9.56 mg/kg (FR) and 0.15 mg/kg (SE) for AMPA. No RAC are available for sediment and no comparison could be done.

Within the open literature review, there are seven published peer-reviewed papers submitted for sediment monitoring data. Detailed results are reported in volume 3CA_B8 in the dedicated section for monitoring data.

Summary of air monitoring data

Regarding the collection of public monitoring data for the air compartment, no data was identified by the applicant from requests to and from searches of online data of regional/national environment agencies for the compartment air.

RMS completes the overview of air compartment with results from a FR national exploratory pesticide campaign[27] that was likely not published at the time the applicant conducted its review. This sampling campaign lasted 12 months, from June 2018 to June 2019 and focused on the monitoring of 74 substances and 1 metabolite (AMPA). It included 50 sites, but for glyphosate and AMPA, due to specific material needed to sample these substances, sampling was performed on 8 sites. There were 3 urban/peri-uban areas and 5 rural areas. Six sites had different agricultural profile (field crops, vineyards, orchards, market gardening and breeding). Two sites were indicated without agricultural profile, due to the very low proportion of surfaces agricultural fields within a radius of 1 and 5 km.
Overall, Glyphosate was quantified in 56% of the analyses (LOQ 0.009 ng/m^3). AMPA was quantified in 1.3% of the analyses (LOQ 0.009 ng/m^3). In details within the different agricultural typology, the frequency of quantification was as follow for glyphosate: 65% of quantification for field crops areas, 75.5% for orchards, 76.9% in vineyards areas, 24.5% in breeding areas, 41.2% in market gardening areas and 54,1% for areas without agricultural profile.
Maximum concentration for glyphosate was 1.225 ng/m^3. The 25th percentile concentration is 0.004 ng/m^3 and 95th percentile concentration is 0.088 ng/m^3. Most of the concentrations (99.5th percentile) are below 0.25 ng/m^3 and mainly in vineyard sites. The maximum concentration of 1.25 ng/m^3 is observed on the orchard site of Cavaillon and is a unique high value.
These results were obtained in a national exploratory campaign on a limited number of sites and duration. Although the frequency of quantification for glyphosate is quite high and unexpected when considering its intrinsic properties (vapour pressure, DT50 in air), further data would be necessary to confirm these observations.

Within the open literature review for the air compartment, a single publication gave reliable results. It describes the results of a monitoring exercise of glyphosate and AMPA in the air of four different sites in the southeast of France where glyphosate is applied intensively. AMPA was not found in the samples. Glyphosate was detected at a global frequency of 7% with frequencies ranging from 0% (Nice) to 23% (Cavaillon), according to the sampling site. These results highlight a higher detection frequency of glyphosate in rural areas than in urban areas. Glyphosate concentration reached a maximum level of 1.04 ng/m^3 in the rural site of Cavaillon. Glyphosate concentration

[27] Résultats de la Campagne Nationale Exploratoire des résidus de Pesticides dans l'air ambiant (2018-2019) - DRC-20-172794-02007A – Ineris, Juin 2020

reached a maximum level of 1.04 ng/m3 in the rural site of Cavaillon. This is despite the physicochemical characteristics of glyphosate, which are not favourable to its passage into the atmosphere. In this study, the absence of simultaneous detection of glyphosate and AMPA suggests that drift during spraying operation is the main atmospheric source of glyphosate and that resuspension from soil particles is minor.

2.8.5 Definition of the residues in the environment requiring further assessment

The following residue definition for risk assessment in environmental compartments is proposed:

Soil: Glyphosate and AMPA
Groundwater: Glyphosate and AMPA
Surface water: Glyphosate, AMPA and HMPA
Sediment: Glyphosate, AMPA and 1-oxo-AMPA
Air: Glyphosate

2.8.6 Summary of exposure calculations and product assessment

Soil

PECsoil were calculated by RMS for the active substance glyphosate and its soil metabolite AMPA, considering a single application of 3600 g/ha, 2880 g/ha and 2160 g/ha, with no interception (risk envelope approach for, railways, orchards and field uses, respectively).

The non-normalised DT50 leading to worst-case PECsoil were selected from field for glyphosate and from laboratory for its metabolite AMPA.

Accumulation was estimated for the two compounds.

PECsoil are available in Volume 3 CP B.8 under B.8.2.

Groundwater

Calculations provided by the applicant were not considered acceptable since some of the input parameters used were not validated. As a 1st informative estimation of PECgw for the peer review, PECgw were performed by RMS for glyphosate and AMPA with both models FOCUS PELMO 5.5.3 and FOCUS PEARL 4.4.4 for 2 examples of uses (perennial and field crops):
- orchards (1 x 2880 g/ha; no interception) at the absolute application date of October 1st,
- potatoes (1 x 2160 g/ha, no interception) at the relative application date of 7 days after FOCUS harvest date.

PECgw were also estimated by RMS for uses on railways (1 x 3600 g/ha) with the model HardsPEC.

Input parameters related to soil degradation were selected taking into account biphasic degradation of glyphosate and pH dependence of soil degradation of glyphosate and AMPA. The resulting PECgw values are expected to be conservative.

All PECgw values for glyphosate and AMPA are below 0.1 µg/L for the 2 simulated uses. Please refer to Volume 3 CP B.8 under B.8.3 for further details.

A data gap is set for the applicant to provide updated PECgw values for all the intended uses, considering the application schemes initially proposed, the endpoints agreed during the peer review and all relevant models.

Surface water and sediment

Calculations provided by the applicant were not considered acceptable since some of the input parameters used were not validated. As a 1st informative estimation of PECsw/PECsed for the peer review, PECsw/PECsed were performed by RMS for glyphosate, AMPA and HMPA with models FOCUS STEP 1-2 v3.2 for the worst case use currently identified (2 x 1440 g/ha, no interception).
PECsw/PECsed were also estimated by RMS for uses on railways (1 x 3600 g/ha) with the model HardsPEC.

Input parameters related to soil degradation were selected taking into account biphasic degradation of glyphosate and pH dependence of soil degradation of glyphosate and AMPA.

PECsw/PECsed are available in Volume 3 CP B.8 under B.8.5.

A data gap is set for the applicant to provide updated PECsw/PECsed values for all the intended uses, considering the application schemes initially proposed, the endpoints agreed during the peer review and all relevant models. As

previously indicated, it should be considered in more details whether metabolite 1-oxo-AMPA exceeds the trigger for further assessment in sediment. Unless it is shown that the trigger is not exceeded or the ecotoxicological risk can be addressed qualitatively, PECsed calculations should be provided for 1-oxo-AMPA, based on default conservative substance properties in the absence of data.

Air

Based on the available data, glyphosate is unlikely to undergo significant volatilisation and any residues reaching air will be rapidly degraded. Therefore the compound will not be subject to significant concerns related to long range atmospheric transport and atmospheric accumulation. No PEC calculations are considered necessary.

Other routes of exposure

No other routes of exposure were identified.

2.9 EFFECTS ON NON-TARGET SPECIES

Ecotoxicological studies conducted with the active substance glyphosate, glyphosate acid, glyphosate salts and its metabolites are evaluated in section B.9 CA of the RAR. Irrespective of test item, all endpoints relevant for the risk assessment of the active substance are thereafter reported in glyphosate acid equivalents (i.e. recalculated to acid equivalents a.e.).

2.9.1 Summary of effects on birds and other terrestrial vertebrates

2.9.1.1 Acute toxicity to birds

Studies considering the acute toxicity to birds were assessed for their validity to current and relevant guidelines for glyphosate, glyphosate salts and the metabolite AMPA and are presented in the following table. Studies previously evaluated in either the monograph 2001 or the RAR 2015 were also included in this assessment. Study summaries for all studies are presented in Volume 3CA, Section B.9.1.

Table 2.9.1.1-1: Studies on acute oral toxicity of glyphosate and its metabolites to birds assessed as reliable by the RMS

Annex point	Study/ Report No.	Study type	Test species	Substance(s)	Status	Remark
CA 8.1.1.1/001	[redacted] 2003; 139-461	Acute oral	*Colinus virginianus*	Glyphosate K-salt (MON 78623)	Valid	-
CA 8.1.1.1/002	[redacted] 1997; [redacted] 400/963858	Acute oral	*Colinus virginianus*	Glyphosate acid	Valid	-
CA 8.1.1.1/003	[redacted] 1991; [redacted] 48/91266	Acute oral	*Colinus virginianus*	Glyphosate technical	Valid	-
CA 8.1.1.1/004	[redacted] 1999; D8.1–382/99	Acute oral	*Coturnix coturnix japonica*	Glyphosate technical	Valid	non GLP
CA 8.1.1.1/005	[redacted] 1996; 1413/4-1011	Acute oral	*Coturnix coturnix japonica*	Glyphosate technical	Valid	-
CA 8.1.1.1/006	[redacted] 1996; 1413/5-1011	Acute oral	*Anas platyrhynchos*	Glyphosate technical	Valid	-
CA 8.1.1.1/007	[redacted] 1992; [redacted] 49/91843	Acute oral	*Anas platyrhynchos*	Glyphosate technical	Valid	-

CA 8.1.1.1/008	[redacted] 1983; [redacted] 95-00214	Acute oral	*Pigeon*	Glyphosate technical	Not valid	-
CA 8.1.1.1/009	[redacted] 1991; 139-277	Acute oral	*Colinus virginianus*	AMPA	Valid	-

Endpoints of available studies considered as valid are shown in the table below. Glyphosate is an acid molecule, so it is often formulated as a salt. Ecotoxicological studies have been conducted with various forms of glyphosate, namely IPA salt, K-salt, glyphosate technical and glyphosate acid. In order to make a direct comparison of toxicity between studies, all endpoints from these studies have been converted to acid equivalents (a.e.). This conversion has been made either by the acid equivalent purity of the test item if stated in the report or by a conversion factor of 0.741 (for IPA salt). By nature of the glyphosate, endpoints of glyphosate or glyphosate technical are automatically expressed as acid equivalent.

Table 2.9.1.1-2: Reliable endpoints: Acute oral toxicity of glyphosate to birds

Reference	Test item	Species	Test design/ GLP	LD_{50} (mg a.e./kg bw)
[redacted] 2003 CA 8.1.1.1/001	Glyphosate K- salt	*Colinus virginianus*	Acute oral	> 2241
[redacted] 1997 CA 8.1.1.1/002	Glyphosate acid	*Colinus virginianus*	Acute oral	> 2000
[redacted] 1991 CA 8.1.1.1/003	Glyphosate technical	*Colinus virginianus*	Acute oral	> 2000
[redacted] 1999 CA 8.1.1.1/004	Glyphosate technical	*Coturnix coturnix japonica*	Acute oral / non GLP	> 2000
[redacted] 1996 CA 8.1.1.1/005	Glyphosate technical	*Coturnix coturnix japonica*	Acute oral	> 2000
[redacted] 1996 CA 8.1.1.1/006	Glyphosate technical	*Anas platyrhynchos*	Acute oral	> 2000
[redacted] 1992 CA 8.1.1.1/007	Glyphosate technical	*Anas platyrhynchos*	Acute oral	> 2000
Proposed endpoint for risk assessment				
Extrapolated	Glyphosate acid	bird	Acute, 14 days ≥ 20 birds per limit/maximum dose group without effects	4334*

a.e.: acid equivalents

* Extrapolated with a factor of 2.167 as recommended by EFSA guidance document 1438/2009 and as described above.

A number of acute studies in birds without any mortality at a limit dose/maximum dose of 2000 mg a.e./kg bw are submitted. According to EFSA Journal 7(12): 1438 (2009) it is possible to extrapolate an LD_{50} value in cases where there is no mortality or a single mortality at a limit dose in an acute avian toxicity study. Therefore, an acute LD_{50} for risk assessment of 2000 × 2.167 = 4334 mg a.e./kg bw was proposed. Although each study did not include the required 20 individuals to allow for the highest extrapolation factor, this is considered reasonable by the RMS given that several studies were available without mortality at the highest treatment level.

A study considering the acute toxicity of the metabolite AMPA to birds is available and reported in the following table. This study was assessed to be valid according to current and relevant guidelines and the corresponding study summary is available below. This acute study with the metabolite AMPA shows similar acute toxicity as the parent.

Table 2.9.1.1-3: Endpoints: Acute oral toxicity of AMPA to birds

Reference	Test item	Species	Test design/ GLP	LD_{50} (mg/kg bw)
[redacted] 1991 CA 8.1.1.1/009	AMPA	*Colinus virginianus*	Acute oral	>2250

2.9.1.2 Short-term dietary toxicity to birds

The assessment of short term dietary toxicity data for birds is not considered to be necessary following the guidance document on Risk Assessment for Birds and Mammals (EFSA Journal 2009; 7(12): 1438), in particular if there are no indications that the dietary LD_{50} will be lower than the LD_{50} based on an acute oral study.

In order to compare the toxicity from acute oral study with the available short term dietary data, a conversion of dietary endpoint to daily dose was needed. A summary of the short term dietary studies are presented in the table below. Studies where validity according to OECD TG 205 could not be confirmed were excluded from the evaluation by the RMS.

Table 2.9.1.2-1: Available short-term dietary toxicity studies of glyphosate and AMPA

Annex point	Study/ Report No.	Study type	Test species	Substance	Status
KCA 8.1.1.2/01	[redacted] 1973; 241-106	8-d dietary	*Colinus virginianus*	Technical CP67573	Excluded by RMS
KCA 8.1.1.2/02	[redacted] 1997; [redacted] 395/963857	8-d dietary	*Colinus virginianus*	Glyphosate acid	Acceptable
KCA 8.1.1.2/03	[redacted] 1989; 1085	8-d dietary	*Coturnix coturnix japonica*	Glyphosate technical	Excluded by RMS
KCA 8.1.1.2/04	[redacted] 1973; 241-107	8-d dietary	*Anas platyrhynchos*	Technical CP67573	Excluded by RMS
KCA 8.1.1.2/05	[redacted] 1997; ZCA 23	8-d dietary	*Anas platyrhynchos*	Glyphosate acid	Acceptable
KCA 8.1.1.2/06	[redacted] 1991; 139-275	8-d dietary	*Colinus virginianus*	AMPA	Excluded by RMS
KCA 8.1.1.2/07	[redacted] 1991; 139-276	8-d dietary	*Anas platyrhynchos*	AMPA	Excluded by RMS

In each short term dietary exposure study, where dietary exposure levels are expressed in terms of mg/kg feed (or ppm), the actual exposure levels are determined by considering the mean amount of diet consumed (g feed) by each bird in each of the exposure groups over the exposure period and the mean bodyweight achieved for each bird over the exposure period, from which a daily dose level is then determined in accordance with the EFSA guidance document (2009).

Where data for day 5 bodyweights was unavailable in the study reports, day 8 bodyweights were used. If no difference in bodyweights (nor in the rates of consumption in the treatment groups relative to the control groups during the post exposure period) on day 8 across the exposure levels and control was observed, use of the day 8 bodyweight in the calculation is considered appropriate.

A summary of the daily dose calculations for each treatment level for each of the short-term dietary studies is provided below.

Table 2.9.1.2-2: Conversion to daily dose for short-term avian studies

Dietary Dose Level (mg a.s./kg feed) 1	Total food consumption over 5 days [g]	Group Mean Feed Consumption (g/bird/day)	Group Mean Body Weight over 5 days (g)	Daily Dose (mg a.s./kg bw/day)
Studies with glyphosate				
KCA: 8.1.1.2/02: Bobwhite quail dietary study				
325	-	5.0	18.5	87.8
650	-	5.3	15.5	185.7
1300	-	4.9	18.0	353.9
2600	-	5.5	19.1	746.7
5200	-	5.2	17.9	1510.6
KCA: 8.1.1.2/05: Mallard duck dietary study				
325	-	63.0	191.5	106.9
650	-	60.0	190.0	205.3
1300	-	58.0	179.5	420.1
2600	-	61.0	186.0	852.7
5200	-	63.0	191.0	1715.2

A summary of endpoints is provided in the table below. No mortality was seen in any of the dietary studies and so the LC_{50} endpoints are all greater than the highest dose tested in the study and also represent a no-mortality concentration.

Table 2.9.1.2-3: Endpoints for dietary toxicity of glyphosate to birds

Reference	Test item	Species	Test design/ GLP	LC_{50} Dietary dose (mg/kg feed)	LDD_{50} Daily dietary dose (mg/kg bw/day)
[redacted] 1997 KCA 8.1.1.2/02	Glyphosate acid	*Colinus virginianus*	8-d dietary	> 5200	>1511
[redacted] 1997 KCA 8.1.1.2/05	Glyphosate acid	*Anas platyrhynchos*	8-d dietary	> 5200	>1715

For the acute oral studies where a single dose is administered by oral gavage, all endpoints are LD_{50} > 2000 mg/kg bw based on 14 day study. The short-term dietary studies described above also have endpoints that are all greater than the highest concentration tested with endpoints ranging from LD_{50} > 1511 mg/kg bw/day to LD_{50} > 1715 mg/kg bw/day based on a five-day dietary exposure. No mortality was observed in the studies. Although the endpoints from the acute oral studies are higher compared to the dietary data, there is no indication that dietary exposure is more severe compared to gavage data. Therefore, the acute oral endpoints are considered appropriate for the risk assessment to assess the acute effects of glyphosate on avian species.

2.9.1.3 Sub-chronic and reproductive toxicity to birds

Studies considering the reproductive toxicity to birds were assessed for their validity to current and relevant guidelines for glyphosate and glyphosate salts are presented in the following table. Studies previously evaluated in either the monograph 2001 or the RAR 2015 were also included in this assessment. Study summaries for all studies are presented in Volume 3CA, section B.9.1.

Table 2.9.1.3-1: Studies on reproductive toxicity of glyphosate to birds

Annex point	Study/ Report No.	Study type	Test species	Substance(s)	Status	Remark
CA 8.1.1.3/001	[redacted] 1999; 123-186	Reproduction	*Colinus virginianus*	Glyphosate acid	Valid	Control mortality exceeds 10%
CA 8.1.1.3/002	[redacted] 2013; letter regarding 123-186	Position paper				Letter regarding control mortality in [redacted] *et al* study CA 8.1.1.3/001

CA 8.1.1.3/003	[redacted], 1978; 139-141	Reproduction	*Colinus virginianus*	Glyphosate technical	Not valid	-
CA 8.1.1.3/004	[redacted] 1999; 123-187	Reproduction	*Anas platyrhynchos*	Glyphosate acid	Valid	-
CA 8.1.1.3/005	[redacted] 1978; 139-143	Reproduction	*Anas platyrhynchos*	Glyphosate technical	Valid	-

Endpoints of studies considered valid are shown in the table below. It should be noted that the previously agreed reproductive endpoint (96 mg a.e./kg bw per day) was derived from a study ([redacted] 1978; Report No. 139-141; CA 8.1.1.3/003) where validity according to OECD TG 206 could not be confirmed. Therefore, the RMS propose that these data are not used for the risk assessment.

Table 2.9.1.3-2: Valid endpoints: Reproductive toxicity of glyphosate to birds

Reference	Test item	Species	Test design	NOAEC (mg a.e./kg feed)	NOAEL (mg a.e./kg bw/d)
[redacted] 1999 CA 8.1.1.3/001	Glyphosate acid	*Colinus virginianus*	20 weeks reproduction	2250	201
[redacted] 1999 CA 8.1.1.3/004	Glyphosate acid	*Anas platyrhynchos*	21 weeks reproduction	1000	**116**
[redacted] 1978 CA 8.1.1.3/005	Glyphosate technical	*Anas platyrhynchos*	17 weeks reproduction	1000	125

a.e.: acid equivalents
Endpoint in bold is used for risk assessment.

In conclusion, the endpoint (NOAEL 116 mg a.e./kg bw/d) derived from the mallard study by [redacted] 1999, is proposed by the RMS to be used for the risk assessment.

Further to the standard studies presented above, the study by Ruuskanen *et al*. (2020); dossier no. CA 9 from the open literature provides additional information on chronic effects to birds. In this study, a dietary treatment with 164 mg glyphosate/kg food for 8 weeks resulted in reductions of flight feather moult and plumage development in juvenile quails. In addition, there seems to be a preference for treated food among female birds. The RMS proposes that the results from this study need to be further considered in the risk assessment.

2.9.1.4 Acute toxicity to mammals

Acute toxicity to mammals is summarised and evaluated by the RMS in Volume 3CA, section B.6.1.

In the previous evaluation, the risk assessment was based on the lowest limit LD_{50} of >2000 mg/kg bw/d as a precautionary approach in order to take into account the observed strong clinical signs which were observed among studies at higher doses; ataxia, convulsions, muscle tremors, red nasal discharge, clear oral discharge, urinary staining of the abdomen, diarrhoea, piloerection, lethargy, and fecal staining of the abdomen, decreased spontaneous motor activity and sedation and crouching position.

The applicant argued that the clinical sublethal effects were similar in nature and extent, and that in all cases the observed clinical effects were transient, and all animals appeared normal at the end of the study - for both species tested. Therefore, it was stated that, as the endpoint required for use in the acute mammalian risk assessment is an acute lethality endpoint, the transient symptomology observed in these studies is not relevant to an acute wild mammal risk assessment, especially where considering that it was not sustained for the duration of the studies.

For the present evaluation, the available data have been re-considered by the RMS, including a more detailed evaluation of the observed sublethal effects, in order to explore the possibility to use a geomean endpoint for the risk assessment, in accordance with the EFSA guidance (2009). An overview of the data is given below.

Table 2.9.1.4-1: Overview of acute oral toxicity studies for glyphosate acid in rats and mice. Sublethal effect LC_{50} (severe effect defined by RMS as >50% affected, duration >1d). Studies assessed as Supportive (Reliable with restrictions) are shown in *italics*

Study/Report No.	Species / Study type / Test substance(s)	Clinical observations (incidence and duration of effects)	Result [mortality LD_{50}]	Result (sublethal effects LC_{50}) RMS remark
CA 5.2.1/002 [redacted] 2011; 10/218-001P	Rat RjHan:WI, females (up and down procedure) Age: 10-11 weeks Guideline: OECD 425 (2008); OPPTS 870.1100 (2002) Study Duration: 14 days Groups / No. rats: 1 gp of 3 Glyphosate technical (Batch:569753(BX20070911) Purity: 96.3 %) Vehicle: 0.5 % Carboxymethyl-cellulose (CMC)	At 5000 mg/kg bw None	>5000 mg/kg bw (females)	5000 (no effects)
CA 5.2.1/006 [redacted] 2009; C22864	Rat HanRcc: WIST (SPF), females (ATC method) Age: 11 weeks Guideline: OECD 423 (2001) Study Duration: 14 day Groups / No. rats: 2 gp of 3 Glyphosate technical (Batch: GI-1045, Purity: 96.66 %) Vehicle: Purified water	At 2000 mg/kg bw None	>2000 mg/kg bw (females)	2000 (no effects)
CA 5.2.1/007 [redacted] 2009; 12170-08	Rat Sprague-Dawley, females (up and down procedure) Age: 8 weeks Guideline: OPPTS 870.1100 (equiv. OECD 425(2008)) Study Duration: 14 days Groups / No. rats: 1 gp of 3 Glyphosate tech grade mixed 5-batch (Batch: 080704-1 thru 5, Purity: 96.40 %) Vehicle: Deionised water	At 5000 mg/kg bw: Slight activity decr. (d5 only) Diarrhoea (d1-5) Piloerection (d1-7) Polyuria (d2-5) Salivation (d1-2) All signs observed in 1 ind out of 3.	>5000 mg/kg bw (females)	5000 (<50% ind. affected)
CA 5.2.1/008 [redacted] 2008; [redacted]- 3996.305.475.07	Rat Wistar Hannover, females (ATC method) Age: 8 weeks Guideline: OECD 423 (2001) Study Duration: 14 days Groups / No. rats: 1 gp of 3 Glyphosate technical (Batch: 20070606, Purity: 98.05 %) Vehicle: Deionised water	At 2000 mg/kg bw None	>2000 mg/kg bw (females)	2000 (no effects)
CA 5.2.1/009 [redacted] 2007; B02755	Rat HanRcc: WIST (SPF), females (up and down procedure) Age: 11 weeks	At 5000 mg/kg bw: Hunched posture (1 out of 3 ind; 2-5h) Slight ruffled fur (3 out of 3 ind.; 5h for 2 rats, 3d for one rat)	>5000 mg/kg bw (females)	5000 (all ind. but slight and in general transient effect)

Study/Report No.	Species / Study type / Test substance(s)	Clinical observations (incidence and duration of effects)	Result [mortality LD_{50}]	Result (sublethal effects LC_{50}) RMS remark
	Guideline: OECD 425 (2001), OPPTS 870.1100 (2002) & JMAFF No. 8147 (2001) Study Duration: 14 days Groups / No. rats: 1 gp of 3 Glyphosate technical material (Batch: 0507, Purity: 96.1 %) Vehicle: Purified water			
CA 5.2.1/010 [redacted] 2007; B02272	Rat HanRcc: WIST (SPF), females (ATC method) Age: 11 weeks Guideline: OECD 423 (2001), Directive 2004173/EC, 8.1 tris 'acute oral toxicity- acute toxic class method ' (2009) & JMAFF No. 8147 (2001) Study Duration: 14 days Groups / No. rats 1 gp of 6 Glyphosate technical (Batch: 200609062, Purity: 95.1 %) Vehicle: Polyethylene glycol 300 (PEG 300)	At 2000 mg/kg bw: Slight ruffled fur (6 out of 6 ind; 1-5h)	>2000 mg/kg bw (females)	2000 (all ind. but slight and transient effect)
CA 5.2.1/011 [redacted] 2005; 15274	Rat Sprague-Dawley derived, females (up and down procedure) Age: 11 weeks Guideline: OPPTS 870.1100 (2002) & OECD 425 (2001) Study Duration: 14 days Groups / No. rats: 1 gp of 3 Glyphosate acid technical (Batch: 040205, Purity: 97.23 %) Vehicle: Distilled water	At 5000 mg/kg bw: Diarrhea (1 out of 3 ind.; 5h) Ano-genital staining (3 out of 3 ind.; 5h-3d) Facial staining (1 out of 3 ind.; 3h) Reduced feces (1 out of 3 ind.; 1d)	>5000 mg/kg bw (females)	5000 (>50% ind. ano-genital staining, not severe)
CA 5.2.1/012 [redacted] 1999; 7907	Rat Sprague-Dawley derived, albino, males/females Age: Not specified Guideline: US EPA, OPPTS 870.1100 (1998) Duration: 14 days Groups/No. rats: 5 ind/sex/dose NUP5a99 (Batch: Drum Sample E, Purity: 62 %) IPA salt Vehicle: None	At 5000 mg/kg bw: Diarrhoea or soft faeces (2 out of 5 females, at 22h only) Anogenital staining (4 out of 5 females, observed at 3 and 22 h)	>5000 mg/kg bw	5000 (>50% females ano-genital staining, transient and not severe)
CA 5.2.1/013 [redacted] 1996; [redacted]/P/4660	Rat Alpk:APfSD (Wistar-derived) rats, males/females Age: Young adult	At 5000 mg/kg bw No clinical observations. Necropsy:	>5000 mg/kg bw	5000 (necropsy findings but no clinical effects, less

Study/Report No.	Species / Study type / Test substance(s)	Clinical observations (incidence and duration of effects)	Result [mortality LD_{50}]	Result (sublethal effects LC_{50}) RMS remark
	Guideline: OECD 401 (1987) & OPPTS 870.1100 (2002) Study Duration: 14 days Groups / No. rats: 5 ind/sex/dose Glyphosate acid, (Batch: P24, Purity: 95.6 %) Vehicle: Deionised water	slight red areas in lung (2 out of 5 per sex) red areas on thymus (1 out of 5 males)		relevant for populations of wild mammals)
CA 5.2.1/014 [redacted] 1995; B-3101	Mouse Crj:CD-1(ICR), males/females Age: 6 weeks Guideline: JMAFF No. 4200 (1985) Study Duration: 14 days Groups / No. rats: 5 ind/sex/dose MON 0139 (Batch: LBRV-11092, Purity: 62.34 %) IPA salt Vehicle: Water	At 5000 mg/kg bw: Slight retardation in body weight gain in males from 7d after treatment	>5000 mg/kg bw	5000 (body weight effects covered by long term endpoint)
CA 5.2.1/015 [redacted], 1995; [redacted] 94-0134	Rat Sprague-Dawley (Crj:CD), SPF, albino, males/females Age: 5 weeks Guideline: OECD 401 (1987), JMAFF No. 4200 (1985) & US EPA (1984) Study Duration: 14 days Groups / No. rats: 5 ind/sex/dose Glyphosate technical, HR-001 (Batch: 940908-1, Purity: 95.68 %) Vehicle: 0.5% carboxymethyl- cellulose	At 5000 mg/kg bw: Decreased spontaneous motor activity; (6 out of 10 ind.; 1-3h) Salivation: (1 ind. out of 10; 1h) No effect at 6h.	>5000 mg/kg bw	5000 (>50% effect, but transient)
CA 5.2.1/016 [redacted], 1995; [redacted] 94-0133	Mouse ICR (Crj:CD-1), SPF, males/females Age: 6 weeks Guideline: OECD 401 (1987), JMAFF No. 4200 (1985) & US EPA (1984) Study Duration: 14 days Groups / No. rats: 1 gp 5 males + 1 gp 5 females Glyphosate technical, HR-001 (Batch: 940908-1, Purity: 95.68 %) Vehicle: 0.5% carboxymethyl- cellulose	At 5000 mg/kg bw: Decreased spontaneous motor activity (1 ind. out of 5 per sex; 1h) Sedation and crouching position (1 male out of 5; 1-3h) No effect at 6h.	>5000 mg/kg bw	5000 (<50% effect, transient)
CA 5.2.1/017 *[redacted] 1995; 00917*	*Rat* *(limit test)* *Age: Not stated – starting bodyweights suggest age estimated to be 5-8 weeks.*	*At 2000 mg/kg bw* *No clinical signs.* *Necropsy: slightly congested lungs, splenomegaly and*	*>2000 mg/kg bw*	*2000* *(necropsy findings but no clinical effects, less relevant for*

Study/Report No.	Species / Study type / Test substance(s)	Clinical observations (incidence and duration of effects)	Result [mortality LD_{50}]	Result (sublethal effects LC_{50}) RMS remark
	Guideline: OECD 401 (1987) & US EPA (1984) *Study Duration: 14 days* *Groups / No. rats: 1 gp 5 males + 1 gp 5 females* *Glyphosate acid technical (Batch: 1073, Purity: 97.6 %)* *Vehicle: Cotton seed oil*	*centrilobular hepatic congestion*		*populations of wild mammals)*
CA 5.2.1/018 [redacted] *1995; 00926*	*Rat* *(limit test)* *Age: Not stated.* *Guideline: OECD 401 (1987) & US EPA (1984)* *Study Duration: 14 days* *Groups / No. rats: 1 gp 5 males + 1 gp 5 females* *Glyphosate (Batch: 940950, Purity: 62 % IPA)* *Vehicle: None (62.2% glyphosate IPA salt)*	*At 2000 mg/kg bw* *No clinical signs.* *Necropsy: severe lung congestion, splenomegaly, hepatomegaly with centrilobular congestion and subcapsular renal petechiae in all male and female animals*	*>2000 mg/kg bw*	*2000* *(necropsy findings but no clinical effects, less relevant for populations of wild mammals)*
CA 5.2.1/019 [redacted] 1995 ; 10670	Rat - Age: 6-8 weeks Guideline: US EPA Subdivision F, 81- 1 (in accordance with OECD guidelines) Study duration: 14 days Groups/No. rats: Male and female 5/sex/dose - Vehicle: CMC	At 5000 mg/kg bw: Piloerection Subdued behaviour Hunched appearance 2h – 1 d after treatment	> 5000 mg/kg bw	5000 (no info on no. ind. affected, transient effect)
CA 5.2.1/020 [redacted] 1994; 545/37	Rat Sprague-Dawley, males/females Age: 5 – 8 weeks Guideline: EPA OTS 798.1175, EPA OPP 81-1 (in accordance with OECD guidelines) Study duration: 14 days Groups/No. rats: Male and female 5/sex/dose Glyphosate Premix (Batch: 290-JaK-146-4, Purity: 46.1 % (Glyphosate), 62.2 % (IPA salt) Vehicle: -	At 5000 mg/kg bw: No effects observed.	> 5000 mg/kg bw	5000 (no effect)
CA 5.2.1/021 [redacted] 1995; 710/14	Rat, Sprague-Dawley (limit test) Age: 5-8 weeks Guideline: OECD 401 Study duration: 14 days Groups/No. rats: 5 animals/sex/dose Glyphosate	At 2000 mg/kg bw: No observed effects	> 2000 mg/kg bw	2000 (no effects)

Study/Report No.	Species / Study type / Test substance(s)	Clinical observations (incidence and duration of effects)	Result [mortality LD_{50}]	Result (sublethal effects LC_{50}) RMS remark
	Vehicle: arachis oil B.P.			
CA 5.2.1/022 [redacted] 1994; [redacted]-94-401/R	Rat Wistar, males/females Age: Not stated – starting bodyweights suggest age estimated to be 5-8 weeks.. Guideline: OECD 401 (1987) Study Duration: 14 days Animals/group Groups / No. rats: 1 gp 5 males+ 1 gp 5 females Glyphosate Technical (Batch: 36300892, Purity: 99.6 %) Vehicle: 3 % carboxymethyl-cellulose in water	No clinical signs. At 5000 mg/kg bw: Statistically significant decrease in weight gain during 2nd week (males only) Necroscopy: heart weight significantly lower (males only).	>5000 mg/kg bw	5000 (necropsy findings but no clinical effects, less relevant for populations of wild mammals; Effects on bw gain covered by chronic data)
CA 5.2.1/023 [redacted] 1994; 940020	Mouse, Crl:CD-1 (ICR)Age: 34-40 days Guideline: OECD 401, GLP Duration: 14 days Groups/No. mice: Male and female 5/sex/dose Glyphosate technical Vehicle: 0.5% methylcellulose in water	At 2000 mg/kg bw: Piloerection (all treated mice) Hunched posture (all treated mice) Hypoactivity (all treated mice) (2-24h after dosing, recovery at 48h).	> 2000 mg/kg bw	2000 (all ind. affected, but less severe, recovery confirmed at 48h)
CA 5.2.1/024 [redacted] 1992; 134/37	Rat Sprague-Dawley, males/females Age: 5-8 weeks Guideline: OECD 401 (1987) Study Duration: 14 days Groups / No. rats: 1 gp 5 males + 1 gp 5 females Glyphosate (Batch: L3258; purity: not specified) Vehicle: Distilled water	At 2000 mg/kg bw: None	>2000 mg/kg bw	2000 (no effects)
CA 5.2.1/025 [redacted] 1991; 12321	Mouse Bom:NMRI, males/females Age: 4-5 weeks Guideline: OECD 401 (1987) Study Duration:14 days Groups / No. rats: 1 gp 5 males + 1 gp 5 females Glyphosate Technical (PMG) (Batch: 206-JaK-25-1, Purity: 98.6 %) Vehicle: Distilled water	At 2000 mg/kg bw: Piloerection (10 out of 10 ind.; 1-6h, no effects at 1d) Sedation (10 out of 10 ind. 1-3h, no effect at 6h)	>2000 mg/kg bw	2000 (all ind. affected, but transient effect)
CA 5.2.1/026 [redacted] *1991;* [redacted] *874.AOR*	*Rat* *Wistar, males/females* *Age: 11 weeks* *Guideline: OECD 401 (1987)* *Study Duration: 14 days* *Groups / No. rats: 1 gp 5 males + 1 gp 5 females per*	*At 2500 mg/kg bw:* *No effects* *At 5000 mg/kg bw:* *No effects* *At 7500 mg/kg bw:* *Mortality (2 out of 5 females, 2 out of 5 males)* *Lethargy (3 out of 5 females)*	*>7500 mg/kg bw*	*5000* *(mortality+adverse sublethal effects at 7500)*

Study/Report No.	Species / Study type / Test substance(s)	Clinical observations (incidence and duration of effects)	Result [mortality LD_{50}]	Result (sublethal effects LC_{50}) RMS remark
	dose gp (2500, 5000 & 7500 mg/kg bw) *Glyphosate Technical (Batch: 60, Purity: 96.80 %)* *Vehicle: Refined groundnut (peanut) oil*	*Ataxia and dyspnea (1 out of 5 females)* *Duration of effects were not stated.*		
CA 5.2.1/027 *[redacted] 1991;* *[redacted]875.AOM*	*Mouse* *Swiss albino, males/females* *Age: 14 weeks* *Guideline: OECD 401 (1987)* *Study Duration: 14 days* *Groups / No. rats: 1 gp 5 males + 1 gp 5 females per dose gp (2500, 5000 & 7500 mg/kg bw)* *Glyphosate Technical (Batch: 60, Purity: 96.80 %)* *Vehicle: Refined groundnut (peanut) oil*	*At 2500 mg/kg bw:* *Mortality (1 male out of 5)* *Lethargy (3 male out of 5)* *At 5000 mg/kg bw:* *Mortality (1 male and 1 female out of 5)* *Lethargy (1 female out of 5)* *Lethargy and urine incontinence (1 male out of 5)* *At 7500 mg/kg bw:* *Mortality (3 out of 5 males, 1 out of 5 females)* *Lethargy (3 out of 5 males, 2 out of 5 females)* *Lethargy, ataxia and dyspnea (1 out of 5 males)* *Duration of effects were not stated.*	*>7500 mg/kg bw*	*5000* *(mortality+adverse sublethal effects at 7500)*
CA 5.2.1/028 *[redacted] 1990;* *[redacted]-900823B*	*Rat* *CD, males/females* *Age: Young adults – starting bodyweights suggests an estimated age of 5-8 weeks.* *Guideline: OECD 401 (1987)* *Study Duration: 14 days* *Groups / No. rats: 1 gp 5 males + 1 gp 5 females per dose gp (3000, 5000 & 8000 mg/kg bw)* *Glyphosate Technical (Batch: 0190 A, Purity: 98.1 %)* *Vehicle: 1% methylcellulose*	*At 3000 mg/kg bw:* *No effects* *At 5000 mg/kg bw:* *Decreased activity (5 out of 5 males, 1 out of 5 females, 1h for 4 ind. 6h for 1 ind.)* *Abnormal body posture (1 out of 5 males)* *Abnormal gait (2 out of 5 males)* *At 8000 mg/Kg bw:* *Decreased activity (5 out of 5 males, 2 out of 5 females, 1h)* *Abnormal body posture (2 out of 5 females, 3h)* *Abnormal gait (1 out of 5 females, 1h)* *Abnormal limb position (1 out of 5 females, 1h)* *No effects after 6h.*	*>8000 mg/kg bw*	*5000* *(all males affected at 5000 and 8000, but transient; Lower value selected for precautionary reasons due to various effects)*
CA 5.2.1/029 [redacted] [redacted]1989; 5883	Rat Sprague-Dawley, males/females Age: 6-8 weeks Guidelines: OECD, EEC, EPA guidelines (not further specified), GLP Duration: 14 days Groups: Male and female 5/sex/dose	At 5000 mg/kg bw: Piloerection (5 out of 5 rats per sex, for 7-8d) Reduced activity (5 out of 5 rats per sex, for 7-9d) Ataxia (1 out of 5 males, d9 only)	> 5000 mg/kg bw	<5000 (all ind. affected for 9 days, although some effects less severe) For precautionary reasons, RMS proposes that LD_{50} for this study is set to the lowest available value

Study/Report No.	Species / Study type / Test substance(s)	Clinical observations (incidence and duration of effects)	Result [mortality LD_{50}]	Result (sublethal effects LC_{50}) RMS remark
	Glyphosate Technical (PMG) (Batch: 206-JaK-25-1, Purity: 98.6 %) Vehicle: 0.5 % Carboxymethylcellulose (CMC)			(2000) from other data.
CA 5.2.1/030 [redacted] 1989; PRO439 / 238050	Rat - Age: 9-11 weeks Guidelines: OECD 401 Duration: 14 days Groups: Male and female 5/sex/dose Glyphosate technical (IPA salt 62 %) Vehicle: (IPA salt 62 % in water equivalent of 46 % of glyphosate)	At 2000 mg/kg bw: No effects observed.	> 2000 mg/kg bw	2000 (no effects)
CA 5.2.1/031 [redacted] 1988; 88.2053.007	Rat Sprague-Dawley, males/females Age: Not specificed– *starting bodyweights suggests an estimated age of 7-8 weeks.* Guideline: US EPA 81-1 (equiv. to OECD 420 (2001) Study Duration: 14 days Groups / No. rats: 1 gp 5 males + 1 gp 5 females Glyphosate (Batch: XLI-55, Purity: 97.76 %) Vehicle: Distilled water	At 5000 mg/kg bw: Diarrhoea/wet abdomen (5 out of 5 males, 5 out of 5 females at d2 only, 1 male at d3) Hair loss on abdomen (1 male and 1 female for the 14d duration of study)	>5000 mg/kg bw	5000 (all ind. affected, but transient and/or less severe effect)
CA 5.2.1/032 [redacted]1987; [redacted]-86-431/9308A	Rat Sprague-Dawley, males/females Age: Not specificed– *starting bodyweights suggests an estimated age of 5-8 weeks.* Guideline: US EPA 81-1 (equiv. to OECD 420 (2001) Study Duration: 14 days Groups / No. rats: 1 gp 5 males + 1 gp 5 females Glyphosate (MON8750) (Batch: XLG-255, Purity: 90.8 %, ammonium salt) Vehicle: Distilled / deionized water	At 2222 mg/kg bw: Diarrhoea (2 out of 5 males, 4 out of 5 females, d1) At 5000 mg/kg bw: Ataxia (2 out of 5 males, d1, 1 out of 5 females d1-3) Decreased activity (5 out of 5 males d1, 1-4 out of 5 females d1-3) Diarrhea (1-3 out of 5 males d1-4, 1-2 out of 5 females d1-3) Hair loss (1 out of 4 males d4-15) Labored breathing (1 out of 5 females d1-3) Rales (1 female out of 1 d3) Sores base of tail (1 out of 4 males d5-7) Wet abdomen (1 out of 4 males d3-4) Necropsy:	4613 mg/kg bw	2222 for males and females (at 2222, >50% females affected, but transient; At 5000, various effects in >50% ind., various duration; At 7500, >50% mortality and severe sublethal effects)

Study/Report No.	Species / Study type / Test substance(s)	Clinical observations (incidence and duration of effects)	Result [mortality LD_{50}]	Result (sublethal effects LC_{50}) RMS remark
		Lungs-dark/red areas (2 out of 5 males and 2 out of 5 females) Stomach (1 out of 5 males and 1(-3) out of 5 females) At 7500 mg/kg bw: Mortality (4 out of 5 males d1-3, 5 out of 5 females d2-3) Ataxia (1-2 out of 5 males, d1-5, 1-3 out of 5 females d1-2) Decreased activity (5 out of 5 males d1, 1-5 out of 5 females d1-2) Diarrhea (1-2 out of 5 males d1-4, 1 out of 1 females d2) Labored breathing (1-2 out of 2 males d2-5, 1-4 out of 5 females d1-2) Wet abdomen (1 out of 1 females d2) Necropsy: Stomach (4 out of 5 males and 5 out of 5 females)		
CA 5.2.1/033 [redacted] 1987; [redacted]- 86-430/93087A	Rat Sprague-Dawley Age: Not stated Guidelines: EPA (in accordance to OECD guidelines) Duration: 14 days Groups: Male and female 5/sex/dose MON8722 Vehicle: water	At 5000 mg/kg bw: Ataxia (3 out of 5 females, d1) Decreased activity (4 out of 5 males and 2 out of 5 females d1, 1 out of 5 males d2) Diarrhoea (5 out of 5 males and 1 out of 5 females d1, 2 out of 5 males and 1 out of 5 females d2) Rectal sores (1 male and 1 female on d5-7)	> 5000 mg/kg bw	5000 (>50% ind. affected, but transient effects)

At 2000 mg/kg bw, seven available tests on rat are considered fully reliable (see table above). Except for slightly ruffled fur for the first 5 hours after treatment in one of these studies (CA 5.2.1/010 [redacted] 2007; B02272), no clinical signs were observed. In two studies assessed as reliable with restrictions ([redacted] 1995a and b; Report No. 00917 and 00926), necropsy evaluations revealed histopathological changes in lungs, spleen and liver, however, these observations seems to be of unclear relevance since no clinical signs were observed during the study and no similar findings were made at higher dose levels in other studies.

At doses from 2222 – 3000 mg/kg bw, one reliable study is available on rat where diarrhea was observed in 80% of female and 40% of male rats during the first day after treatment (CA 5.2.1/032 [redacted] 1987; [redacted]-86-431/9308A). Although >50% of the male rats were affected, severity of this effect is not considered comparable with mortality in the field, especially due to the fast recovery of the affected animals (within 1 day). In two additional studies (CA 5.2.1/026 [redacted] 1991; [redacted]874.AOR and CA 5.2.1/028 [redacted] 1990; [redacted]-900823B), assessed as reliable with restrictions, no clinical signs were observed at these dose levels.

A dose of 5000 mg/kg bw is tested in 15 of the available studies on rat, and of these, two studies were considered as supportive (reliable with restriction). In two of the reliable studies (CA 5.2.1/002 [redacted] 2011; 10/218-001P and CA 5.2.1/020 [redacted] 1994; Report no. 545/37), no clinical signs or necropsy findings were observed at this dose level. In other studies, a range of sublethal effects were reported, mostly related to decreased activity and/or stomach problems. Clinical signs such as decreased activity/hunched posture/piloerection/slight ruffled fur and/or

diarrhoea/soft feces/reduced feces/anogenital staining were observed. However, in the majority of the studies these effects were either slight, observed in less than 50% of the test animals, and/or transient with recovery within a few hours or up to 1 day. In two studies at 5000 mg/kg bw (CA 5.2.1/029 [redacted],1989; Report no. 5883 and CA 5.2.1/033 [redacted] 1987 Report no.: [redacted]- 86-430/93087A), ano-genital staining, piloerection and reduced activity were observed in >50% of the test animals and persisted >1 day. Since these effects may have an impact on the overall fitness and survival of individuals in a field situation, the surrogate LD_{50} from these studies should be set with precaution. In the RMS calculation of the geomean, the LD_{50} was set to a lower dose level tested in the same study (where available) or, when only one dose was tested, to the lowest overall LD_{50} value from the available data.

For mouse, a dose level of 2000 mg/kg bw were tested in two reliable studies. In CA 5.2.1/023 [redacted]1994 Report no.: 940020, all treated mice showed piloerection, hunched posture and hypoactivity for 2-24 h after dosing (recovery at 48 h), and in CA 5.2.1/025 [redacted], 1991, Report No. 12321, piloerection and sedation were observed in all animals for 6 hours after dosing. In one study on mouse (CA 5.2.1/027 [redacted], 1991; Report No. [redacted]875.AOM), assessed as reliable with restrictions, dose levels of 2500, 5000 and 7500 mg/kg bw were tested. Mortality and lethargy were seen at all doses, although dose response was not evident. At the lowest dose, beside one dead male, 60% of males showed lethargy, while at the mid dose, incidence of lethargy and mortality was below 50%. At the highest dose, 7500 mg/kg bw, mortality was 60% in males.

In two reliable studies on mouse (CA 5.2.1/014 [redacted], 1995; Report No. B-3101 and CA 5.2.1/015 [redacted] 1995; Report No. [redacted] 94-0134), dose levels of 5000 mg/kg bw were tested. [redacted] (1995) observed decreased spontaneous motor activity, sedation and crouching position but the effects were transient.

In two studies on rat and mouse, respectively, slight effects were seen on body weight gain at 5000 mg/kg bw (CA 5.2.1/022 [redacted], 1994; Report No. [redacted]-94-401/R; CA 5.2.1/014 [redacted], 1995; Report No. B-3101). Such effects are however considered to be covered by the long term risk assessment, and does not need to be taken into account for the acute endpoint.

Overall, given that most of the clinical signs described in the available data were observed in less than 50% of the test animals, and the slight and/or transient nature of the observations (recovery within 24 hours in most cases), it is considered that the acute oral toxicity of glyphosate is in most cases sufficiently covered by the reported LD_{50} values from each study. This is in line with the standard procedure for such studies, where the acute oral toxicity is based on mortality. Although it is noted that there is an uncertainty as to whether the relevance of the sublethal effects being observed might be higher under in-field conditions compared to controlled laboratory conditions, it is proposed that the geomean approach for derivation of the acute endpoint in accordance with the EFSA guidance (2009) can be appropriate for the risk assessment for wild mammals.

The geometric mean endpoint was calculated in accordance with the EFSA (2009) guidance document by firstly considering endpoints according to species (mouse or rat), which were then combined to give an overall geometric mean endpoint.

The endpoints used, and the calculated geometric mean values are presented in the table below.

Table 2.9.1.4-2: Calculated geomean acute endpoint for mammals according to EFSA (2009)

Study	Species	Endpoint (sublethal effects LC_{50}), mg/kg bw	Geomean	
CA 5.2.1/002 [redacted] 2011	Rat	5000	Mouse:	3466±1643
CA 5.2.1/006 [redacted] 2009	Rat	2000	Rat:	3428±1500
CA 5.2.1/007 [redacted] 2009	Rat	5000	**OVERALL:**	**3447 mg/kg bw**
CA 5.2.1/008 [redacted] 2008	Rat	2000		
CA 5.2.1/009 [redacted] 2007	Rat	5000		
CA 5.2.1/010 [redacted] 2007	Rat	2000		
CA 5.2.1/011 [redacted] 2005	Rat	5000		
CA 5.2.1/012 [redacted] 1999	Rat	5000		
CA 5.2.1/013 [redacted]1996	Rat	5000		
CA 5.2.1/014 [redacted] 1995	Mouse	5000		

Study	Species	Endpoint (sublethal effects LC_{50}), mg/kg bw	Geomean
CA 5.2.1/015 [redacted] 1995	Rat	5000	
CA 5.2.1/016 [redacted] 1995	Mouse	5000	
CA 5.2.1/017 [redacted] 1995	Rat	*2000*	
CA 5.2.1/018 [redacted] 1995	Rat	*2000*	
CA 5.2.1/019 [redacted] 1995	Rat	5000	
CA 5.2.1/020 [redacted] 1994	Rat	5000	
CA 5.2.1/021 [redacted] 1995	Rat	2000	
CA 5.2.1/022 [redacted] 1994	Rat	5000	
CA 5.2.1/023 [redacted], 1994	Mouse	2000	
CA 5.2.1/024 [redacted] 1992	Rat	2000	
CA 5.2.1/025 [redacted] 1991	Mouse	2000	
CA 5.2.1/026 [redacted] 1991	Rat	*5000*	
CA 5.2.1/027 [redacted] 1991	Mouse	*5000*	
CA 5.2.1/028 [redacted] 1990	Rat	*5000*	
CA 5.2.1/029 [redacted] 1989	Rat	2000*	
CA 5.2.1/030 [redacted] 1989	Rat	2000	
CA 5.2.1/031 Reagan & Laveglia, 1988	Rat	5000	
CA 5.2.1/032 [redacted] 1987	Rat	2222	
CA 5.2.1/033 [redacted] 1987	Rat	5000	

*due to potential adverse effects at the limit dose in this study, the proposed surrogate LD_{50} was set to the overall lowest value from available data by the RMS.

For mouse, the overall geometric mean acute endpoint value was determined to be 3466 mg/kg bw.

For rat, the overall geometric mean acute endpoint value was determined to be 3428 mg/kg bw.

When combined, the overall geometric mean LD_{50} value calculated by the RMS is 3447 mg/kg bw. This value is more conservative compared to the geomean value of 3923 mg/kg bw originally proposed by the applicant.

For the acute environmental mammal risk assessment, the lowest available acute oral endpoint (LD_{50} >2000 mg/kg bw) was used at the screening and Tier I levels of the EU assessment. At the refinement step, a geometric mean acute endpoint is considered appropriate.

Regarding the representative product (MON 52276) and the environmental metabolite AMPA, the available toxicological data indicate similar or lower acute oral toxicity compared to the active ingredient, both with LD_{50} values >5000 mg a.e./kg bw. These values are relevant for the risk assessment. Please refer to the toxicology section for more detailed information.

The overall approach for selection of acute endpoints for wild mammals is in line with the proposal by the applicant.

2.9.1.5 Long term toxicity to wild mammals

Reproductive toxicity to mammals is summarised and evaluated by the RMS in Volume 3CA, section B.6.6. An overview of the available studies is presented in the tables below.

Table 2.9.1.5-1: Available data on developmental toxicity to rat and rabbit (for transparency, effects not considered adverse but observed in several studies are included in italics). Only acceptable or supportive studies

Reference/ Report No. Method, Study quality	Species no/group duration	Test substance Dose levels	NOAEL	LOAEL	Targets/ Main effects
5.6.2/001 [redacted] 1996; [redacted]/P/4819 OECD 414 (1981) Acceptable	Rat 24/group Gavage GD 7-16	Glyphosate acid Technical 95.6% 0, 250, 500, 1000 mg/kg bw/d In deionised water	Maternal and developmental: 1000 mg/kg bw/d	>1000 mg/kg bw/d	None
5.6.2/002 [redacted] 1995; [redacted] 94-0152 OECD 414 (1981) Acceptable	Rat 24/group Gavage GD 6-15	HR-001 95.68% 0, 30, 300, 1000 mg/kg bw/d in purified water with 0.5 % sodium CMC	Maternal: 300 mg/kg bw/d Developmental: 1000 mg/kg bw/d	Maternal: 1000 mg/kg bw/d Developmental: >1000 mg/kg bw/d	Maternal: Loose faeces 20/22 Development: *skeletal variations not considered treatment-related*
5.6.2/003 [redacted] 1991, [redacted] 43 & 41/90716 OECD 414 (1981) Acceptable	Rat, gavage, GD 6-15	Glyphosate Technical 98.6% 0, 300, 1000, 3500 mg/kg bw/d In purified water with 1% sodium methylcellulose (MC)	Maternal >300 mg/kg bw/d Developmental 300 mg/kg bw/d	Maternal: 1000 mg/kg bw/d Developmental 1000 mg/kg bw/d	Maternal 3500 mg/kg bw/d: Mortality 2/24 Bw gain (d 6-16) ↓32% Loose faeces (22/24) Noisy respiration (15/22) Salivation (22/24) Gaseous distension in GI tract *Maternal 1000 mg/kg bw/d:* *Noisy respiration (2/25)* *Bw gain (d 6-16) ↓3%* Development: 3500 mg/kg bw/d: Mean foetal weight: ↓6% Reduced ossification 35.7% compared to 11.7% in control 1000 mg/kg bw/d: Reduced ossification 28.4% compared to 11.7% in control, skeletal variations at low incidences
5.6.2/004 and 5.6.2/005 [redacted] 1991; [redacted].883.TER-R Guideline not stated Supportive	Rat, gavage, 30 x controls, GD 6-15	Glyphosate Technical 96.8% 0, 1000 mg/kg bw/d in Postman brand refined groundnut (peanut) oil	Maternal: 1000 mg/kg bw/d Developmental: not applicable (<1000 mg/kg bw/d)	Maternal 1000 mg/kg bw/d Developmental: not applicable (<1000 mg/kg bw/d)	Maternal: no effects Development: *reduced ossification (toxicological significance unknown)*

Reference/ Report No. Method, Study quality	Species no/group duration	Test substance Dose levels	NOAEL	LOAEL	Targets/ Main effects
5.6.2/008 [redacted] 1980; 401-054 OECD 414 (1981) Acceptable	Rat, gavage, GD 6-19	Glyphosate Technical 98.7% 0, 300, 1000, 3500 mg/kg bw/d in 0.5 % aqueous Methocel®	Maternal and developmental: 1000 mg/kg bw/d	Maternal and developmental: 3500 mg/kg bw/d	<u>Maternal 3500 mg/kg bw/d:</u> Mortality 6/25 Soft stool/diarrhoea: 22/25 <u>Development 3500 mg/kg bw/d:</u> ↓ implantations ↑ post-implantation loss Reduced mean foetal body weight (↓9%) ↓mean number of viable foetuses (20%) ↑visceral and skeletal malformations/variations
5.6.2/009 [redacted] 1996; [redacted]/P/5009 OECD 414 (1981) Acceptable	Rabbit 20 ind/dose Gavage GD 8-20	Glyphosate Technical 95.6%. 0, 100, 175, 300 mg/kg bw/d in deionised water	Maternal: 100 mg/kg bw/d Developmental: 175 mg/kg bw/d	Maternal: 175 mg/kg bw/d Developmental: 300 mg/kg bw/d	<u>Maternal 300 mg/kg bw/d:</u> *Mortality: 2/20 compared to 1/20 in control* Food intake during treatment (d 8-20) ↓19-43% Bw gain (days 8-20) ↓32% ↑Diarrhoea (19/20 compared to 4 in control) <u>Maternal 175 mg/kg bw/d:</u> *Mortality: 2/20* ↓Food intake (19-43%) ↑Diarrhoea (11/20) <u>Maternal 100 mg/kg bw/d:</u> *Mortality: 2/20* <u>Developmental 300 mg/kg bw/d:</u> Foetal wt ↓8% *Delayed ossification* *Minor skeletal defects*
5.6.2/010 [redacted] 1996; 434/020 OECD 414 (1981) Acceptable	Rabbit, 18 ind/dose Gavage GD 7-19	Glyphosate Technical 95.3%. 0, 50, 200, 400 mg/kg bw/d in 1 % CMC	Maternal 50 mg/kg bw/d Developmental: >400 mg/kg bw/day	Maternal: 200 mg/kg bw/d Developmental: >400 mg/kg bw/day	<u>Maternal 400 mg/kg bw/d:</u> Mortality: 2/18 Scours 16/16 (compared to 5/14 in controls) Food intake during treatment (d 7-19) ↓26-38% Bw gain (days 7-19) ↓>90%

Reference/ Report No. Method, Study quality	Species no/group duration	Test substance Dose levels	NOAEL	LOAEL	Targets/ Main effects
					↑Diarrhoea (10/16) Maternal 200 mg/kg bw/d: Bw gain (days 7-19) ↓24-29%, n.s.s *Developmental 400 mg/kg bw/d:* ↑ *post-implantation loss 12.1 compared to 3.7 in control (n.s.s)* *Developmental 200 mg/kg bw/d:* ↑ *post-implantation loss 11.5 compared to 3.7 in control (stat.sign)*
5.6.2/011 [redacted] 1995, [redacted] 94-0153 OECD 414 (1981) Acceptable	Rabbit 18 ind/dose Gavage GD 6-18	Glyphosate Technical 97.56%. 0, 10, 100, 300 mg/kg bw/d In purified water with 0.5% CMC	Maternal: 100 mg/kg bw/d; Developmental: >300 mg/kg bw/d	Maternal: 300 mg/kg bw/d; Developmental: >300 mg/kg bw/d	Maternal 300 mg/kg bw/d: *Mortality: 1/18* Loose faeces (4/17) Abortions (2/18). *Maternal 10 mg/kg bw/d:* *Abortions 2/18* *Developmental 300 mg/kg bw/d:* ↑ *Number of litters with malformations 5//14 compared to 1/18 in controls)* *(parietal bone, hemivertebra)*
5.6.2/012/013 [redacted] 1993; TOXI: 884-TER-RB OECD 414 (1981) Supplementary	Rabbit 26 ind/contr. 15-17 ind/dose Gavage GD 6-18,	Glyphosate Technical 96.8% 0, 20, 100, 500 mg/kg bw/d in 0.5% aqueous carboxy methyl cellulose	Maternal: 20 mg/kg bw/d; Developmental: not applicable	Maternal: 100 mg/kg bw/d; Developmental: not established due to low number of foetuses at top dose	Maternal 500 mg/kg bw/d: Mortality: 8/15 Soft/liquid faeces; 12/15 Food consumption (treatment, d 6-19) ↓31% No bwg during treatment compared to 100 g in controls. Maternal 100 mg/kg bw/d: Mortality: 4/16 Developmental, all doses: *stat. sign. increase in viceral malformations ("dilated hearts")*

Reference/ Report No. Method, Study quality	Species no/group duration	Test substance Dose levels	NOAEL	LOAEL	Targets/ Main effects
5.6.2/014 [redacted] 1991; [redacted] 45, 39 and 40 /901303 OECD 414 (1981) Acceptable	Rabbit 16-20 ind/dose Gavage GD 7-19	Glyphosate Technical 98.6% 0, 50, 150, 450 mg/kg bw/d in 1 % methylcellulose	Maternal: 50 mg/kg bw/d Developmental: 150 mg/kg bw/d	Maternal: 150 mg/kg bw/d Developmental: 450 mg/kg bw/d	<u>Maternal 450 mg/kg bw/d:</u> Mortality: 1/20 Soft/liquid stool; 13/20 Food consumption (treatment, d 7-19) ↓6-17%, n.s.s Body weight gain (day 11-29) ↓10%, n.s.s <u>Maternal 150 mg/kg bw/d:</u> Soft/liquid stool; 5/16 Food consumption (d 11-19) ↓12%, n.s.s Body weight gain (day 11-29) ↓21%, n.s.s <u>Developmental 450 mg/kg bw/d:</u> Late embryonic deaths 1.3 compared to 0.2 in controls (HCD 0.1 – 1.3 (0.7)) Post-implantation loss 21% compared to 5,7 % in controls (HCD 6.5 – 17.5 (12.9)) Cardiac malformations 11//95 compared to 1/163 in controls. *<u>Developmental 150 mg/kg bw/d:</u>* *Post-implantation loss 15.3% compared to 5,7 % in controls* *(HCD 6.5 – 17.5 (12.9))* *<u>Developmental 50 mg/kg bw/d:</u>* *Post-implantation loss 19.5% compared to 5,7 % in controls* *(HCD 6.5 – 17.5 (12.9)*
5.6.2/019 [redacted] 1980; 401-056 Supportive	Rabbit 16 ind/dose Gavage GD 6-27	Technical glyphosate 98.7%. 0, 75, 175, 350 mg/kg bw/d in 1% aqueous Methocel®	Maternal: 75 mg/kg bw/d; Developmental: ≥75 mg/kg bw/d	Maternal: 175 mg/kg bw/d Developmental: not established due to low number of foetuses	<u>Maternal, 350 mg/kg bw/d:</u> Mortality: 10/17 (59%) Soft stool/diarrhoea reported but no incidences presented <u>Maternal, 175 mg/kg</u> Mortality: 2/16 (12.5%)

Reference/ Report No. Method, Study quality	Species no/group duration	Test substance Dose levels	NOAEL	LOAEL	Targets/ Main effects
					Soft stool/diarrhoea reported but no incidences presented No effects on maternal bw and bw gain. <u>Development, 350, 175 mg/kg bw/d:</u> Not possible to assess due to high mortality in dam.

Table 2.9.1.5-2: Summary of available generational studies in rat. Only studies assessed as acceptable or supportive are included in the table

Reference Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results - NOAEL
5.6.1/001/002/003 [redacted] (2007) Report No. 2060/0013 Two generation reproduction study (dietary) OECD TG 416 (2001) Rat Acceptable	Glyphosate technical Purity: 95.7% (w/w) Lot/Batch#: H05H016A 0, 1500, 5000, 15000 ppm (equivalent to mean achieved dose levels of 0, 104, 351 and 1063 mg/kg bw/day for males, and 0, 162, 530 and 1634 mg/kg bw/day for females)	NOAEL for parental, offspring and reproductive toxicity: 5000 ppm (351 mg/kg bw/day).
5.6.1/004 [redacted] (2000) Report No.: [redacted]/P/6332 Two generation reproduction study (dietary) OECD TG 416 Rat Acceptable	Glyphosate acid Purity: 97.6% (w/w) Lot/Batch#: Y04707/082 0, 1000, 3000, 10000 ppm equivalent to mean achieved dose levels of: F0: 0, 99.4, 292.6, 984.7 mg/kg bw/day for males and 0, 104.4, 322.8, 1054.3 mg/kg bw/day for females during pre-mating period F1: 0, 116.5, 351 and 1161 mg/kg bw/day for males, and 0, 123.3, 370.8 and 1218.1 mg/kg bw/day for females, during the premating period.	NOAEL for offspring toxicity: 3000 ppm (293 mg/kg bw/day, mean daily intake of glyphosate during pre-mating phase in F0 males of 3000 ppm group) NOAEL for parental and reproductive toxicity: 10000 ppm (985 mg/kg bw/day, mean daily intake of glyphosate during pre-mating phase in F0 males of 10000 ppm group)
5.6.1/005 [redacted] (1997) Report No.: [redacted] 96-0031 Two generation reproduction study (dietary) OECD TG 416 Rat Acceptable	Glyphosate technical Purity: 94.61 % (w/w) Lot No.: T-950308 0, 1200, 6000, 30000 ppm Equivalent to: F0: 0, 83.6, 417, 2151 and 0, 96.9, 485, 2532 mg/kg bw/day in males and females, respectively F1: 0, 91.7, 458, 2411 and 0, 104.8, 530, 2760 mg/kg bw/day in males and females, respectively)	NOAEL for parental, reproductive and offspring toxicity: 6000 ppm (417 mg/kg bw/day)

Reference **Method, guideline, deviations if any, species, strain, sex, no/group**	**Test substance, dose levels duration of exposure**	**Results** **- NOAEL**
5.6.1/006 [redacted] (1993) Report No.: TOXI 885-RP-G2 Two generation reproduction study (dietary) OECD TG 416 (1983) Rat Supplementary only (effect dose lacking, limited parameters investigated in study)	Glyphosate technical Purity: 96.8 % (w/w) Batch No.: 60 0, 100, 1000, 10000 ppm Dietary level would correspond to a mean daily compound intake of 0, 7.7, 77 and 770 mg/kg bw/day. [The mean daily intake was not reported for all dietary levels, but for the low level of 100 ppm a corresponding average value of 7.7 mg/kg bw/d was given in the original report].	NOAEL for parental, offspring and reproductive toxicity: 10000 ppm (would correspond to a mean daily compound intake of 700-800 mg/kg bw/d)
5.6.1/007/008 [redacted] (1992) Report No.: [redacted] 47/911129 Two generation reproduction study (dietary) OECD TG 416 (1983) Rat Acceptable	Glyphosate technical Purity: 99.2 % (w/w) Batch No.: 206-JaK-119-1 0, 1000, 3000, 10000 ppm Equivalent to: F0: 0, 66, 197, 668 and 0, 75, 226, 752 mg/kg bw/day in males and females, respectively F1: 0, 76, 230, 771 and 0, 82, 245, 841 mg/kg bw/day in males and females, respectively)	The NOAEL for offspring and reproductive toxicity was set at 10000 ppm (668 mg/kg bw/d). (Note: NOAEL for parental toxicity set to 1000 ppm (66 mg/kg bw/day) based on histopathological changes in salivary glands; not considered ecologically relevant)
5.6.1/009 [redacted] (1991) Report No. [redacted] 42/90619 One-generation range finding study (dietary) No guideline Rat Supplementary only (low number of animals, limited parameters investigated, no statistics)	Glyphosate technical Purity: 98.6 % (w/w) Lot/Batch No.: 206-Jak-25-1 0, 3000, 10000, 30000 ppm Equivalent to: F0 females: 0, 236-311, 799-1010 and 2515-2789 mg/kg bw/day F1 offspring: 0, 368-390, 1291-1335 and 3918-4453 mg/kg/day for males and 355-402, 1191-1271 and 3961-4397 mg/kg/day for females	The study is not suitable for NOAEL setting. Study acceptable as dose range finding study only (low number of animals, limited parameters investigated, no statistics)
5.6.1/010 [redacted] (1990) Report No. [redacted]-10387 Two generation reproduction study (dietary) No guideline Rat Acceptable	Glyphosate Purity: 97.67 % (w/w) Lot No.: XLI-203 0, 2000, 10000, 30000 ppm Corresponding to 132-140, 666-711, 1983-2230 mg/kg bw/day for males and 160-163, 777-804, 2322-2536 mg/kg bw/day for females) (calculated for F0 and F1A adults)	NOAEL for parental, offspring and reproductive toxicity: 10000 ppm (666-711 mg/kg bw/day for males and 777-804 mg/kg bw/day for females)

The available reproductive data indicate that rabbit is more sensitive than the rodent species tested. Therefore, the selection of endpoint for the reproductive risk assessment will mainly focus on this species. In the previous evaluation, an overall NOAEL of 50 mg/kg bw/d (from rabbit developmental study 5.6.2/014 [redacted] 1991;

[redacted] 45, 39 and 40 /901303); maternal and developmental effects) was agreed for the mammalian long-term reproductive risk assessment. This endpoint was the lowest NOAEL from all available developmental toxicity studies performed in rabbit (lagomorph) dosed via the oral gavage route.

The applicant proposed that this endpoint is overly-conservative, due to dose spacing in 5.6.2/014 [redacted] 1991; [redacted] 45, 39 and 40 /901303study, as there are higher NOAELs in other studies that fall below the lowest LOAEL (considering all the available rabbit developmental toxicity data). Instead, it was proposed to consider all available rabbit developmental toxicity study data together, as if derived in a single study, and then, from the larger dataset, to select the highest NOAEL value that falls below all LOAEL values, in accordance with section 2.4.3 of the EFSA Guidance on Risk Assessment for Birds and Mammals (2009).

The mammalian reproductive endpoint was thoroughly discussed during the previous evaluation, including the conservativism of the selected value of 50 mg/kg bw per day in the light of all available data. Although the proposal to merge developmental data sets from several studies is an acceptable approach according to the EFSA guidance, it was during the previous evaluation not considered feasible in this case due to the maternal mortality at 100 mg/kg bw (4 out of 16 rabbits died) in the developmental rabbit study CA 5.6.2/012 [redacted], 1993; TOXI: 884-TER-RB. It should be noted that in the previous evaluation as well as for the renewal, that this study was regarded as supportive information. This was mainly due to the high maternal mortality which complicated the evaluation of developmental toxicity, but also due to methodological deficiencies of the study. Given that other (acceptable) developmental studies in rabbit did not show similar high mortality at corresponding or higher dose levels (<10% maternal mortality up to 200 mg/kg bw/d), it seems reasonable to conclude that the observations by CA 5.6.2/012 [redacted] 1993; TOXI: 884-TER-RB are not consistently dose related and below doses at which sub-lethal effects were observed among the dams. The study 5.6.2/019 [redacted] 1980; Report No. 401-056was also considered as supportive only. However, the maternal mortality observed at 175 mg/kg bw/d in 5.6.2/019 [redacted] 1980; Report No. 401-056 can be considered as a more reliable/robust result, since sub-lethal maternal effects were observed at the same and lower dose levels. Regarding mortality observed in the 5.6.2/009 [redacted] 1996; [redacted]/P/5009 study, the toxicologists did not consider this as treatment related since there was no dose response; there was 5% mortality also in the control group, and 10% mortality was observed at all treatment doses (100, 175 and 300 mg/kg bw/d), i.e. no increase at higher exposure levels.

An overview of the available studies is given in the table below.

Table 2.9.1.5-3: Overview of significant observations in available developmental studies on rabbit. Studies marked with * are regarded as supportive information. Ecologically relevant endpoint levels for developmental effects, sublethal maternal effects and maternal mortality are marked in bold

Study	Dose level, mg/kg bw/d	Developmental effects	Maternal sublethal effects	Mortality >10% (maternal)
5.6.2/011 [redacted] 1995	10	no	no	no
5.6.2/012/13 [redacted] 1993*	20	-	no	no
5.6.2/010 [redacted] 1996	50	no	no	no
5.6.2/014 [redacted] 1991	50	no	no	no
5.6.2/019 [redacted] 1980*	75	no	no	no
5.6.2/009 [redacted], 1996	100	no	no	no
5.6.2/011 [redacted] 1995	100	no	**no**	no
5.6.2/012/13 [redacted] 1993*	100	-	-	yes
5.6.2/014 [redacted] 1991	150	no	yes	no
5.6.2/009 [redacted] 1996	175	no	yes	no
5.6.2/019 [redacted] 1980*	175	-	yes	yes
5.6.2/010 [redacted] 1996	200	**no**	yes	no
5.6.2/009 [redacted] 1996	300	yes	yes	**no**
5.6.2/011 [redacted] 1995	300	no	yes	no
5.6.2/019 [redacted] 1980*	350	-	yes	yes
5.6.2/010 [redacted] 1996	400	no	yes	yes
5.6.2/014 [redacted] 1991	450	yes	yes	no

Study	Dose level, mg/kg bw/d	Developmental effects	Maternal sublethal effects	Mortality >10% (maternal)
5.6.2/012/13 [redacted] 1993*	500	-	yes	yes

Based on the overall assessment of the available data, it is proposed to set the mammalian reproductive endpoint to 100 mg/kg bw/d. This is in line with the proposal of the applicant above based on rabbit data.

The applicant further argued that dosing via oral gavage as used in the developmental studies is from an ecotoxicological perspective less representative of dietary exposure expected in the field. This was because the use of gavage dosing can result in high systemic levels that may induce adverse findings that cannot be reproduced when equivalent doses (in mg/kg bw/d) are given via the diet (see EFSA Guidance on Risk Assessment for Birds and Mammals (2009), section 2.3). In contrast to the oral gavage route of exposure, dietary exposure gives information on uptake and toxicokinetic processes (absorption, distribution, metabolism and excretion). However, from the RMS point of view, data from gavage exposure should not be disregarded, especially since the available data demonstrate a higher sensitivity of rabbit compared to rodents and no further studies on dietary exposure or ADME are presented for rabbit. Therefore, a lower toxicity from the dietary route of exposure cannot be demonstrated for this species. Further details on the relevance of the rabbit developmental toxicity study for use in risk assessment is presented in the toxicology section.

Refined endpoint for rodent species

It was also proposed by the applicant that since acceptable long-term risk for lagomorphs like the rabbit can already be demonstrated using the most conservative NOAEL of 50 mg/kg bw/d at the Screening and Tier 1 steps, a higher tier risk assessment for the protection of wild mammals is only required for rodents. Therefore, it was proposed that the refined risk assessment is based on NOAEL for rodents only.

The applicant's presentation on selection of NOAEL for rats is included here for information:

For maternal effects;

- the lowest LOAEL value is **3500 mg/kg bw/d** (5.6.1/009 [redacted]. (1991); Report No. [redacted] 42/90619 and 5.6.2/008 [redacted] 1980; Report No. 401-054).
- the highest NOAEL value below the lowest LOAEL is **1000 mg/kg bw/d**
- achieved in three studies, 5.6.2/001 [redacted] 1996; Report no. [redacted]/P/4819, 5.6.2/008 [redacted] 1980; Report No. 401-054 and CA 5.2.1/026 [redacted] 1991; Report No. [redacted].874.AOR

For developmental or offspring effects;

- the lowest LOAEL value is **3500 mg/kg bw/d** (5.6.1/009 [redacted] (1991); Report No. [redacted] 42/90619 and 5.6.2/008 [redacted] 1980; Report No. 401-054)
- the highest NOAEL value below the lowest LOAEL is **1000 mg/kg bw/d**
- achieved in four studies, 5.6.2/001 [redacted] 1996; Report no. [redacted]/P/4819, 5.6.2/002 [redacted] 1995; Report No. [redacted] 94-0152, CA 5.2.1/026 [redacted] 1991; Report No. [redacted].874.AOR and 5.6.2/008 [redacted] 1980; Report No. 401-054

In the toxicology section B.6.6, further evaluation of the study findings is presented. Overall, the available studies on developmental toxicity in rats consistently revealed that in utero exposure to glyphosate did not result in teratogenicity in rats. If observed, test substance-related effects, including maternal toxicity and developmental effects occur at 1000 mg/kg bw/day. Thus, based on the available data a NOEL of 300 mg/kg bw/day was derived for both maternal and developmental toxicity in rats.

Relevant endpoints for the survival of wild mammal populations, such as pup development, exposure via lactation, reproductive success of offspring is also addressed in 2-3 generation rat reproduction studies, with dosing via the dietary route. Therefore, in addition to the refined approach on endpoint selection presented above for the rat an alternative endpoint selection approach based on the available multi-generational data was presented by the applicant.

There are nine multi-generational reproduction studies available, and it was again proposed to compare the NOAEL with the LOAEL values and to determine the highest NOAEL below the lowest LOAEL. The lowest LOAEL for

offspring effects was 1000 mg/kg bw/d (5.6.1/001/002/003 [redacted] (2007); Report No. 2060/0013), whilst the highest NOAEL below the lowest LOAEL was 700 mg/kg bw/d, achieved in the 5.6.1/006 [redacted] (1993) Report No.: TOXI 885-RP-G2 study.

From the RMS point of view, however, there is no evidence that all rodents in the field would be of more similar sensitivity to rat and mouse compared to rabbits. Instead, each tested species is regarded as representatives of the overall mammalian community and therefore the risk assessment should normally be based on the most sensitive species. Using differentiated endpoints for taxonomic groups of mammals is generally not accepted within the EU process.

Overall, the RMS proposes that the NOAEL of 100 mg/kg bw/d, derived from the available data on rabbit, is selected for the reproductive risk assessment for mammals. This value is considered relevant for all species scenarios included in the assessment.

Regarding the environmental metabolite AMPA, the available toxicologal data indicate similar or lower reproductive toxicity compared to the active ingredient, with developmental NOAEL from 400 mg/kg bw/day and corresponding maternal NOAEL from 150 mg/kg bw/day. The RMS proposed that lower of these values is used for the risk assessment of the metabolite. Please refer to the toxicology section for more detailed information.

2.9.1.6 Literature data

Regarding the literature search from the previous evaluation (RAR 2015), it was proposed that there was no critical data on terrestrial vertebrates that must be included in the environmental risk assessment. From these data, it was not possible to distinguish between the effect of the technical glyphosate and the surfactant added to the commercial formulations by the experimental designs used. No adverse data were identified from studies using test materials without the forbidden surfactant (POEA), compared to results from the current literature search. Hence, these results have not been considered further.

A review of published literature data on glyphosate for the period 2010-2020 was presented by the applicant, who proposed that two studies should be considered as relevant and six studies as relevant but supplementary. Study summaries and RMS evaluation of data from the literature search for the renewal dossier in 2020 are presented in the Appendix to Volume 3CA, section B.9.1.

The applicant considered that only studies on glyphosate and formulations identical to the representative product MON 52276 should be included in the risk assessment. However, for a weight of evidence, the RMS took into consideration results on all tested formulations, as long as they did not include substances that are not allowed within the EU (Regulation (EU) 2016/1313 and DRAFT Regulation amending Annex III of Regulation (EC) 1107/2009). Such data were considered by the RMS to be "less relevant but supplementary". In addition, the applicant justified the non-relevance of some studies by stating that the observations are caused by a "mixture of compounds / potentially causal factors and thus not attributable to a substance of concern (e.g. mixture toxicity)", which is not accurate, as those studies also included experimental groups treated with glyphosate or glyphosate-based formulations alone. Moreover, the RMS does not agree with the applicant's reasoning that results cannot be extrapolated to the EU conditions when studies are performed with other species not found within the EU or under other "geo-climatic properties, land-uses or agricultural practices, non-EU monitoring data, residue definitions differing from EU". The RMS' view on this is that between-species extrapolation is needed in ecotoxicology, and since the exposure pathways were realistic and the biological endpoints were coupled to the *measured* concentrations of glyphosate, such studies are relevant for the risk assessment.

In cases where the active substance and a formulated product were tested in the same study, the results from the active ingredient test were considered the most relevant.

A summary of the available published literature data, considered by the RMS as relevant for the risk assessment, is presented in the table below (Table 2.9.1-14.). Moreover, studies that were considered less relevant but supplementary, and that will be used in a WoE are listed in Table 2.9.1-15.

Table 2.9.1.6-1: Summary and overall discussion on literature data considered as relevant for the risk assessment

Reference	Species/life stage	Test substance/ product	Time scale	Endpoint	Toxicity	Status (RMS)
Birds						
CA 9 Ruuskanen S. *et al*. 2020	*Coturnix japonica* (10 days-old juveniles, and 1 year-old adults)	Roundup Flex	8 weeks	LOEC (NOEC n.d.[a])	164 mg a.s,/kg food (flight feather moult in females and plumage development in all juveniles)	Reliable
			30 min/day x 4 consecutive days	LOEC (NOEC n.d.[a])	20 mg a.s./kg food (adult female behaviour: preference for treated food)	
			20 h	LOEL (NOEC n.d.[a])	164 mg a.s./kg body weight/day (feed consumption by females)	
Amphibians						
CA 8.2.8/001; Daam, M.A. *et al*. 2019	*Physalaemus cuvieri* (tadpoles Gs 25)	glyphosate	96 h	LC_{50}	115 mg a.s./L	Reliable with restrictions
	Hypsiboas pardalis (tadpoles Gs 25)		96 h	LC_{50}	106 mg a.s./L	
CA 8.1.4 Turhan D. Ö *et al*. 2020	*Xenopus laevis* (embryos stage 8 and tadpoles Gs 46)	glyphosate	96 h	LC_{50}	>403 mg glyphosate/L	Reliable with restrictions
CA 8.1.4 Bach N. C. *et al*. 2016	*Leptodactylus latrans* (tadpoles Gs 25 and 36)	glyphosate	96 h	LC_{50}	>300 mg glyphosate/L	Reliable
			96 h	LOEC	15 mg glyphosate/L (development and growth, Gs 25) 30 mg glyphosate/L (morphological abnormalities, Gs 25 and 36)	
CA 9 Babalola O. O. *et al*. 2019	*Xenopus laevis* (embryos)	Enviro	96 h	LC_{50}	446 mg a.e./L	Reliable
CA 9 Lajmanovich R. C. *et al*. 2011	*Rhinella arenarum* tadpoles Gs 36-38)	Roundup Ultra-Max	48 h	LC_{50}	2.42 mg a.e./L	Reliable with restrictions
CA 9 Lajmanovich R. C. *et al*. 2013	*Rhinella arenarum* (tadpoles Gs 29-30)	Ultra-Max®	48 h	LC_{50}	13.20 mg a.i./L	Reliable with restrictions
CA 9 Wagner N. *et al*. 2017	*Xenopus laevis* (larvae NF stage 47)	Roundup® UltraMax	96 h	LC_{50}	7.04 mg a.i./L	Reliable
	Xenopus laevis (embryos NF stage 8-11)		96 h	LC_{50}	25.82 mg a.i./L	
	Discoglossus pictus (larvae Gs 25)		96 h	LC_{50}	18.29 mg a.i./L	
	Discoglossus pictus (embryos Gs 8-9)		96 h	LC_{50}	128.2 mg a.i./L	

Reference	Species/life stage	Test substance/ product	Time scale	Endpoint	Toxicity	Status (RMS)
CA 9 Rissoli Zanelli R. *et al*. 2016	*Lithobates catesbeianus* (tadpoles Gs 25)	glyphosate	96 h	LOEC (NOEC n.d.[a])	1 mg a.e/L (epidermis morphology and O_2 uptake)	Reliable with restrictions
CA 9 Slaby S. *et al*. 2019	*Xenopus laevis* (stage VI oocytes)	glyphosate	overnight	NOEC	1480 µM a.e. (cell abnormalities)	Reliable with restrictions*

NOEC could not be determined (n.d.) because:
a only one glyphosate concentration was tested and resulted in a significant effect;
*Not relevant for the standard risk assessment, but for ED-assessment.

Table 2.9.1.6-2: Summary and overall discussion on literature data considered as less relevant but supplementary (to be used in a WoE)

Reference	Species/life stage		Test substance/ product	Time scale	Endpoint	Toxicity	Status (RMS)
Amphibians							
CA 8.1.4. Lenkowski J. R. *et al*. 2010	*Xenopus laevis* (embryo NF stage 41)		Roundup	48 h	NOEC	1 mg a.i./L (intestinal malformations)	Reliable with restrictions
CA 8.1.4. Williams B. K. *et al*. 2010	Tadpoles Gs 25	*Pseudacris triseriata*	Roundup WeatherMax	Chronic (not specified)	NOEC	0.0006 mg a.i. (survival)	Reliable with restrictions
		Bufo americanus			NOEC	0.0006 mg a.i. (time to metamorphosis) 0.7 mg a.i (survival)	
		Hyla versicolor			NOEC	0.7 mg a.i. (survival)	
	Tadpoles Gs 25	*Pseudacris triseriata*	Roundup Original Max	Chronic (not specified)	NOEC	0.7 mg a.i. (survival and time to metamorphosis)	
		Bufo americanus			NOEC	0.0006 mg a.i. (time to metamorphosis) 0.7 mg a.i (survival)	
		Hyla versicolor			NOEC	0.700 mg a.i. (survival)	
CP 10.1.1 & 10.1.2 Edge C. *et al* 2011	*Lithobates clamitans* (juveniles)		VisionMax®	14 days	Correlation	2.03 - 10.21 kg a.e./ha (application rate negatively correlated to liver somatic index and fungal infection rates)	Reliable (see Annex biodiversity)
CP 10.1.1 & 10.1.2 Edge C. 2013	*Lithobates clamitans* (juveniles)		Roundup WeatherMax™	16 days	NOED	8.64 kg a.e./ha (survival, body condition, liver somatic index)	Reliable with restrictions (see Annex biodiversity)
	Lithobates pipiens (juveniles)						
CA 9 Agostini M. G. *et al*. 2020	*Boana pulchellus* (tadpoles Gs 37-42)		Glyphosate commercial formulation (unspecified)	24 h	NOEC	179.3 µg glyphosate/L (survival)	Reliable
					LOEC (NOEC n.d.[b])	54.5 µg glyphosate/L (mobility)	

Reference	Species/life stage	Test substance/ product	Time scale	Endpoint	Toxicity	Status (RMS)
	Rhinella arenarum (tadpoles Gs 37-40)		24 h	NOEC	315.5 µg glyphosate/L (survival)	
				LOEC (NOEC n.d.[b])	214.5 µg glyphosate/L (mobility)	
CA 9 Babalola O. O. *et al*. 2019	*Xenopus laevis* (embryos)	Kilo Max	96 h	LC_{50}	207 mg a.e./L	Reliable
CA 9 Edge C. *et al*. 2014	*Lithobates sylvaticus* (tadpoles Gs 25)	Roundup WeatherMax	96 h	LC_{50}	6.01 mg a.e./L (geomean of 4 populations)	Reliable
		Roundup Weed and Grass Control	96 h	LC_{50}	0.65 mg a.e./L (geomean of 4 populations)	
CA 9 Fuentes L. *et al*. 2011	Tadpoles Gs 25: *Rana sphenocephala*	Roundup® WeatherMAX	96 h	LC_{50}	1.33 mg a.e./L	Reliable
	Tadpoles Gs 25: *Bufo fowleri*		96 h	LC_{50}	1.96 mg a.e./L	
	Tadpoles Gs 25: *Rana catesbeiana*		96 h	LC_{50}	1.97 mg a.e./L	
	Tadpoles Gs 25: *Rana pipiens*		96 h	LC_{50}	2.27 mg a.e./L	
	Tadpoles Gs 25: *Rana clamitans*		96 h	LC_{50}	2.77 mg a.e./L	
	Tadpoles Gs 25: *Hyla chrysoscelis*		96 h	LC_{50}	3.26 mg a.e./L	
CA 9 Jones D. K. *et al*. 2010	*Rana sylvatica* (tadpoles Gs 26)	Roundup Original MAX®	18 days exposure, with product application on day 0, 7 or 14	LC_{50}	2.10 mg a.e./L (application day 0) 2.44 mg a.e./L (application day 7) 4.27 mg a.e./L (application day 14)	Reliable
				NOEC	1 mg a.e./L (body mass, application day 14)	
	Bufo americanus (tadpoles Gs 25)		Product application on day 0, 7 or 14, observations on day 18	LC_{50}	2.31 mg a.e./L (application day 0) 2.30 mg a.e./L (application day 7) 3.93 mg a.e./L (application day 14)	
				NOEC	1 mg a.e./L (body mass, days 7 and 14)	
CA 9 Jones D. K. *et al*. 2011	*Rana catesbeiana*	Roundup Original MAX®	Product application on day 7, mortality assessed day 16	LC_{50} (at 'low' competition)	2.18 mg a.e./L	Reliable
	Hyla versicolor				2.04 mg a.e./L	
	Rana clamitans				2.58 mg a.e./L	

Reference	Species/life stage	Test substance/ product	Time scale	Endpoint	Toxicity	Status (RMS)
CA 9 Krynak K. L. *et al.*2017	*Acris blanchardi* (tadpoles Gs 42, juveniles 11-18 days old)	Rodeo™ Original MAX®	12 days exposure, observations depending on life stage	NOEC	1.5 mg a.e./L (mortality and skin bacterial community, tadpoles) 2.5 mg a.e./L (mortality, juveniles)	Reliable with restrictions
CA 9 Lajmanovich R. C. *et al.* 2011	*Rhinella arenarum* tadpoles Gs 36-38)	Infosato	48 h	LC_{50}	38.76 mg a.e./L	Reliable with restrictions
		Glifoglex	48 h	LC_{50}	73.77 mg a.e./L	
		C-K YUYOS FAV	48 h	LC_{50}	77.52 mg a.e./L	
CA 9 Lanctot C. *et al.* 2014	*Lithobates sylvaticus* (tadpoles Gs 25 - 1st pulse and Gs 30-2nd pulse)	Roundup WeatherMax®	96 h exposure (1st pulse), observation after 16 days	NOEC	0.21 mg a.e./L (mortality)	Reliable
			96 h exposure (1st pulse), observation after 18 days	LOEC (NOEC n.d.c)	≤0.21 mg a.e./L (weight increase)	
			2 x 96 h exposure (2nd pulse), observation after > 18 days	LOEC (NOEC n.d.c)	≤0.21 mg a.e./L (snout-vent length)	
CA 9 Munoz L. M. H. *et al.* 2015	Tadpoles Gs 25 *Hypsiboas crepitans*	Roundup® Active	96 h	LC_{50}	1.41 mg a.e./L	Reliable with restrictions
	Tadpoles Gs 25 *Rhinella marina*		96 h	LC_{50}	1.42 mg a.e./L	
	Tadpoles Gs 25 *Rhinella humboldti*		96 h	LC_{50}	2.44 mg a.e./L	
	Tadpoles Gs 25 *Engystomops pustulosus*		96 h	LC_{50}	2.79 mg a.e./L	
CA 9 Triana Velasquez T. M. *et al.* 2013	Embryos st. 10 *Rhinella marina*	Roundup® Active	96 h	LC_{50}	1.42 mg a.e./L	Reliable with restrictions
	Embryos st. 10 *Hypsiboas crepitans*		96 h	LC_{50}	2.15 mg a.e./L	
	Embryos st. 10 *Rhinella humboldti*		96 h	LC_{50}	2.9 mg a.e./L (lab) 40.8 mg a.e./L (microcosm)	
	Embryos st. 10 *Engystomops pustulosus*		96 h	LC_{50}	3.03 mg a.e./L (lab) 74.7 mg a.e./L (microcosm)	
CA 9 Brodeur J. C. *et al.* 2014	*Rhinella arenarum* (tadpoles Gs 25)	Glifosato Atanor	96 h	LC_{50}	19.4 mg a.e./L	Reliable
		Glifoglex	96 h	LC_{50}	72.8 mg a.e./L	
Ca 9 Navarro-Martín L. *et al.* 2014	*Lithobates sylvaticus* (tadpoles Gs 25)	VisionMax®	~41 days (average time to reach Gs 46)	NOEC	1.1 mg a.e./L (mortality, development rate and metamorphic success)	Reliable
CA 9	Tad *Hyla versicolor*		17 days	LC_{50}	2.3 mg a.e./L	Reliable

Reference	Species/life stage	Test substance/ product	Time scale	Endpoint	Toxicity	Status (RMS)
Relyea R. A. 2018	*Rana catesbeiana*	Roundup Original Max	17 days	LC_{50}	3 mg a.e./L	
	Rana clamitans		17 days	LC_{50}	>3 mg a.e./L	
Reptiles						
CA 9 Poletta G. L. *et al*.2011	*Caiman latirostris* (embryos)	Roundup Full II (sprayed)	5 days exposure; observations after 3 months	LOEC (NOEC n.d.[a])	17.25 g glyphosate/L (total length and SVL)	Reliable

NOEC could not be determined (n.d.) because:
a only one glyphosate concentration was tested and resulted in a significant effect;
b significant effects were observed in all treated ponds. Thus, LOEC is based on the lowest measured concentration of glyphosate;
c significant effects were observed in the lowest tested concentration.

Only one of the literature studies on birds considered as relevant involved dietary exposure to glyphosate, namely Ruuskanen *et al*., 2020. In this study, a dietary treatment with 164 mg glyphosate/kg food for 8 weeks resulted in reductions of flight feather moult and plumage development in juvenile quails. This study also showed that glyphosate residues were present in quail muscles and liver, as well as in eggs from exposed parents (average concentrations 0.17, 2.05 and 0.76 mg/kg, respectively) following chronic exposure to food contaminated with glyphosate-based herbicide (164 mg glyphosate/kg food). In addition, there seems to be a preference for treated food among female birds. The RMS proposes that the results from Ruuskanen *et al*. (2020) need to be further considered in the risk assessment.

Regarding **amphibian data** from the open literature, results are available for a wide range of species and life stages. Acute exposure (up to 96 hours), resulted in LC_{50} values ranging from 0.75 mg a.e./L to >403 mg glyphosate/L for 19 species tested. Sublethal effects observed in these short-term studies included reduced mobility and malformations, with an overall lowest acute NOEC of <0.54 mg glyphosate/L.

Several studies (e.g., Williams *et al*. 2010, Jones *et al*. 2010 and Navarro Martín *et al*. 2014) involved chronic exposure and observations were made on growth and development. The most sensitive parameters were time to metamorphosis and survival, with a NOEC of 0.0006 mg glyphosate/L. In some studies (e.g., Edge and Houlahan 2012, Lanctot *et al*. 2014 and Rissoli Zanelli *et al*. 2016), effects were seen at all treatment levels and no NOEC could be determined.

It should be noted that all amphibian studies tested the aquatic life stages and should therefore be assessed in relation to the available data on aquatic organisms.

In a study on the **reptile species** *Caiman latirostris* (Poletta *et al*. 2011) the test material was applied as a spray solution on the eggshell surface. It seems less likely that eggs of reptile species would be present on the ground surface of treated fields, and therefore this exposure pathway is considered extreme. However, the study showed effects on total length and snout vent length (SVL) at a treatment level of 17.25 g glyphosate/L, which is only slightly higher than the highest recommended concentration of glyphosate in the spray liquid according to the representative GAP (1.35 – 14.4 g a.e./L). Hence, based on the available data, further consideration is needed to exclude the potential risk to reptiles exposed via overspray in the treated field.

2.9.2 Summary of effects on aquatic organisms [section 11.5 of the CLH report]

2.9.2.1 Bioaccumulation [equivalent to section 11.4 of the CLH report template]

Table 69: Summary of relevant information on bioaccumulation

Method	Species	Results	Key or Supportive study[1]	Remarks	Reference
Guideline 72-6	Bluegill Sunfish (*Lepomis macrochirus)*	No reliable BCF value. Indication of	Supportive	Provide evidence that the potential for	[redacted] (1989a), CA 8.2.2.3/001 [redacted]

		low bioaccumulation potential.		bioaccumulation of glyphosate is low. Test item: ^{14}C-glyphosate (N-phosphonomethylglycine-methyl-^{14}C, 99.2% purity)	(1989), CA 8.2.2.3/002
OPPTS 830.7550 OECD Guideline 107 (shake flask method) GLP	Not applicable	Log P_{ow} at 25 °C: -5.39 (pH 5) -6.28 (pH 7) -5.83 (pH 9)	Valid	Test item: Glyphosate acid (99.9% purity)	[redacted] (2020a), Report no. 139K-101 CA 2.7/001
OECD Guideline 107 (shake flask method) GLP	Not applicable	Log P_{ow} at 20 °C: < -3.4	Valid	Test item: Glyphosate acid (99.5% purity)	[redacted] (1990), Report no. 238498 CA 2.7/002
US EPA Guideline CG-1400 Non-GLP	Not applicable	Log P_{ow} at 25 °C: < -3.2	Supportive	Test item: Glyphosate acid (99.9% purity)	[redacted] (1987), Report no. Amended MSL-7241 CA 2.7/003
OECD Guideline 107 OECD Guideline 117 EEC A.8 GLP	Not applicable	Log P_{ow} at 20 °C: -4.16 (pH 4.3-6.2)	Supportive	Test item: Glyphosate IPA-salt (98.1% purity)	[redacted] (1995), Report no. 134224 CA 2.7/004
OECD Guideline 107 GLP	Not applicable	Log P_{ow} at 20 °C: < -3.7 (pH 3.16)	Supportive	Test item: Glyphosate NH_4-salt (97.9% purity)	[redacted] (1993b), Report no. 93/MONO32/0343 CA 2.7/005
OECD Guideline 107 EEC A.8 OPPTS 830.7550 GLP	Not applicable	Log P_{ow} at 20 °C: Shake flask method: < -0.7 (pH 3.16) Estimation method: < -4.0	Valid	Test item: Glyphosate K-salt (91.8% purity)	[redacted] (2012), Report no. 497741 CA 2.7/006
OPPTS 830.7550 OECD Guideline 107 (shake flask	Not applicable	Log P_{ow} at 25 °C: -6.29 (pH 5) -6.26 (pH 7)	Valid	Test item: N-acetyl glyphosate (93% purity)	[redacted] (2020b), Report no. 139K-104 CA 2.7/008

method) GLP		-6.86 (pH 9)			

[1] The status of the studies as reported in Volume B.9 CA are reported here.
"Valid" is used for a regulatory study that meets the validity criteria of the guideline and provides reliable endpoint,
"Supportive" is used for a regulatory study that could not be considered fully valid or fully reliable.

2.9.2.1.1 Estimated bioaccumulation

None

2.9.2.1.2 Measured partition coefficient and bioaccumulation test data

One standard laboratory study on bioconcentration potential performed according to US EPA guideline OPP 72-6 is available [redacted] (1989a), CA 8.2.2.3/001, [redacted] (1989), CA 8.2.2.3/002). In this study, bluegill sunfish (*Lepomis macrochirus*) has been exposed to ^{14}C-glyphosate during 35 days via a flow-through used to maintain a mean measured water concentration of 12 ± 0.7 mg/L. Subsequently, the fish were exposed for 21-days to flowing uncontaminated well water. Six of the control and treated fish were dissected into fillet/edible (body muscle, skin and skeleton) and viscera/non-edible (fins, head and internal organs). Four fish of the control and treated samples per sampling date were used for whole fish analysis. For metabolite characterisation, 12 fish from the control and treatment group from each aquarium were sampled and dissected on days 7, 14, 21 and 28 of the uptake phase.
The daily bioconcentration factor ranged from <0.11 to 0.38 for fillet, from <0.11 to 0.52 for whole fish, and from <0.11 to 0.63 for viscera, respectively. Uptake tissue concentrations of 14C-glyphosate ranged from <1.4 to 4.6 mg a.e./kg for fillet, from <1.3 to 6.2 mg a.e./kg for whole fish, and from <1.3 to 7.6 mg a.e./kg for viscera, respectively. 14C-residue levels were below minimum quantifiable limits until day 21 for fillet and day 7 for whole fish and viscera samples. Radio-analysis on day 21 of the depuration period indicated 35%, 52% and 51% depuration from fillet, whole fish and viscera, respectively. Water samples from treatment days 1, 28, and 35 of the bioconcentration phase were analyzed by HPLC and found to contain 95-97% glyphosate with 1.1-1.9% chromatographing as aminomethylphosphonic acid (AMPA).
The uptake rate constant (k1) of 14C-glyphosate was estimated to be 0.022 ± 0.004 mg a.s./kg in fish/mg/L per day while the depuration rate constant (k2) was of 0.020 ± 0.01/day. The 50% clearance was estimated to be to 35 ± 18 days. All validity criteria according to the OECD guideline 305 were fulfilled. The autors concluded that time to reach 90% of steady state was estimated to be 120 ± 59 days. The bioconcentration factor (BCF) was estimated to be 1.1 ± 0.61. The derived BCF is clearly below the trigger value of 500.

However the test was conducted long before the revised OECD 305 guideline. Since then, experience has shown that biological factors such as growth and fish lipid content can have a strong impact on the results and may need to be taken into account (as recommended in the actual guideline).
RMS highlights that:
- fish lipid content was not measured.
- BCFk s may have not been corrected for growth dilution. (This was not done/reported in this study).

RMS notes that a steady-state could not be observed (concentrations in fish still increasing at the end of the uptake phase). However in view of the very low concentrations levels in fish tissues and the slow increase, RMS is of the opinion that this would have led to an impractically long uptake phase to reach steady-state, so a kinetic approach is preferred in such a case (as it was proposed in this study).
The OECD 305 recommends the use of reference substances of known bioconcentration potential and low metabolism to verify the experimental procedure, when required (e.g. when a laboratory has no previous experience with the test or experimental conditions have been changed). No data was reported in the study report.
Fish loading range of 0.1 g – 1.0g/L is recommended in the guideline. Actual loading was of 1.5 g/L. RMS considers this is acceptable as higher fish-to-water loading rates can be used if it is shown that the required concentration of test substance was maintained within ± 20% limits, and that the concentration of dissolved oxygen did not fall below 60% saturation (these 2 criteria were fulfilled).
Only one concentration was tested. The test guideline was originally designed for non-polar organic substances. For this type of substance, the exposure of fish to a single concentration was expected to be sufficient, as no concentration effects are expected. However the guideline hence states that: "if substances outside this domain are tested, or other indications of possible concentration dependence are known, the test should be run with two or more concentrations. If only one concentration is tested, justification for the use of one concentration should be given". Glyphosate is a polar compound. Therefore, more than one concentrations should have been tested.

The concentration tested was of 12 mg/L. The guideline recommends that "the concentration(s) of the test substance should be selected to be below its chronic effect level or 1% of its acute asymptotic LC50, within an environmentally relevant range…". Based on current knowledge on the substance, RMS considers that the tested concentration is too high.

Overall RMS considers that the study is not robust enough to derive a BCF value. However in view of low lipophilicity of the substance (Log Pow below -4 as shown in the table above), a bioaccumulation study is not required. Even if no BCF can be set, the results are considered informative and provide evidence that the potential for bioaccumulation of glyphosate is low.

2.9.2.2 Acute aquatic hazard [equivalent to section 11.5 of the CLH report template]

Table 70: Summary of relevant information on acute aquatic toxicity

Method	Species	Test material	Results[1]	Key or Supportive study[1]	Remarks	Reference
Fish						
US EPA Guideline, FIFRA subdivision E, section 71-1 GLP	*Lepomis macrochirus*	Glyphosate acid (95.6% purity)	LC_{50} (96 h): >32 mg a.e./L (nom)	Valid with restrictions	pH issue (pH outside the recommended range at all tested concentration. Endpoints set at the highest dose without mortality)	[redacted] (1995a), Report no.: [redacted]5553/B (CA 8.2.1/009)
US EPA Guideline, FIFRA subdivision E, section 71-1 GLP	*Oncorhynchus mykiss*	Glyphosate acid (95.6% purity)	LC_{50} (96 h)> 100 mg a.e./L (nom)	Valid with restrictions	pH induced effects at 180 mg/L	[redacted] (1995b), Report no.: AB0503/D (CA 8.2.1/002)
In accordance with OECD- or equivalent guidelines. Non-GLP	*Cyprinus carpio*	Glyphosate acid	LC_{50} (96 h) = 115 mg a.e./L	Not assessed	No study report available. Data from RAR (2015)	[redacted] (1973), Report no.: 95-00015 (CA 8.2.1/014)
In accordance with OECD- or equivalent guidelines. GLP	*Oncorhynchus mykiss*	Technical glyphosate (> 94% purity)	LC_{50} (96 h) > 100 mg a.e./L (nom)	Not assessed	No study report available. Data from RAR (2015)	[redacted] (1995a), Report no.: 710/21 (CA 8.2.1/003)
OECD Guideline 203 (1983) GLP	*Oncorhynchus mykiss*	Technical glyphosate (purity 98.9%)	LC_{50} (96 h) > 87.7 mg a.e./L (gm)	Valid with restrictions	pH issue (pH of 5.6 at 87.7 mg/L with no mortality)	[redacted] (1990b), Report no.: 271631 (CA 8.2.1/005)
EEC directive	*Lepomis*	Technical glyphosate	LC_{50} (96 h) > 119 mg a.e./L	Supportive	Results can not be	[redacted] (1991),

92/69, Part C.1 OECD Guideline 203 (1992) EPA 540/9-82-024 GLP	*macrochirus*	(purity 98.9%)	(gm)		considered for acute risk assessment as fish are bigger than recommended. pH issue (endpoint set at highest concentration without effets)	Report no.: 271642 (CA 8.2.1/010)
OECD Guideline 203 (1992) JMAFF Testing Guideline for Toxicology Studies, 12 NohSan No. 8147, Guideline 2-7-1 (2000) GLP	*Cyprinus carpio*	Technical glyphosate (purity 95.7%)	LC_{50} (96 h) > 100 mg a.e./L(nom)	Valid	-	[redacted] (2006), Report no.: 2060/015 (CA 8.2.1/013)
OECD Guideline 203 OPPTS 850.1075 GLP	*Oncorhynchus mykiss*	Glyphosate K-salt (47.7% purity)	LC_{50} (96 h) > 1193 mg a.e./L (nom)	Valid	-	[redacted] (2003a), Report no.: 139A-310C (CA 8.2.1/001)
OECD Guideline 203 EEC Directive 92/69 GLP	*Oncorhynchus mykiss*	Glyphosate isopropylamine salt (61.6% purity)	LC_{50} (96 h) = 1001 mg a.e./L (nom)	Valid	-	[redacted] (1993a), Report no.: 80-91-2328-03-93 (CA 8.2.1/004)
OECD Guideline 203 EEC Directive 92/69 GLP	*Leuciscus idus*	Glyphosate isopropylamine salt (61.6% purity)	LC_{50} (96 h) > 2282 mg a.e./L (nom)	Supportive	Not listed in the recommended species of OECD 203. Sensitivity of individuals of that size (5.90 cm) is not known.	[redacted] (1993b), Report no.: 80-91-2328-02-93 (CA 8.2.1/016)
Committee on Methods for Toxicity Tests with Aquatic	*Salmo gairdneri* (*Oncorhynchus mykiss*)	Glyphosate isopropylamine salt (62.49%	LC_{50} (96 h) > 463 mg a.e./L (nom)	Supportive	No analytical test verifications, exposure cannot be	[redacted] (1981a),

Organisms GLP		purity)			confirmed. Other small deviations (pH, fish lengths)	Report no.: 27202 (CA 8.2.1/006)
Committee on Methods for Toxicity Tests with Aquatic Organisms Non-GLP	*Salmo gairdneri* (*Oncorhynchus mykiss*)	Glyphosate technical (83% purity)	LC_{50} (96 h) = 71.4 mg a.e./L (nom)	Supportive	No analytical test verifications, exposure cannot be confirmed	[redacted] (1978), Report no.: [redacted] 78-165 (CA 8.2.1/007)
Committee on Methods for Toxicity Tests with Aquatic Organisms Non-GLP	*Lepomis macrochirus*	Glyphosate technical (technical grade)	100 mg a.e./L<LC_{50} (96 h)< 140 mg a.e./L (nom)	Supportive	No analytical test verifications, exposure cannot be confirmed	[redacted] (1978a), Report no.: [redacted] 78-123 (CA 8.2.1/012)
OECD Guideline 203 (1993) GLP	*Brachydanio rerio* (*Danio rerio*)	Glyphosate technical (95% purity)	LC_{50} (96 h) = 123 mg a.e./L (nom)	Supportive	Insufficient analytical test verifications, exposure cannot be confirmed	[redacted] (2000a), Report no.: [redacted]-D61.47/99 (CA 8.2.1/015)
No guideline followed Non-GLP Literature data	*Poecilia reticulata*	Glyphosate (96% purity)	LC_{50} (96 h): Males: 68.78 mg/L (nom) Females: 70.87 mg/L (nom)	Supportive	No analytical verification. Mature individual used.	Antunes A. M. *et al.* (2017), Report no.: DOI 10.1002/jat.3461, E-ISSN: 1099-1263 (CA 8.2.1/021)
OECD Guideline 203 Non-GLP Literature data	*Cyprinus carpio*	Glyphosate (purity not reported)	LC_{50} (96 h): 6.75 mg/L Cholinesterase activity was inhibited in the fingerlings treated with sublethal concentrations of glyphosate	Supportive	No analytical verification. Control mortality not reported (validity of results questionable).	Gholami *et al.* (2013), Report no.: ISSN: 2008-2525 (CA 8.2.1/022 and CA 8.2.1/023)
No information on followed guideline Non-GLP	*Oncorhynchus mykiss* *Lepomis macrochirus*	Glyphosate acid (purity not reported)	-	Not reliable	No analytics. Dissolved oxygen <60%	Russell, M. (1972), Report no.: [redacted]-72-104 (CA 8.2.1/008)

Committee on Methods for Toxicity Tests with Aquatic Organisms Non-GLP	*Lepomis macrochirus*	Glyphosate isopropylamine salt (62.49% purity)	-	Invalid	No analytical test verifications, exposure cannot be confirmed and some validity criteria not met. Dissolved oxygen < 60%.	[redacted] (1981b), Report no.: 27201 (CA 8.2.1/011)
Aquatic invertebrates						
EPA FIFRA, Subdivision E, Guideline 72-3 ASTM (1989) E724/9-85-012 (OPPTS 850.1055) GLP	*Crassostrea gigas*	Glyphosate acid (95.6% purity)	EC_{50} (48 h): 40 mg a.s./L (nom)	Valid	-	[redacted] (1996a), Report no.: AB0503/G (CA 8.2.4.2/003)
OECD Guideline 202 (1984) EPA FIFRA, Subdivision E, Guideline 72-2 GLP	*Daphnia magna*	Glyphosate acid (95.6% purity)	EC_{50} (48 h) >100 mg a.s./L (nom)	Valid with restrictions	pH issues (endpoints set at doses without mortality/effects)	[redacted] (1996), Report no.: AB0503/C (CA 8.2.4.1/004)
OECD Guideline 202 (1984) OPPTS 850.1010 (1996) EU Directive 67/548/EEC Method C2 (1992) GLP	*Daphnia magna*	Glyphosate K-salt (47.7% purity)	EC_{50} (48 h)= 278 mg a.e./L (am)	Valid	-	[redacted] 2003b, Report no.: 139A-309 (CA 8.2.4.1/001)
OECD Guideline 202 (1984) GLP	*Daphnia magna*	Glyphosate isopropylamine salt (612.7 g/kg salt equivalent)	EC_{50} (48 h) > 471 mg a.e./L (im)	Valid	-	[redacted] (2000a), Report no.: RF-D51.017/00 (CA 8.2.4.1/002)
OECD Guideline	*Daphnia magna*	Glyphosate technical	EC_{50} (48 h)>334 mg a.e./L (im)	Valid with restrictions	pH issues (endpoints set	[redacted]

202 (1984) GLP		(95% purity)			at doses without mortality/effects)	(2000b), Report no.: RF-D51.39/99 (CA 8.2.4.1/003)
OECD Guideline 202 (1984) GLP	*Daphnia magna*	Glyphosate (96% purity)	EC_{50} (48 h) > 100 mg a.e./L (nom)	Valid	-	[redacted] (1995a), Report no.: 141863 (CA 8.2.4.1/006)
OECD Guideline 202 GLP	*Daphnia magna*	Glyphosate isopropylamine salt (61.6% purity)	EC_{50} (48 h): > 45.64 mg a.e./L (nom)	Valid	Limit test	[redacted] (1994), Report no.: 83-91-0737-00-93 (CA 8.2.4.1/007)
OECD Guideline 202 (1984) GLP	*Daphnia magna*	Glyphosate technical (98.9% purity)	EC_{50} (48 h) >62.5 mg a.e./L (nom)	Valid with restrictions	pH issue Endpoints set at doses with no effects due to impact of pH	[redacted] (1990c), Report no.: 272968 (CA 8.2.4.1/009)
EPA FIFRA, Subdivision E, Guideline 72-3 GLP	*Mysidopsis bahia*	Glyphosate acid (95.6% purity)	LC_{50} (96 h) = 80 mg a.s./L (nom)	Valid with restrictions	pH issue (endpoints based with pH of 6 at 100mg/L and 4.5 at 180 mg/L	[redacted] (1996b), Report no.: AB0503/H (CA 8.2.4.2/001)
Data were generated in accordance with OECD- or equivalent guidelines GLP	*Daphnia magna*	Technical glyphosate (> 94% purity)	LC_{50} (48 h) = 40 mg a.e./L	Not assessed but used as critical for Daphnids	Report not available. The endpoint measured in this study is the lowest acute toxicity endpoint for Daphnia magna.	[redacted] (1995b), Report no.: 710/22 (CA 8.2.4.1/005)
Data were generated in accordance with OECD- or equivalent guidelines GLP	*Daphnia magna*	Glyphosate isopropylamine salt (61 – 65% purity)	LC_{50} (48 h): > 1000 mg a.s./L	Not assessed	Report is not available. Data from RAR (2015)	[redacted] (1993c), Report no.: 94-00549 (CA 8.2.4.1/008)
Methods of Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians,	*Daphnia magna*	Glyphosate isopropylamine salt (62.49% purity)	LC_{50} (48 h) = 581 mg a.e./L (nom)	Supportive	No analytical verification of test concentrations	[redacted] (1981), Report no.: 27203 (CA 8.2.4.1/010)

US EPA, Ecol Res. Ser. 660/3-75009 GLP						
Committee on methods for toxicity tests with aquatic organisms Non-GLP	*Daphnia magna*	Technical glyphosate (83.0% purity)	-	Not reliable	No analytical verification of test concentrations. No pH values available.	[redacted] (1978b), Report no.: AB 78-201 (CA 8.2.4.1/011)
Committee on Methods for Toxicity Tests with Aquatic Organisms (1975) Non-GLP	*Mysidopsis bahia*	Several glyphosate related test items (purity not stated). Solid test materials: Glyphosate, BN-78-44 and Glyphosate intermediate, BN-78-45 Liquid test materials: Comp. #1, BN-78-46; Comp. #2, BN-78-47; Comp. #3A, BN-78-48; Comp. #4, BN-78-49 and Comp. 5A.	-	Not reliable	No analytical verification of test concentrations. Only one replicate per treatment. Age of shrimps (6-8 days old). Temperature at 20°C. heterogenous salinity. Low dissolved oxygen.	[redacted] (1978a), Report no.: BP-78-4-032 (CA 8.2.4.2/002)
Woelke, C. E. - "Measurement of Water Quality with the Pacific Oyster Bioassay." Water Quality Criteria, ASTM Spec. Tech. Publ. 416, Am. Soc. Testing	*Crassostrea virginica*	Glyphosate technical (96.7% purity)	-	Not reliable	No analytical verification of test concentrations. No information about dissolve oxygen. pH values not available.	[redacted] (1985), Report no.: BN-73-79 (CA 8.2.4.2/004)

Mats, 1967, p. 112-120 Non-GLP						
No guideline followed Non-GLP Literature data	*Hydra attenuate*	Round up Max (74.4% glyphosate)	LC_{50} (96h) = 18.2 mg a.e./L	Reliable with restrictions	Results insufficiently detailed.	Demetrio P. M. et al., 2012 (CA 8.2.4) Literature data
No guideline followed Non-GLP Literature data	*Crassostrea gigas*	Glyphosate (97% purity)	LC_{50} (48h) > 100 mg a.e./L EC_{50} (48h) = 27.1 mg a.e./L	Reliable	-	Mottier A. et al., 2013 (CA 8.2.8) Literature data
No guideline followed Non-GLP Literature data	*Pomacea canaliculata*	Glyphosate (98% purity)	LC_{50} (96h) = 174.7 mg a.e./L	Reliable with restrictions	No analytical verification	Xu Yanggui et al., 2017 (CA 9) Literature data
Algae						
OECD Guideline 201 (1984) US EPA Guideline 540/09-82-020 (1982) GLP	*Skeletonema costatum*	Glyphosate acid (95.6% purity)	72h ErC50 = 13.5 mg a.e./L (nom) 72h EyC50 = 9.00 mg a.e./L (nom)	Valid	-	[redacted] (1996a), Report no.: AB0503/I (CA 8.2.6.2/006) [redacted] (2020a), Report no.: 110054-007 (updated statistical evaluation) (CA 8.2.6.2/007)
OECD Guideline 201 EEC Directive 92/69 C.3 GLP	*Pseudokirchneriella subcapitata* (*Raphidocelis subcapitata*)	Glyphosate isopropylamine salt (62.66% purity)	96h ErC50 = 23.7 mg a.e./L (mm) 72h EyC50 = 6.85 mg a.e./L (mm) 96h EyC50 = 7.63 mg a.e./L (mm)	Valid	-	[redacted] (2002), Report no.: A-99-02-04 (CA 8.2.6.1/001)
OECD Guideline 201 (1984) US EPA Guideline	*Selenastrum capricornutum* (*Raphidocelis subcapitata*)	Glyphosate acid (95.6% purity)	72h ErC50 = 17.3 mg a.e./L (nom) 72h EyC50 = 16.4 mg a.e./L	Valid	-	[redacted] (1995), Report no.: AB0503/B (CA

540/09-82-020 (1982) GLP			(nom)			8.2.6.1/005) [redacted] (2020b), Report no.: 110054-002 (updated statistical evaluation) (CA 8.2.6.1/006)
US EPA Guideline 123-2 – FIFRA GLP	*Selenastrum capricornutum* (*Raphidocelis subcapitata*)	Glyphosate technical (96.6% purity)	72h ErC50 = 20.1 mg a.e./L (nom) 72h EyC50 = 12.11 mg a.e./L (nom)	Valid	-	[redacted] (1987a), Report no.: 1092-02-1100-1 (CA 8.2.6.1/009) [redacted] (2020c), Report no.: 110054-003 (updated statistical evaluation) (CA 8.2.6.1/010)
Guideline 123-2, U.S. EPA – FIFRA (Growth and Reproduction of Aquatic Plants, Tier 2) GLP	*Anabaena flos-aquae*	Glyphosate technical (96.6% purity)	72h ErC50 = 33.4 mg a.e./L (nom) 72h EyC50 = 16.4 mg a.e./L (nom) **Data gap for 96h endpoints**	Valid	-	[redacted] (1987b), Report no: 1092-02-1100-4 (CA 8.2.6.2/002) [redacted] (2020d), Report no.: 110054-006 (updated statistical evaluation) (CA 8.2.6.2/003)
OECD Guideline 201 (1993) GLP	*Selenastrum caprocornutum* (*Raphidocelis subcapitata*)	Glyphosate technical (white powder, 954.9 g/kg)	72h ErC50 = 469 mg a.e./L 72h EyC50 = 75.9 mg a.e./L	Supportive	No analytical verification of test concentrations throughout the test	[redacted] C.M. (2000b), Report no. RF-D2.44/99 (CA 8.2.6.1/003) [redacted] (2020e), Report no.: 110054-001 (updated statistical evaluation)

						(CA 8.2.6.1/004)
No information on followed guideline GLP	*Desmodesmus subspicatus*	Glyphosate acid (no information on purity)	-	Not assessed Invalid	Not assessed. Report not available. Data from DAR (2001) considered relied upon in RAR (2015)	[redacted] (1995c), Report no.: 710/12 (CA 8.2.6.1/011)
Data were generated in accordance with OECD- or equivalent guidelines GLP	*Desmodesmus subspicatus*	Glyphosate isopropylamin-salt (61-65% purity)	-	Not assessed	Report is not available. Data from DAR (2001) considered relied upon in RAR (2015)	[redacted] (1994), Report no.: 95-00554 (CA 8.2.6.1/012)
OECD Guideline 201 (1984) US EPA Guideline 540/09-82-020 (1982) GLP	*Anabaena flos-aquae*	Glyphosate acid (95.6% purity)	-	Not reliable	Correlation between biomass and optical density cannot be demonstrated.	[redacted] (1996b), Report no.: AB0503/J (CA 8.2.6.2/001)
OECD Guideline 201 (1984) EU Directive 92/69/EEC, Method C.3. (1992) ASTM Standard Guide 1218-90E (1990) GLP	*Pseudokirchneriella subcapitata* (*Raphidocelis subcapitata*)	Glyphosate K-salt (47.7% purity)	-	Invalid	Coefficient of variation for section specific growth rate: > 35%	[redacted] (2003), Report no.: 139A-311 (CA 8.2.6.1/002)
OECD Guideline 201 (1984) EEC Directive 92/69, Part C-3 (1992) ISO International Standard 8692 (1989) GLP	*Pseudokirchneriella subcapitata* (*Raphidocelis subcapitata*)	Glyphosate (96% purity)	72h ErC50 = 54 mg a.e./L (nom) 72h EbC50 = 48 mg a.e./L (nom)	Valid	-	[redacted] (1995b), Report no.: 141896 (CA 8.2.6.1/007)
No information	*Pseudokirchneriella*	Glyphosate (> 94%	-	Not assessed	Report is not available	[redacted] (1995c),

on followed guideline GLP	*subcapitata* (*Raphidocelis subcapitata*)	purity)			Data from DAR (2001) considered relied upon in RAR (2015)	Report no.: R481 (CA 8.2.6.1/008)
OECD Guideline 201 (1984) "Hemmung der Zellvermehrung bei Grünalge *Scenedesmus subspicatus* – Verfahrensvorschlag der ad hoc Arbeitsgruppe des Umweltbundesamtes Berlin" GLP	*Scenedesmus subspicatus* (*Desmodesmus subspicatus*)	Glyphosate isopropylamine-salt (61.6% purity)	-	Invalid	Coefficient of variation for section specific growth rate: > 35%, coefficient of variation of average specific growth rates: >7%	[redacted] (1993d), Report no.: 80-91-2328-01-93 (CA 8.2.6.1/013)
OECD Guideline 201 GLP	*Scenedesmus subspicatus* (*Desmodesmus subspicatus*)	Glyphosate (95% purity)	-	Invalid	Validity criteria could not be checked, no analytical measurements.	[redacted] (1990), Report no.: 1-7-46-90 (CA 8.2.6.1/014)
OECD Guideline 201 (1984) GLP	*Scenedesmus subspicatus* (*Desmodesmus subspicatus*)	Glyphosate (98.7% purity)	-	Invalid	Coefficient of variation for section specific growth rate: > 35%	[redacted] (1990d), Report no.: 250773 (CA 8.2.6.1/015)
OECD Guideline 201 (1984) US EPA Guideline 540/09-82-020 (1982) GLP	*Navicula pelliculosa*	Glyphosate acid (95.6% purity)	-	Invalid	Coefficient of variation for section specific growth rate: > 35%	[redacted] (1996c), Report no.: AB0503/K (CA 8.2.6.2/004)
Guideline 123-2, U.S. EPA – FIFRA (Growth and Reproduction of Aquatic Plants, Tier 2)	*Navicula pelliculosa*	Glyphosate technical (96.6% purity)	**Data gap (EC10, EC20 and EC50 values should be calculated for 72h based on yield and growth rate)**	Valid	RMS considered that validity criteria are met.	[redacted] (1987c), Report no.: 1092-02-1100-2 (CA 8.2.6.2/005)

GLP						
Guideline 123-2, U.S. EPA – FIFRA (Growth and Reproduction of Aquatic Plants, Tier 2) GLP	*Skeletonema costatum*	Glyphosate technical (96.6% purity)	-	Invalid	Biomass increase in control cultures: <16 and coefficient of variation for section specific growth rate: > 35%	[redacted] (1987d), Report no.: 1092-02-1100-3 (CA 8.2.6.2/008)
Environmental Protection Agency: Bioassay procedures for the ocean disposal permit program (1976) Non-GLP	*Skeletonema costatum*	Several glyphosate related test items (purity not stated): Solid test materials (TM): Glyphosate, BN-78-44 and Glyphosate intermediate, BN-78-45 Liquid TM: Comp. #1, BN-78-46; Comp. #2, BN-78-47; Comp. #3A, BN-78-48; Comp. #4, BN-78-49 and Comp. 5A.	-	Invalid	No information on validity criteria. No analytical measurments.	[redacted] (1978b), Report no.: BP-78-4-031 (CA 8.2.6.2/009)
OECD Guideline 201 (1984) Non-GLP	*Nitzschia palea*	Glyphosate technical (96.7% purity)	-	Invalid	validity criteria not met	[redacted] (1996), Report No.: 960606FH (CA 8.2.6.2/010)
Aquatic plants						
Maltby, L., *et al.* (2008): Aquatic Macrophyte Risk Assessment for	*Myriophyllum aquaticum*	Glyphosate acid (85.2% purity)	-	Invalid	coefficient of variation for yield based on measurements of shoot	[redacted] (2012), Report no.: CHE-015/4-80/A (CA 8.2.7/010)

Pesticides, SETAC AMRAP GLP					fresh weight > 35%	
OECD Guideline 221 GLP	*Lemna minor*	Glyphosate isopropylamine salt (97.1% purity)	Frond number 7d ErC50 = 30.3 mg a.e./L (nom) 7d EyC50 = 16.5 mg a.e./L (nom) Dry weight 7d EyC50 = 32.1 mg a.e./L (nom) Phytotoxicity NOEC = 8.65 mg a.e./L (nom)	Valid	Results based on statistical re-evaluation	[redacted] (2002), Report no.: CEMR-1873 (CA 8.2.7/001) [redacted] (2020f), Report no.: 110054-008 (updated statistical evaluation) (CA 8.2.7/002)
Guideline ASTM E 1415-91 (June 1991) GLP	*Lemna gibba*	Glyphosate isopropylamine salt (62% purity)	-	Valid but not reliable	Validity criteria were met but actual exposure questionable	[redacted] (1999), Report no.: 980909FH (CA 8.2.7/003) [redacted] (2020g), Report no.: 110054-009 (updated statistical evaluation) (CA 8.2.7/004)
EPA FIFRA Subdivision J Guideline 123-2 GLP	*Lemna gibba*	Glyphosate acid (95.6% purity)	Frond number 7d ErC50 = 36.0 mg a.e./L (nom) 7d EyC50 = 24.0 mg a.e./L (nom) Phytotoxicity NOEC = 1.5 mg a.e./L (nom)	Valid	Results based on statistical re-evaluation	[redacted] (1996d), Report no.: AB0503/L (CA 8.2.7/005) [redacted] (2020h), Report no.: 110054-010 (updated statistical evaluation) (CA 8.2.7/006)
Guideline 123-2, U.S. EPA –	*Lemna gibba*	Glyphosate (96.6%	Frond number 7d ErC50 > 49.4 mg a.e./L (mm)	Valid	Results based on statistical	[redacted] (1987e), Report no.:

FIFRA (Growth and Reproduction of Aquatic Plants, Tier 2) GLP		purity)	7d EyC50 = 25 mg a.e./L (mm) Phytotoxicity Not recorded		re-evaluation	1092-02-1100-5 (CA 8.2.7/007) [redacted] (2020i), Report no.: 110054-010 (updated statistical evaluation) (CA 8.2.7/008)
OECD Guideline 221 Non-GLP	*Spirodela polyrhiza*	Glyphosate (96.8% purity)	EC_{50} (7 d): 12.817 mg/L (nom)	Relevant but reliability not assignable (data gap : provide an English certified translation) .	Literature article Report in chinese. No translation available. no analytical test verifications	Yanhui *et al.* (2015), Document no.: ISSN: 1002-5480 (CA 8.2.7/013)
No information on followed guideline Non-GLP	*Lemna gibba*	Glyphosate technical (> 94% purity)	No information on results available.	Invalid	Report not available. Study reported as not acceptable in DAR (2001)	[redacted] (1987f), Report no.: XX-88-416 (CA 8.2.7/009)
Other aquatic organisms						
OECD Guideline 241 ASTM E1439-12 Non-GLP	*Physalaemus cuvieri* and *Hypsiboas pardalis*	Glyphosate (99.2% purity)	*Physalaemus cuvieri*: LC_{50} (96 h): 115 mg a.s./L (nom) *Hypsiboas pardalis*: LC_{50} (96 h): 106 mg a.s./L (nom)	Reliable with restrictions .	Literature article Validity criteria not reported. No analytical test item verifications	Daam, M. A *et al*. (2019), Document no.: doi.org/10.1007/s10646-019-02067-5, ISSN: 0963-9292 (CA 8.2.8/001)

am: arithmetic mean measured, gm: geometric mean measured, im: initial measured, nom: nominal, n.d.: not determined
[1] The status of the studies as reported in Volume 3 B.9 CA are reported here.
"Valid" is used for a regulatory study that meets the validity criteria of the guideline and provides reliable endpoint,
"Valid with restriction" is used for a regulatory study that meet the validity criteria of the guideline but for which issue such as potential influence of pH on the results gave been identified,
"Valid but not reliable" or "not reliable" is used for a regulatory study that is valid but the endpoint is not considered reliable
"Supportive" is used for a regulatory study that could not be considered fully valid or fully reliable.
"Invalid" is used for a regulatory study that does not meet the validity criteria
"Reliable" is used for literature study considered reliable
"Reliable with restriction" is used for literature study for which some uncertainties are seen that lowered their reliability status
"Not assessed" is used for a study that has been assessed in the previous DAR/RAR but for which no study report has been made available (see volume 3 B.9 for details)

2.9.2.2.1 Acute (short-term) toxicity to fish

Numerous studies testing the acute toxicity of glyphosate, glyphosate acid and glyphosate salts on a range of fish species are available and summarized in the following section. Endpoints range from 96-hour LC_{50} >32 mg a.e./L to > 2282 mg a.e./L.

Valid studies

The lowest valid effect value was derived in a 96-hour static toxicity study testing the acute effects of glyphosate acid to bluegill sunfish (*Lepomis macrochirus*) at nominal test concentrations between 10 and 180 mg a.s./L ([redacted] 1995a). The overall mean measured concentrations of glyphosate acid in the treatment groups ranged from 98 to 100% of nominal concentrations. By 96 hours, there was 90% mortality in the 56 mg a.s./L treatment and 100% mortality in the 100 and 180 mg a.s./L treatments. All validity criteria according to the OECD guideline 203 were fulfilled. As pH values were below the range required to maintain the health of the organisms tested (here 6.5-8.5) recommended by OECD guideline for the concentrations showing mortality, it was proposed to set the endpoint at the highest tested dose that did not induce mortality with a tolerable pH value. The 96-hour LC_{50} value for bluegill sunfish (*L. macrochirus*) exposed to glyphosate acid was >32 mg a.s./L (nominal concentration).

The acute effects of glyphosate acid to rainbow trout (*Oncorhynchus mykiss*) was evaluated in a 96-hour static toxicity test conducted at nominal test concentrations of 32, 56, 100, 180, 320 and 560 mg glyphosate acid/L ([redacted] 1995b). The overall mean measured concentrations of glyphosate acid in the treatment groups ranged from 91 to 100% of nominal concentrations. All validity criteria according to the guideline OECD guideline 203 were fulfilled. As pH values were below the range required to maintain the health of the organisms tested (here 6.5-8.5) recommended by OECD guideline for the concentrations showing mortality, it was proposed to set the endpoint at the highest tested dose that did not induce mortality with a tolerable pH value. The 96-hour LC_{50} value for rainbow trout exposed to glyphosate acid was determined to be >100 mg a.s./L (nominal).

The effects of glyphosate technical on rainbow trout (*O. mykiss*) were evaluated further in a 96-hour static toxicity test according to OECD 203 ([redacted] 1990b). Groups of ten fish each were exposed to glyphosate technical at concentrations of 95, 171, 309, 556, and 1000 mg a.s./L (nominal concentrations), corresponding to 87.7, 135, 188, 497 and 1019 mg a.e/L based on geometric mean measured concentrations.
All validity criteria according to the guideline OECD guideline 203 were fulfilled. As pH values were below the range required to maintain the health of the organisms tested (here 6.5-8.5) recommended by OECD guideline for the concentrations showing mortality, it was proposed to set the endpoint at the highest tested dose that did not induce mortality with a tolerable pH value. The 96-h LC_{50} for *O. mykiss* exposed to glyphosate technical was estimated to be > 87.7 mg a.e./L based on (geometric) mean measured concentration.

The effects of technical glyphosate to common carp (*Cyprinus carpio*) were evaluated in a 96-hour semi-static toxicity test (48 hour renewal of test media) conducted as limit test at a nominal test concentration of 100 mg a.e./L ([redacted] 2006). No mortality or sub-lethal effects to common carp were observed, when exposed to glyphosate technical at the nominal concentration of 100 mg a.e./L. All validity criteria according to the OECD guideline 203 were fulfilled. Measured concentrations ranged from 90% to 98% of nominal test concentrations throughout the test. The 96-hour LC_{50} value for common carp exposed to technical glyphosate was determined to be > 100 mg a.e./L, the highest concentration tested.

In a another valid study, the toxicity of glyphosate potassium (K) salt on rainbow trout (*O. mykiss*) was determined in a 96-hour static toxicity test conducted at nominal test concentrations between 156 and 2500 mg glyphosate K-salt/L, corresponding to 74.4, 149, 298, 596 and 1193 mg a.e./L ([redacted] 2003). All validity criteria according to the guideline OECD 203 were fulfilled. Measured concentrations ranged from 99.8% to 109% of nominal test concentrations throughout the test. The 96-hour LC_{50} for rainbow trout (*O. mykiss*) exposed to glyphosate K-salt was determined to be > 2500 mg a.s./L, equivalent to >1193 mg a.e./L (nominal).

[redacted] (1993a) assessed the effects of glyphosate isopropylamine (IPA) salt on rainbow trout (*O. mykiss*) in a 96-hour static toxicity test. The toxicity test was performed using nominal concentrations of 107, 235, 517, 1136 and 2500 mg test item/L, corresponding to 65.9, 145, 318, 700 and 1540 mg glyphosate IPA salt/L (mg a.s./L) or 48.8, 107, 236, 519 and 1141 mg a.e./L. All validity criteria according to the guideline OECD 203 were fulfilled. Some deviations from the recommendations of the guideline have been noted (pH, t°C, fish size, fish loading…). This does not seem to have affected the outcome of the study. The study is valid according to validity criteria. Analytic verifications are available for only three concentrations (out of 5) but appear consistent between them. Measured concentrations ranged from 85.1% to 102.7% of nominal test concentration throughout the test. The 96-hour LC_{50} was determined to be 2192 mg test item/L, corresponding to 1350 mg a.s./L or 1001 mg a.e./L (nominal).

Supportive studies

Furthermore, several studies considered as supportive are available. These studies assessed the effects of glyphosate and glyphosate salts on a variety of fish species.

In a static acute fish toxicity test ([redacted] 1981a), the 96-hour LC_{50} for rainbow trout (*Salmo gairdneri*) exposed to glyphosate IPA salt was determined to be >1000 mg test item/L (nominal), corresponding to > 625 mg a.s./L or 463 mg a.e./L (nominal). No analytical verification of test concentrations are available in this study. [redacted] (1978a) derived a 96-hour LC_{50} of 71.4 mg a.e. /L for rainbow trout (Salmo gairdneri) in a non-GLP study. Since no analytical test verifications were performed, exposure to the test item cannot be confirmed and the study is only considered supportive. In [redacted] (1978b), the LC_{50} for *Lepomis macrochirus* was estimated between 100 and 140 mg a.e./L in a 96-hour static toxicity test without analytical measurements of the tested glyphosate technical. This estimation is similar to a study performed by [redacted] (2000a) with *Danio rerio* and glyphosate technical. The derived 96-hour LC_{50} was 123 mg a.e./L. However, exposure to the test item cannot be confirmed due to insufficient analytical test verifications. [redacted] (1991) derived an LC_{50} >119 mg a.e./L in a 96-hour static toxicity test with *Lepomis macrochirus* but fish were too big compared to recommended fish size in OECD 203.

In a further supportive study by [redacted] (1993b), effects of glyphosate IPA salt on golden orfe (*Leuciscus idus*) were evaluated. The 96-hour LC_{50} was determined to be > 5000 mg test item/L, corresponding to 3080 mg glyphosate IPA salt/L (mg a.s./L) or 2282 mg a.e./L (nominal). As this species is not listed in the recommended species of OECD 203, the sensitivity of the individuals is not known.

Additionally, two literature articles investigating the short-term toxicity of glyphosate to fish are considered reliable with restrictions (summary and assessment of literature studies could be found in the appendix of Volume 3 CA B.9 related to literature data on ecotoxicology).
In the study by Antunes A. M. *et al.* (2017), the ecotoxicity of glyphosate was investigated in guppies (*Poecilia reticulata*). The acute 96-hour LC_{50} of glyphosate obtained for male and female guppies were 68.78 mg/L and 70.87 mg/L, respectively.
Gholami *et al.* (2013) determined a 96-hour LC_{50} of 6.75 mg/L for common carp fingerlings by static exposure to glyphosate at five test concentrations between 5.5 and 9.5 mg/L.

Invalid studies
Further LC_{50} values were derived in two non-GLP studies considered as invalid for assessment. The values were 78 mg a.e./L for the bluegill and 38 mg a.e. for the rainbow trout from a study performed with glyphosate acid ([redacted] 1972) and 463 mg a.e./L for *Lepomis macrochirus* exposed to glyphosate IPA salt ([redacted] 1981b), respectively.
In [redacted] 1973 (non-GLP), the LC_{50} of technical glyphosate to *Cyprinus carpio* was determined to be 115 mg a.e./L. [redacted] (1995a) tested the toxicity of technical glyphosate to *O mykiss* in a 96 hours static test. The derived LC_{50} was >100 mg a.e./L. For both studies, the full study reports were not available. However, these data were provided in the Monograph 2001 and relied upon in the previous evaluation, RAR (2015).

In conclusion the most sensitive acute endpoint of glyphosate towards fish resulted in the study by Kent *et al.* (1995a) with a 96-hour LC_{50} >32 mg a.e./L (bluegill sunfish, *Lepomis macrochirus)*.

2.9.2.2.2 Acute (short-term) toxicity to aquatic invertebrates

Numerous studies are available investigating the acute toxicity of glyphosate, glyphosate acid and its salts to aquatic invertebrates. Results of the available studies are summarized in the following section; endpoints range from 48-hour EC_{50} at 40 mg a.e./L to > 471 mg a.e./L.

Valid studies
The lowest effect value was derived in a 48-hour static toxicity study assessing the effects of glyphosate acid to the pacific oyster (*Crassostrea gigas*) according to OPPTS 850.1055 at nominal test concentrations 3.2, 5.6, 10, 18, 32, 56, 100 and 180 mg/L ([redacted] 1996a). To each test vessel 0.535 mL inoculum containing 22 embryos/mL was added. Mean measured concentrations ranged from 91 to 100% of nominal concentrations. No significant reduction of development up to nominal concentrations of 32 mg test item/L was found. The 48-hour LC_{50} for *C. gigas* was 40 mg a.s./L (nominal). All validity criteria were fulfilled.

A further study assessed the effects of glyphosate acid on *Daphnia magna* in a 48-hour static toxicity test ([redacted] 1996). Twenty *Daphnia* per concentration were exposed to nominal 10, 18, 32, 56, 100 and 180 mg/L of glyphosate acid and a pH-adjusted 1000 mg/L test concentration of glyphosate acid. The analysed test

concentrations ranged between 85 and 100% of the nominal values. All validity criteria according to the OECD guideline 202 were fulfilled. As pH values were below the range required to maintain the health of the organisms tested (here 6-9) recommended by OECD guideline for the concentrations showing mortality, it was proposed to set the endpoint at the highest tested dose that did not induce mortality with a tolerable pH value.Therefore, the 48-hour EC_{50} is >100 mg a.s./L.

In another valid study performed by [redacted] (2003b), the effects of Glyphosate K-salt on *D. magna* were evaluated in a 48-hour static toxicity test performed using nominal concentrations of 74, 149, 298, 596 and 1193 mg a.e./L. Mean overall measured concentrations of acid equivalents ranged between 100 and 106% of the nominal values. Based on lethargy, RAR 2015 recalculated the 48-hour EC_{50} to be 278 mg a.e./L (arithmetic mean measured).

The effects of glyphosate IPA-salt on *D. magna* were evaluated in a 48-hour static toxicity test by [redacted] (2000a). *Daphnia* were exposed to nominal concentrations of 100, 180, 320, 560, and 1000 mg a.s./L. The concentration of the test substance in the test media was measured only at the beginning of the study. Based on the other acute toxicity tests on daphnia for which analytical verifications are available, it could be confirmed that glyphosate is satisfactorily maintained in 48h daphnid test. Thus RMS considers that it is likely that the targeted concentrations were sufficiently maintained during the test duration (48h). The analysed test concentrations ranged between 75.90 and 139.70% of the nominal values. Therefore, the results reported are related to initial measured concentrations of the test item. The 48-h EC_{50} was > 1397 mg test item/L (corresponding to >471 mg a.e./L). All validity criteria according to the OECD guideline 202 were fulfilled.

[redacted](2000b) assessed the effects of glyphosate technical on *D. magna* in a 48-hour static toxicity test according to OECD guideline 202. Nominal concentrations in a range of 100 and 1000 mg a.e./L were tested. The analysed test concentrations ranged between 99.75 and 106.61% of the nominal values. As pH values were below the range required to maintain the health of the organisms tested (here 6-9) recommended by OECD guideline for the concentrations showing mortality, it was proposed to set the endpoint at the highest tested dose that did not induce mortality with a tolerable pH value. A 48-hour EC_{50} of >334 mg a.e./L was determined based on initial measured concentrations. All validity criteria according to the OECD guideline 202 were fulfilled.

[redacted] (1995a) derived a 48-hour EC_{50} of >100 mg a.e./L in a 48-hour static toxicity test with *D. magna*. At or below the highest nominal test concentration of 100 mg glyphosate/L, no immobilisation was observed. Measured concentrations ranged from 109% to 112% of nominal test concentrations throughout the test. All validity criteria according to OECD guideline 202 were fulfilled.

In a 48-hour static toxicity test performed as limit test using only one test concentration of 100 mg test item/L nominal, equivalent to 61.6 mg glyphosate IPA-salt/L or 45.64 mg glyphosate/L none of the *D. magna* was found to be immobilised ([redacted] 1994). The 48-hour EC_{50} was determined to be >100 mg test item/L, equivalent to 61.6 mg glyphosate IPA-salt/L or 45.6 mg a.e./L (nominal). Measured concentrations ranged from 103.2% to 103.7% of nominal test concentrations throughout the test. All validity criteria according to OECD guideline 202 were fulfilled.

[redacted] (1990c) evaluated the effects of glyphosate technical on *D. magna* in a 48-hour static toxicity test using five nominal concentrations, 62.5, 125, 250, 500 and 1000 mg test item/L. The daphnids were exposed to a mean concentration of 86.1% of nominal concentration. The immobilisation of *D. magna* increased with increasing test concentration, while at increasing test concentrations, the pH decreases beyond the pH range of 6 - 9 as given in the guideline. As pH values were below the range required to maintain the health of the organisms tested (here 6-9) recommended by OECD guideline for the concentrations showing mortality, it was proposed to set the endpoint at the highest tested dose that did not induce mortality with a tolerable pH value. A 48-hour EC_{50} of >62.5 mg a.e./L. Measured concentrations ranged from 80.9% to 89.1% of nominal test concentration (62.5 mg/L) throughout the test. The validity criteria according to the OECD guideline 202 were fulfilled.

Furthermore, [redacted] (1996b) evaluated effects of glyphosate acid on mysid shrimp *Mysidopsis bahia* in a 96-hour static toxicity test. The shrimps were exposed to nominal 3.2, 5.6, 10, 18, 32, 56, 100 and 180 mg a.s./L, together with pH adjusted 320, 560, and 1000 mg a.s./L. At the lowest test concentration of 3.2 mg/L, analytical results indicated that an error might have occurred during the preparation of the test solution, leading to a value 150% of nominal. Since this was a no effect concentration, and several higher concentrations gave no indication of toxicity, this data point was excluded from all calculations. At 5.6 and 10 mg/L, analytical results indicated recovery percentages below 80% of nominal. Since these low recoveries were only observed at one intermediate time point of this static study (with concentrations at end of study > 80%) and that no effects were seen at these

concentrations, this deviation has no impact on the outcome of the test. The 96-h LC_{50} for *M. bahia* exposed to glyphosate acid was 80 mg a.s./L based on nominal concentration. In test systems dosed with pH adjusted glyphosate acid, no mortalities at a nominal concentration of 560 mg a.s./L and 50% mortality at 1000 mg a.s./L indicated that the 96-hour LC_{50} of 80 mg a.s./L was caused by the low pH of the unneutralised glyphosate acid test solutions.

Supportive studies

In addition, studies considered as supportive for the risk assessment of glyphosate are available.

[redacted] (1995b), assessed the toxicity of glyphosate technical to *D. magna* and derived a 48-hour EC_{50} of 40 mg a.s./L. The study is considered as supportive because the report is not available and therefore it cannot be concluded on the study validity according to the current guideline requirements. However, it was used in the risk assessment as the endpoint measured in this study is the lowest acute toxicity endpoint for Daphnia magna. The study report was neither available for a study performed by [redacted] (1993c). In this acute toxicity test with glyphosate IPA-salt and *D. magna* a 48-hour EC_{50} of >1000 mg a.s./L was derived.

In a further supportive study by [redacted] (1981) the 48-h EC_{50} for *D. magna* exposed to glyphosate IPA-salt was determined to be 930 mg test item/L equivalent to 581 mg a.e./L. No chemical analysis was performed to confirm glyphosate concentrations in the test media.

In a study considered not reliable, the 48-hour EC_{50} for *D. magna* exposed to technical glyphosate was calculated to be 780 mg test item/L, equivalent to 647.4 mg a.e./L (nominal; [redacted] 1978). All validity criteria according to the guideline OECD guideline 202 were fulfilled. However no analytical verification of test concentrations was performed and no pH values were available.

The effects of glyphosate technical on the normal embryonic development of the Atlantic oyster (*Crassostrea virginica*) were evaluated in a 48-hour static toxicity test ([redacted] 1985). The test was performed using nominal concentrations in the range of 0.75 and 10 mg glyphosate/L. No adverse effects of glyphosate on the normal embryonic development of oysters were observed up to the highest concentration tested. The EC_{50} was therefore determined to be > 10 mg glyphosate/L. Since no analytical verification was performed, dissolved oxygen nor pH values were available, the study is considered to be not reliable.

Demetrio P. M. et al., 2012, assessed the lethal effects of glyphosate and glyphosate formulation Roundup® Max on the Hydra attenuate (96 hours). This study indicates relative sensitivity of this species. (96h-LC50 glyphosate a.i =18.2 mg a.i/L, 96h-LC50 RoundupMax® =21.8 mg a.i/L (considered less relevant by RMS due to the different formulation tested)). The study seems well conducted (despite the absence of specific guideline) however there are no details of biological observations reported in the paper. Thus, the observed mortality and the LC50 calculation cannot be confirmed by RMS. This study is reliable with restrictions.

Mottier A. et al., 2013, assessed the toxicity of glyphosate, AMPA and two commercial formulations, Roundup Express® (REX) and Roundup Allées et Terrasses® (RAT), containing glyphosate as the active ingredient, on the early life stages of the Pacific oyster, Crassostrea gigas (marine species). This is an embryotoxicity bioassay. The EC50 values were 27.1 and 46.1 mg/Lfor glyphosate and AMPA, respectively for the parameter development (Abnormality rates in D-shaped larvae, measured concentrations). The EC10 values were 13.457 and 10.299 mg/L for glyphosate and AMPA.

Xu Yanggui et al., 2017, investigated the effect of glyphosate an alien invasive species, the golden apple snail Pomacea canaliculata in China. Snails were kept in the water. An endpoint for mortality was set : 96h LC50 = 174.7 mg/L (95% CI: 174.7-175.6). Long-term exposures to glyphosate at 20 and 120 mg/L caused inhibition of food intake, limitation of growth performance and alterations in metabolic profiles of the snail. Glyphosate at 2 mg/L benefited growth performance in P. canaliculata. The study is considered reliable with restrictions (no analytical verification).

In conclusion the most sensitive acute endpoint of glyphosate towards aquatic invertebrates resulted in the study by [redacted] (1995a) with a 48-hour EC_{50} of 40 mg a.s./L (pacific oyster, *Crassostrea gigas*).

2.9.2.2.3 Acute (short-term) toxicity to algae or aquatic plants

The effects of the active substance glyphosate, glyphosate acid and its salts on aquatic algae and plants have been tested in several studies with a variety of freshwater and marine algal species as well as freshwater macrophytes.

Acute results are discussed in Chapter **Fout! Verwijzingsbron niet gevonden.** 'Chronic toxicity to algae or aquatic plants'.

2.9.2.2.4 Acute (short-term) toxicity to other aquatic organisms

An overview of the data on amphibians are presented under Vol 1, section 2.9.1.6 Literature data.

2.9.2.2.5 Acute (short-term) toxicity data for the metabolites and the representative formulation

Table 2.9.2.2.5-1: Studies on acute toxicity to aquatic organisms of the metabolites of glyphosate and the representative formulation MON 52276

Annex point	Study	Substance(s)	Test species	Study type	LC_{50} (mg a.e./L)	Status /Remark
CA 8.2.1/017	[redacted] 1998	AMPA	*Oncorhynchus mykiss*	Acute / static	> 100 (nom)	Valid -
CA 8.2.1/018	Anonymous, 1994	AMPA	*Oncorhynchus mykiss*	Acute / static	>180	Not assessed.No study report available. Study of DAR 2001. Not mentioned in RAR (2015)
CA 8.2.1/019	[redacted] 1991	AMPA	*Oncorhynchus mykiss*	Acute / static	520	Invalid analytical results not found in separate report ML-90-403, no validation data for analytical method was available (see Volume 3 (AS) B.5).
CA 8.2.1/020	[redacted] 1993	AMPA	*Oncorhynchus mykiss*	Acute / static	> 180 (nom)	Valid
CA 8.2.1/021 Literature data	Antunes et al., 2017.	AMPA	*Poecilia reticulata*	Acute / static	180 mg/L (male) 164.3 mg/L (female	Supportive No analytical verification. Mature individual used.
CA 8.2.4.1/012	[redacted] 1998	AMPA	*Daphnia magna*	48 hour acute static	> 100 nom	Valid

Annex point	Study	Substance(s)	Test species	Study type	LC_{50} (mg a.e./L)	Status /Remark
CA 8.2.4.1/013	[redacted] 1994	AMPA	*Daphnia magna*	48 hour acute static	>180 nom	Valid
CA 8.2.4.1/014	[redacted] 1991	AMPA	*Daphnia magna*	48 hour acute static	690 nom	Supportive Analytical separate report (ML-90-403/EHL-90187-Daphnia) with no results reported on analytics. No validation data for analytical method was available (see Volume 3 (AS) B.5).
CA 8.2.8 Literature data	Mottier A. et al., 2013	AMPA	*Crassostrea gigas*	Acute, 48h	>100 (LC50)	Reliable
				EC50 = 46.1 (Abnormality rates in D-shaped larvae,). The EC10 = 10.299 mg/L		
CA 8.2.4.1/015	[redacted] 2011	HMPA	*Daphnia magna*	48 hour acute static	>100 nom	Valid
CA 8.2.6.1/016 CA 8.2.6.1/017	[redacted] 1998 [redacted] 2020	AMPA	*Pseudokirchneriella subcapitata* *(Raphidocelis subcapitata)*	72 h algae inhibition	72h ErC50 = 191 mg AMPA/L (nom) 72h EyC50 = 110 mg AMPA/L (nom)	valid
CA 8.2.6.1/018	[redacted] 1994	AMPA	*Scenedesmus subspicatus* *(Desmodesmus subspicatus)*	72 h algae inhibition	-	invalid
CA 8.2.6.1/019 CA 8.2.6.1/020	[redacted] 2011 [redacted] 2020	HMPA	*Pseudokirchneriella subcapitata* *(Raphidocelis subcapitata)*	72 h algae inhibition	72h ErC50 >120 mg HMPA/L (nom) 72h EyC50 > 120 mg HMPA/L (nom)	valid
CA 8.2.7/011	[redacted] 2012	AMPA	*Myriophyllum aquaticum*	14-d static	Shoot length 14d ErC50 > 94.6 mg AMPA/L (mm)	valid

Annex point	Study	Substance(s)	Test species	Study type	LC_{50} (mg a.e./L)	Status /Remark
					14d EyC50 > 94.6 mg AMPA/L (mm) <u>Shoot fresh weight</u> 14d ErC50 > 94.6 mg AMPA/L (mm) 14d EyC50 = 70.8 mg AMPA/L (mm) <u>Shoot dry weight</u> 14d ErC50 = 72 mg AMPA/L (mm) 14d EyC50 = 63.2 mg AMPA/L (mm) <u>Root length</u> 14d ErC50 > 94.6 mg AMPA/L (mm) 14d EyC50 = 31.1 mg AMPA/L (mm)	
CA 8.2.7/012	[redacted] 2011	HMPA	*Lemna gibba*	7-d, semi-static	<u>Frond number/biomass dry weight</u> 7d ErC50 > 123 mg HMPA/L (nom) 7d EyC50 > 123 mg HMPA/L (nom)	Valid

Annex point	Study	Substance(s)	Test species	Study type	Endpoints	Status
CP 10.2.1/001	[redacted] 1992	MON-52276	*Oncorhynchus mykiss*	Acute, 96 h, static	LC_{50} > 989 mg MON 52276/L >306 mg a.e./L (am)	Valid
CP 10.2.1/002	[redacted] 1992	MON-52276	*Cyprinus carpio*	Acute, 96 h, static	LC_{50} > 895 mg MON 52276/L > 277 mg a.e./L (am)	Valid

CP 10.2.1/003	[redacted] 1992	MON-52276	*Daphnia magna*	Acute, 48 h flow-through	EC_{50} = 676 mg MON 52276/L =209 mg a.e./L (am)	Valid
CP 10.2.1/004	[redacted] 1992	MON-52276	*Selenastrum capricornutum (Raphidocelis subcapitata)*	Acute, static	- **Data gap: Toxicity study on alga with the representative formulation**	Valid but not reliable*
CP 10.2.1/005	[redacted] 2002	MON 52276	*Lemna gibba*	Acute, semi-static	<u>Frond number</u> 7d-ErC50 > 150 mg MON 52276/L (>46.35 mg a.e./L) (nom) 7d-NOErC = 19.1 mg MON 52276/L (5.90 mg a.e./L). 7d-EyC50 = 66.58 mg MON 52276/L (20.57 mg a.e./L) (nom) 7d-NOEyC = 19.1 mg MON 52276/L (5.90 mg a.e./L). <u>Dry weight</u> 7d-EyC50 = 118.16 mg MON 52276/L (36.51 mg a.e./L) 7d-NOEyC = 19.1 mg MON 52276/L (5.90 mg a.e./L). **Data gap** **(EC10, EC20 and EC50 values should be calculated based on growth rate for dry weight)**	Valid
CP 10.2.1/006	[redacted] 2012	MON 52276	*Myriophyllum aquaticum*	Acute, static	**<u>Shoot length</u>** 14d NOErC = 3.59 mg MON52276/L (1.1 mg a.e./L) (mm) 14d ErC10 = 3.46 mg MON52276/L (1.07 mg a.e./L) (mm) 14d ErC20 = 12.42 mg MON52276/L (3.81 mg a.e./L) (mm) 14d ErC50 = 139.5 mg MON52276/L (42.79 mg a.e./L) (mm) 14d NOEyC = 3.59 mg MON52276/L (1.1 mg a.e./L) (mm) 14d EyC10 = 1.39 mg MON52276/L (0.43 mg a.e./L) (mm) 14d EyC20 = 4.60 mg MON52276/L (1.41 mg a.e./L) (mm) 14d EyC50 = 43.81 mg MON52276/L (13.44 mg a.e./L) (mm)	Valid

					<u>Shoot fresh weight</u> 14d NOErC < 0.98 mg MON52276/L (<0.3 mg a.e./L) (mm) 14d ErC10 = 0.518 mg MON52276/L (0.16 mg a.e./L) (mm) 14d ErC20 = 2.15 mg MON52276/L (0.66 mg a.e./L) (mm) 14d ErC50 = 33.67 mg MON52276/L (10.33 mg a.e./L) (mm) 14d NOEyC < 0.98 mg MON52276/L (<0.3 mg a.e./L) (mm) 14d EyC10 = 0.36 mg MON52276/L (0.11 mg a.e./L) (mm) 14d EyC20 = 1.27 mg MON52276/L (0.39 mg a.e./L) (mm) 14d EyC50 = 14.47 mg MON52276/L mg a.e./L) (mm) <u>Shoot dry weight</u> 14d ErC10 = 1.42 mg MON52276/L (0.44 mg a.e./L) (mm) 14d ErC20 = 10.52 mg MON52276/L (3.23 mg a.e./L) (mm) 14d ErC50 = 467.1 mg MON52276/L (143.3 mg a.e./L) (mm) 14d EyC50 > 473 mg MON52276/L (>145 mg a.e./L) (mm) EyC10 <0.98 mg MON52276/L (< 0.3 mg a.e./L) (mm) <u>Root length</u> 14d NOErC = 3.59 mg MON52276/L (1.1 mg a.e./L) (mm) 14d ErC10 = 7.22 mg MON52276/L (2.23 mg a.e./L) (mm)	

					14d ErC20 = 20.63 mg MON52276/L (6.33 mg a.e./L) (mm) 14d ErC50 = 151.6 mg MON52276/L (46.5 mg a.e./L) (mm) 14d NOEyC = 3.59 mg MON52276/L (1.1 mg a.e./L) (mm) 14d EyC10 = 3.40 mg MON52276/L (1.05 mg a.e./L) (mm) 14d EyC20 = 6.16 mg MON52276/L (1.89 mg a.e./L) (mm) 14d EyC50 = 19.04 mg MON52276/L (5.84 mg a.e./L) (mm)	

* The product study on algae ([redacted] 1992) was performed according to the valid test guideline at the time of conduct. In the last Annex I renewal, this study was evaluated and considered acceptable for use in risk assessment. See study summary for more details (CP 10.2.1/004).

Concerning the product study performed on *Lemna gibba* ([redacted] 2002), the study was conducted according to the draft OECD 221 test guideline from October 2000. The currently adopted test guideline is largely unchanged from the draft guideline. In the last Annex I renewal, this study was evaluated and considered as supportive for use in risk assessment. See study summary for more details (CP 10.2.1/005).

2.9.2.3 Long-term aquatic hazard [equivalent to section 11.6 of the CLH report template]

Table 71: Summary of relevant information on chronic aquatic toxicity

Method	Species	Test material	Results[1]	Relevant study	Remarks	Reference
Fish						
OECD Guideline 210 (1992) GLP	*Oncorhynchus mykiss*	Glyphosate acid (96.03% purity)	-	Valid but not reliable	Validity criteria were met but not to be used as critical endpoint as only 2 replicates were used	[redacted] (2010), Report no.: 1005.029.321 (CA 8.2.2.1/001)
EPA: Recommended bioassay procedures for fathead minnow (*Pimephales promelas*, Rafinesque) chronic tests. By the Bioassay Committee,	*Pimephales promelas*	Glyphosate (87.3% purity)	NOEC (225 d): = 25.7 mg a.e./L (mm)	Supportive)	Analytical method validation not available. Indirect quantification of glyphosate. Some parameters show high	Anonymous (1975), Report no.: BN-75-129 (CA 8.2.2.2/001)

National Water Quality Laboratory, Duluth, USA (1971) Non-GLP					variability. Statistics not reliable.	
OECD Guideline 229 (2009) GLP	*Pimephales promelas*	Glyphosate acid (purity: 85.14% before drying, 95.93% glyphosate acid, dried)	NOEC (21 d); 33 mg a.s./L (mm)	Valid	-	[redacted] (2012a), Report no.: 707A-102A (CA 8.2.3/001)
IBAMA 1990: Manual de testes para avaliacao da ecotoxicidade de agentes quimicos GLP	*Brachydanio rerio*	Glyphosate acid (954.9 g/kg acid equivalent)	NOEC (7 d): 1 mg a.s./L (nom)	Valid	-	[redacted] C.M. (2000c), Report No.: [redacted]-D62.16/99 (CA 8.2.2.1/002)
OECD Guideline 236 Non-GLP Literature data	*Danio rerio* (embryo)	Glyphosate (99% purity)	LC_{50} (96 h): > 100 mg/L	Relevant and reliable with restrictions	No analytical verification	Rodrigues L.B. *et al.* (2019), Document no.: doi.org/10.1016/j.mrgentox.2019.05.002, E-ISSN: 1873-135X (CA 8.2.2.1/005)
Based on OECD 236 Non-GLP Literature data	*Danio rerio* (embryo)	Glyphosate (96% purity)	Effects due to low (glyphosate induced) pH LC_{50} (96 hpf): 98.4 mg a.s./L (unbuffered) Developmental delays: EC_{10} (96 hpf): 21.3 mg a.s./L	reliable with restrictions	Fertilisation rate of the batch of eggs not reported. No analytical verification	Schweizer. M. *et al.* (2019), Document no.: DOI 10.7717/peerj.7094, ISSN: 2167-8359 (CA 8.2.2.1/006)
			Heart rates: EC10 = 7.27 mg a.s./L. Hatching rate: 96 hpf -EC10 and EC50 = 26.2 mg a.s./L and 37.9, respectively. Developmental delays: at 24 hpf the EC10 = 21.3 mg a.s./L. Malformations found in embryos of all glyphosate treatments but with rates below 20%. EC10 = 30.2 mg			

			a.s./L			
No guideline followed Non-GLP Literature data	*Danio rerio* Embryo (5h post fertilisation)	glyphosate	48h-LD50 = 66.04 mg/L	Relevant and reliable with restrictions	No analytical verification	CA 8.2.1 Gaur H. et al. 2019 Biochemical and biophysical research communications (2019) Vol. 513, No. 4, pp. 1070
			50 and 100 mg/L glyphosate showed abnormalities like pericardial edema, yolk sac edema and tail bending in the treated embryos. Hatching was significantly delayed at concentrations of 50 mg/mL and above.			
Literature data	*Danio rerio*	glyphosate	No NOEC 10 mg/L glyphosate reduced egg production but not fertilization rate in breeding colonies. increased early stage embryo mortalities and premature hatching. Effect assumed to be primarily by exposure during gametogenesis.			Uren Webster T. M. et al., 2014
Literature data	*Danio rerio*	glyphosate	NOEC for morphological alterations =10 mg/L (epiboly process and body length, eye and head area) NOEC Surface tension of chorion < 1mg/L (not concentration dependant), the study author claims that it is not significant at concentrations below 1mg/L but the data are not shown in this study NOEC hatching rate = 200mg/L (increase with concentration) NOEC larvae abnormality = 10 mg/L Study considered reliable with restrictions.			Zhang S . et al., 2017
Aquatic invertebrates						
OECD 202, Part II, Reproduction Test (1984) GLP	*Daphnia magna*	Glyphosate acid (97.6% purity)	NOEC (21 d): 12.5 mg a.s. /L (nom) No EC10 could be set for immobility and reproduction. EC10 for length: 94.47 mg/L.	Valid	-	[redacted] (1999), Report no.: AF0497/B (CA 8.2.5.1/001) [redacted] 2020, Report no.: 110054-012 (updated statistical evaluation) (CA 8.2.5.1/009)

OECD Guideline 202 ECC Draft Guideline XI/681/86 "Prolonged Toxicity Study with *Daphnia magna*: Effects on Reproduction " GLP	*Daphnia magna*	Glyphosate (96% purity)	NOEC (21 d): 56 mg a.e./ L (nom)	Valid with restriction	pH issue (pH of 5-6 at 100 mg/L, impact on endpoint considered low)	[redacted] (1995d), Report no.: 141874 (CA 8.2.5.1/002)
OECD Guideline 202, Part I and II GLP	*Daphnia magna*	Glyphosate isopropylamine salt (61.6% purity)	NOEC (21 d): 42.90 mg a. e./L (nom)	Valid	-	[redacted] (1993e), Report no.: 80-91-2328-05-93 (CA 8.2.5.1/003) [redacted] 2020, Report no.: 110054-014 (updated statistical evaluation) (CA 8.2.5.1/011)
OECD Guideline 202, Part II, Reproduction Test (1984) GLP	*Daphnia magna*	Glyphosate (98.7% purity)	EC10 (21d) = 22.65 mg a.e./L (nom)	Valid	-	[redacted] (1990e), Report no.: 250795 (CA 8.2.5.1/004) [redacted] 2020, Report no.: 110054-015 (updated statistical evaluation) (CA 8.2.5.1/012)
OECD Guideline 202 U.S. Guideline 72-4, (EPA-FIFRA, 40 CFR, Section 158.145) GLP	*Daphnia magna*	Glyphosate technical (97.67% purity)	NOEC (21 d): 100 mg a.e./L (nom)	Valid	-	[redacted] (1989b), Report no.: AB 89-58 (CA 8.2.5.1/005)
ASTM Committee, (Draft No. 5, September,1979, E-35.2; Draft No. 3, 1981, E-47.01; Draft No. 2, September, 1979, E-35.21)	*Daphnia magna*	Glyphosate (99.7% purity)	NOEC (21 d): 41 mg a.e./ L (nom)	Valid	-	[redacted] (1982), Report no.: AB 82-036 (CA 8.2.5.1/006) [redacted] 2020, Report no.: 110054-016 (updated statistical evaluation) (CA 8.2.5.1/013)

Non-GLP						
OECD Guideline 219 (2004) GLP	*Chironomus riparius*	Glyphosate acid (97.7% purity)	NOEC (28 d): 1000 mg a.s./L (nom)	Supportive	No alaytical verification in sediment. No report for analytical method was available (see Volume 3 (AS) B.5)	[redacted] (2020), Report no.: 20FV2ME (CA 8.2.5.3/001)
Standard procedures recommended by the APHA et al. (2005) - 10600 Fishes Non-GLP Literature data	*Cherax quadricarinatus* juveniles	Glyphosate acid (99.8% purity)	At 60 days : 33 % mortality at 40 mg/L of glyphosate; 35% decrease in weight gain at 40 mg/L.	Reliable with restrictions	Results insufficiently detailed.	Avigliano L. et al., 2014 (CA 8.2.4)
No guideline followed Non-GLP Literature data	*Neohelice granulate* adult females	Glyphosate acid (99.8% purity)	NOEC (3 months) < 0.02 mg/L for body weight gain	Reliable with restrictions	Results insufficiently detailed.	Avigliano L. et al., 2018 (CA 9)
No guideline followed Non-GLP Literature data	*Neohelice granulate* adult males	Glyphosate acid (99.8% purity)	NOEC (60d) < 1.27 mg/L for body weight gain	Reliable with restrictions	Results insufficiently detailed.	Canosa I. S. et al., 2019 (CA 9)
Algae						
OECD Guideline 201 (1984) US EPA Guideline 540/09-82-020 (1982) GLP	*Skeletonema costatum*	Glyphosate acid (95.6% purity)	72h NOErC = 5.6 mg a.e./L 72h ErC10 = 1.87 mg a.e./L 72h ErC20 = 2.98 mg a.e./L (nom) 72h NOEyC = 5.6 mg a.e./L 72h EyC10 = 5.22 mg a.e./L	Valid	-	[redacted] (1996a), Report no.: AB0503/I (CA 8.2.6.2/006) [redacted] (2020a), Report no.: 110054-007 (updated statistical evaluation) (CA 8.2.6.2/007)

			72h EyC20 = 6.38 mg a.e./L (nom)			
OECD Guideline 201 EEC Directive 92/69 C.3 GLP	*Pseudokirchneriella subcapitata* (*Raphidocelis subcapitata*)	Glyphosate isopropylamine salt (62.66% purity)	72h NOErC = 2.21 mg a.e./L 72h ErC10 = 4.23 mg a.e./L 72h ErC20 = 7.6 mg a.e./L (mm) 96h NOErC = 4.87 mg a.e./L (mm) 96h ErC10 = 7.11 mg a.e./L 96h ErC20 = 10.8 mg a.e./L 72h NOEyC = 2.21 mg a.e./L 72h EyC10 = 2.17 mg a.e./L 72h EyC20 = 3.22 mg a.e./L 96h NOEyC = 2.21 mg a.e./L 96h EyC10 = 3.05 mg a.e./L 96h EyC20 = 4.19 mg a.e./L (mm)	Valid	-	[redacted] [redacted] (2002), Report no.: A-99-02-04 (CA 8.2.6.1/001)
OECD Guideline 201 (1984) US EPA Guideline 540/09-82-020 (1982) GLP	*Selenastrum capricornutum* (*Raphidocelis subcapitata*)	Glyphosate acid (95.6% purity)	72h NOErC = 10 mg a.e./L 72h ErC10 = 5.74 mg a.e./L 72h ErC20 = 8.91 mg a.e./L (nom)	Valid	-	[redacted] (1995), Report no.: AB0503/B (CA 8.2.6.1/005) [redacted] (2020b), Report no.: 110054-002 (updated statistical evaluation) (CA 8.2.6.1/006)

			72h NOEyC = 10 mg a.e./L 72h EyC10 = 4.84 mg a.e./L 72h EyC20 = 7.59 mg a.e./L (nom)			
US EPA Guideline 123-2 – FIFRA GLP	*Selenastrum capricornutum* (*Raphidocelis subcapitata*)	Glyphosate technical (96.6% purity)	72h ErC10 < 10 mg a.e./L 72h ErC20 = 10.8 mg a.e./L (nom) 72h EyC10 < 10 mg a.e./L 72h EyC20 = 10.25 mg a.e./L (nom)	Valid	-	[redacted] (1987a), Report no.: 1092-02-1100-1 (CA 8.2.6.1/009) [redacted] (2020c), Report no.: 110054-003 (updated statistical evaluation) (CA 8.2.6.1/010)
Guideline 123-2, U.S. EPA – FIFRA (Growth and Reproduction of Aquatic Plants, Tier 2) GLP	*Anabaena flos-aquae*	Glyphosate technical (96.6% purity)	72h ErC10 = 7.63 mg a.e./L (nom) 72h ErC20 = 12.7 mg a.e./L (nom) 72h EyC10 = 9.97 mg a.e./L (nom) 72h EyC20 = 11.8 mg a.e./L (nom) **Data gap for 96h endpoints**	Valid	-	[redacted] (1987b), Report no: 1092-02-1100-4 (CA 8.2.6.2/002) [redacted] (2020d), Report no.: 110054-006 (updated statistical evaluation) (CA 8.2.6.2/003)
OECD Guideline 201 (1993) GLP	*Selenastrum caprocornutum* (*Raphidocelis subcapitata*)	Glyphosate technical (white powder, 954.9 g/kg)	72h NOErC= 5.6 mg a.e./L (nom) 72h ErC10 = 62.6 mg a.e./L	Supportive	No analytical verification of test concentrations throughout the test	[redacted] (2000b), Report no. RF-D2.44/99 (CA 8.2.6.1/003) [redacted] (2020e), Report no.: 110054-001 (updated statistical evaluation) (CA

			72h ErC20 = 132 mg a.e./L 72h NOEyC= 5.6 mg a.e./L (nom) 72h EyC10 = 5.54 mg a.e./L 72h EyC20 = 14.6 mg a.e./L			8.2.6.1/004)
No information on followed guideline GLP	*Desmodesmus subspicatus*	Glyphosate acid (no information on purity)	-	Not assessed Invalid	Not assessed. Report not available. Data from DAR (2001) considered relied upon in RAR (2015)	[redacted] (1995c), Report no.: 710/12 (CA 8.2.6.1/011)
Data were generated in accordance with OECD- or equivalent guidelines GLP	*Desmodesmus subspicatus*	Glyphosate isopropylamin-salt (61-65% purity)	-	Not assessed	Report is not available. Data from DAR (2001) considered relied upon in RAR (2015)	[redacted] (1994), Report no.: 95-00554 (CA 8.2.6.1/012)
OECD Guideline 201 (1984) US EPA Guideline 540/09-82-020 (1982) GLP	*Anabaena flos-aquae*	Glyphosate acid (95.6% purity)	-	Not reliable	Correlation between biomass and optical density cannot be demonstrated.	[redacted] (1996b), Report no.: AB0503/J (CA 8.2.6.2/001)
OECD Guideline 201 (1984) EU Directive 92/69/EEC, Method C.3. (1992) ASTM	*Pseudokirchneriella subcapitata* (*Raphidocelis subcapitata*)	Glyphosate K-salt (47.7% purity)	-	Invalid	Coefficient of variation for section specific growth rate: > 35%	[redacted] (2003), Report no.: 139A-311 (CA 8.2.6.1/002)

Standard Guide 1218-90E (1990) GLP						
OECD Guideline 201 (1984) EEC Directive 92/69, Part C-3 (1992) ISO International Standard 8692 (1989) GLP	*Pseudokirchneriella subcapitata* (*Raphidocelis subcapitata*)	Glyphosate (96% purity)	72h NOErC = 32 mg a.e./L (nom) 72h ErC10 = 33 mg a.e./L (nom) 72h NOEbC = 10 mg a.e./L (nom) 72h EbC10 = 18 mg a.e./L (nom)	Valid	-	[redacted] (1995b), Report no.: 141896 (CA 8.2.6.1/007)
No information on followed guideline GLP	*Pseudokirchneriella subcapitata* (*Raphidocelis subcapitata*)	Glyphosate (> 94% purity)	-	Not assessed	Report is not available Data from DAR (2001) considered relied upon in RAR (2015)	[redacted] (1995c), Report no.: R481 (CA 8.2.6.1/008)
OECD Guideline 201 (1984) "Hemmung der Zellvermehrung bei Grünalge *Scenedesmus subspicatus* – Verfahrensvorschlag der ad hoc Arbeitsgruppe des Umweltbundesamtes Berlin" GLP	*Scenedesmus subspicatus* (*Desmodesmus subspicatus*)	Glyphosate isopropylamine-salt (61.6% purity)	-	Invalid	Coefficient of variation for section specific growth rate: > 35%, coefficient of variation of average specific growth rates: >7%	[redacted] (1993d), Report no.: 80-91-2328-01-93 (CA 8.2.6.1/013)
OECD Guideline 201	*Scenedesmus subspicatus* (*Desmodesmu*	Glyphosate (95%	-	Invalid	Validity criteria could not	[redacted] (1990), Report no.: 1-7-46-90

GLP	*s subspicatus*)	purity)			be checked, no analytical measurements.	(CA 8.2.6.1/014)
OECD Guideline 201 (1984) GLP	*Scenedesmus subspicatus* (*Desmodesmus subspicatus*)	Glyphosate (98.7% purity)	-	Invalid	Coefficient of variation for section specific growth rate: > 35%	[redacted] (1990d), Report no.: 250773 (CA 8.2.6.1/015)
OECD Guideline 201 (1984) US EPA Guideline 540/09-82-020 (1982) GLP	*Navicula pelliculosa*	Glyphosate acid (95.6% purity)	-	Invalid	Coefficient of variation for section specific growth rate: > 35%	[redacted] (1996c), Report no.: AB0503/K (CA 8.2.6.2/004)
Guideline 123-2, U.S. EPA – FIFRA (Growth and Reproduction of Aquatic Plants, Tier 2) GLP	*Navicula pelliculosa*	Glyphosate technical (96.6% purity)	**Data gap (EC10, EC20 and EC50 values should be calculated for 72h based on yield and growth rate)**	Valid	RMS considered that validity criteria are met.	[redacted] (1987c), Report no.: 1092-02-1100-2 (CA 8.2.6.2/005)
Guideline 123-2, U.S. EPA – FIFRA (Growth and Reproduction of Aquatic Plants, Tier 2) GLP	*Skeletonema costatum*	Glyphosate technical (96.6% purity)	-	Invalid	Biomass increase in control cultures: <16 and coefficient of variation for section specific growth rate: > 35%	[redacted] (1987d), Report no.: 1092-02-1100-3 (CA 8.2.6.2/008)
Environmental Protection Agency: Bioassay procedures for the ocean disposal permit program	*Skeletonema costatum*	Several glyphosate related test items (purity not stated): Solid test materials	-	Invalid	No information on validity criteria. No analytical measurme	[redacted] (1978b), Report no.: BP-78-4-031 (CA 8.2.6.2/009)

(1976) Non-GLP		(TM): Glyphosate, BN-78-44 and Glyphosate intermediate, BN-78-45 Liquid TM: Comp. #1, BN-78-46; Comp. #2, BN-78-47; Comp. #3A, BN-78-48; Comp. #4, BN-78-49 and Comp. 5A.			nts.	
OECD Guideline 201 (1984) Non-GLP	*Nitzschia palea*	Glyphosate technical (96.7% purity)	-	Invalid	validity criteria not met	[redacted] (1996), Report No.: 960606FH (CA 8.2.6.2/010)
Aquatic plants						
Maltby, L., *et al.* (2008): Aquatic Macrophyte Risk Assessment for Pesticides, SETAC AMRAP GLP	*Myriophyllum aquaticum*	Glyphosate acid (85.2% purity)	-	Invalid	coefficient of variation for yield based on measurements of shoot fresh weight > 35%	[redacted] (2012), Report no.: CHE-015/4-80/A (CA 8.2.7/010)
OECD Guideline 221 GLP	*Lemna minor*	Glyphosate isopropylamine salt (97.1% purity)	Frond number 7d NOErC = 8.65 mg a.e./L (nom) 7d ErC10 = 8.16 mg a.e./L (nom) 7d ErC20 = 12.8 mg a.e./L (nom) 7d NOEyC = 8.65 mg	Valid	Results based on statistical re-evaluation	[redacted] (2002), Report no.: CEMR-1873 (CA 8.2.7/001) [redacted] (2020f), Report no.: 110054-008 (updated statistical evaluation) (CA 8.2.7/002)

			a.e./L (nom) 7d EyC10 = 7.8 mg a.e./L (nom) 7d EyC20 = 10.3 mg a.e./L (nom) Dry weight 7d NOEyC = 8.65 mg a.e./L (nom) 7d EyC10 = 5.72 mg a.e./L (nom) 7d EyC20 = 10.3 mg a.e./L (nom) Phytotoxicity NOEC = 8.65 mg a.e./L (nom)			
Guideline ASTM E 1415-91 (June 1991) GLP	*Lemna gibba*	Glyphosate isopropylamine salt (62% purity)	-	Valid but not reliable	Validity criteria were met but actual exposure questionable	[redacted] (1999), Report no.: 980909FH (CA 8.2.7/003) [redacted] (2020g), Report no.: 110054-009 (updated statistical evaluation) (CA 8.2.7/004)
EPA FIFRA Subdivision J Guideline 123-2 GLP	*Lemna gibba*	Glyphosate acid (95.6% purity)	Frond number 7d NOErC = 12 mg a.e./L (nom) 7d ErC10 = 13.3 mg a.e./L (nom) 7d ErC20 = 18.7 mg	Valid	Results based on statistical re-evaluation	[redacted] (1996d), Report no.: AB0503/L (CA 8.2.7/005) [redacted] (2020h), Report no.: 110054-010 (updated statistical evaluation) (CA 8.2.7/006)

			a.e./L (nom) 7d NOEyC = 6 mg a.e./L (nom) 7d EyC10 = 10.5 mg a.e./L (nom) 7d EyC20 = 14.2 mg a.e./L (nom) Phytotoxicity NOEC = 1.5 mg a.e./L (nom)			
Guideline 123-2, U.S. EPA – FIFRA (Growth and Reproduction of Aquatic Plants, Tier 2) GLP	*Lemna gibba*	Glyphosate (96.6% purity)	Frond number 7d NOErC = 16.6 mg a.e./L (mm) 7d ErC10 = 20.8 mg a.e./L (mm) 7d ErC20 = 31.9 mg a.e./L (mm) 7d NOEyC = 16.6 mg a.e./L (mm) 7d EyC10 = 18.2 mg a.e./L (mm) 7d EyC20 = 20.3 mg a.e./L (mm) Phytotoxicity Not recorded	Valid	Results based on statistical re-evaluation	[redacted] (1987e), Report no.: 1092-02-1100-5 (CA 8.2.7/007) [redacted] (2020i), Report no.: 110054-010 (updated statistical evaluation) (CA 8.2.7/008)
No information on followed guideline Non-GLP	*Lemna gibba*	Glyphosate technical (> 94% purity)	No information on results available.	Invalid	Report not available. Study reported as not acceptable in DAR (2001)	[redacted] (1987f), Report no.: XX-88-416 (CA 8.2.7/009)
Other aquatic organisms						

OECD Guideline 231 (2009) OPPTS/OCSPP Guideline 890.1100 (2009) GLP Note to AGG: this study has been assessed by SE. Results reported are agreed in SE RAR of 01/04/2021	*Xenopus laevis*	Glyphosate acid (85.14% purity)	NOEC (21 d): ≥ 100 mg a.s. /L	Valid	-	[redacted] (2012b), Report no.: 707A-103 (CA 8.2.3/002)

a.e.: acid equivalents, a m.: arithmetic mean measured, gm: geometric mean measured, nom: nominal
n.d.: not determined.

2.9.2.3.1 Chronic toxicity to fish

Four studies testing the chronic toxicity of glyphosate, glyphosate acid and glyphosate salts on fish are available and summarized in the following section. NOECs range from 1 mg a.e./L to 33 mg a.e./L.

Valid studies

A fish short term toxicity test with glyphosate acid with larvae of *Danio rerio* (formerly named *Brachydanio rerio*) was performed under semi-static conditions with test medium renewal each 48 hours ([redacted] 2000). Three replicates with 30 fish per concentration were exposed for 168 hours to seven concentrations of glyphosate acid, ranging from 0.32 to 32 mg a.s./L. Observations for mortality and sub lethal responses were made every 24 hours. Dissolved oxygen, pH and temperature were measured and recorded daily. Glyphosate acid concentrations were measured by liquid chromatography in the stock solutions. Mean measured concentrations were at least 80% of nominal concentrations. A significant increase of mortality was observed at a concentration of 5.6, 10 and 32 mg a.s./L, behavioural responses such as lethargy was observed at 3.2, 5.6, 10 and 32 mg a.s./L. The No-Observed-Effect Concentration (NOEC) for zebra fish (*Danio rerio*) exposed to glyphosate acid was determined to be 3.2 mg a.s./L by the study author. The applicant considered that this study is not relevant for use in EU level ecotoxicological risk assessment. RMS did not agree to consider the study not relevant. In this study several limitations have been identified by comparison with guideline OECD 212 and assessed by RMS (please refer to the study summary and detailed of assessment under Vol 3 CA B.9). However this study was not meant to follow the recommendations of the OECD 212. Moreover in view of the effects reported with the clear dose response on mortality and the time course of effects RMS considered that increase of study duration may have influence the results. Underestimation of effects could not be excluded and should be considered when setting the NOEC.
Significant increase of mortality reported as statistically significant in the study report (Fisher test) was observed at a concentration of 5.6, 10 and 32 mg a.s./L. The LC50 after 168 hours was determined to be 24.71 mg a.s./L.
The No-Observed-Effect Concentration (NOEC) for zebra fish (Danio rerio) exposed to glyphosate acid was determined by the author to be 3.2 mg a.s./L (nominal). Nevertheless, as previously agreed in RAR 2015, the mortality effect in the study with Danio rerio followed a dose response relationship and in the treatment level at 3.2 mg/L a mortality of 10% was observed. Lethargy was also observed at this concentration.
RMS considered that lethargy is a severe effect of biological significance and should therefore be considered. It is acknowledged that the effect was not well described, in particular the number of affected fish was not mentioned. Lethargy occurred at 3.2 mg/L and above at the same time as mortality. There is a clear dose-response on mortality. Moreover, mortality was seen to increase with time at concentrations greater and equal to 3.2 mg/L and lethargy appeared at the same time in these tested concentrations.
Overall, RMS considered appropriate to set the NOEC at 1 mg/L considering that 10% mortality is observed after the 7-day exposure period with clear increase with time.

As lethargy occurred at same time as mortality which exhibit a clear dose-response and increase in time, RMS considered that the 10% effects on mortality should be considered as biologically relevant. RMS considered the NOEC of 1.0 mg/L relevant for the risk assessment. No LC10 was calculated.
A data gap is set to provide a statistical re-analysis (NOEC, LC10/20). Moreover the extent of lethargy should be provided did not agree with the applicant.

In a 21-day short-term reproduction assay performed by [redacted] (2012a), breeding groups of fathead minnows (*P. promelas*) were exposed to glyphosate acid at arithmetic mean measured concentrations of 0.046, 0.23, 1.2, 6.2 and 33 mg a.s./L for 21 days. The endpoints evaluated were adult survival, body length and wet weight, fecundity (cumulative egg production and eggs per female reproductive day), fertilization success, secondary sex characteristics (including fatpad and tubercle scores), GSI, VTG and gonad histopathology. There were no effects observed. The study is considered valid and an overall 21-day NOEC of 33 mg a.s./L (arithmetic mean measured) was derived.

The effects of glyphosate acid on the early life-stages of rainbow trout was determined under flow-through (continuous renewal) exposure conditions ([redacted] 2010). Fertilized eggs of *Oncorhynchus mykiss* were exposed for 85 days to nominal glyphosate acid concentrations of 0.095, 0.305, 0.977, 3.125 and 10.0 mg a.s./L. Mean measured concentrations were substantially achieved and ranged between 85.7 and 96.3% of nominal concentrations.
No statistically significant differences were detected for normal fry at hatch, hatching success, survival at test termination and growth (total length, wet and dry weight), when compared to the control group. The 85-day NOEC was determined to be 9.63 mg a.s./L based on geometric mean measured concentrations. All validity criteria according to OECD guideline 210 were met. However, even if the study is considered valid, the endpoint could not be considered as reliable for risk assessment since only two replicates per test concentration were used and high variability in some parameters were measured (egg viability, hatching success, wet and dry weight), questioning the statistical robustness of the endpoint.

Supportive studies

The effects of glyphosate on fathead minnow (*Pimephales promelas*) were evaluated in a full life cycle test under flow-through test conditions (Anonymous, 1975). The test was performed using mean measured concentrations of 0.7, 2.8, 7.0, 13.0 and 25.7 mg glyphosate/L. During the full life cycle test, adult fecundity (approx. day 112) and survival (day 30, 60 and day 134) were recorded. The egg hatchability was determined as well as total length, total wet weight, sex ratio and gonadal conditions were equally determined for each adult fish. All validity criteria according to EPA guideline OPPTS 850.1500 were fulfilled. None of the parameters studied (adult fecundity, parental and juvenile mortality, total length, wet weight, sex ratio and gonadal conditions), were significantly affected by the chronic exposure to the test item. The 225-day NOEC was therefore ≥ 25.7 mg a.e./L (mean measured). This study is considered supportive, as analytical method validation are not available.

The following literature data were found:
Rodrigues (2019) assessed the acute toxicity of glyphosate in a zebrafish (*Danio rerio*) embryo-larval toxicity test according to OECD guideline 236 at 6 concentrations between 1.7 and 100 mg/L. Glyphosate caused no acute toxic effect up to the highest test concentration (96-hour LC_{50} > 100 mg/L). Morphological abnormalities (from 10 mg/L to 100 mg/L) were observed, including pericardial and yolk sac edemas, spinal curvature, head and tail deformities in different exposure times; not statistically significant. Potential effects on hatching were not investigated. The results from this study are considered reliable with restrictions (no analytical verification).

A further literature study assessing the effects of glyphosate on zebrafish embryos according to OECD guideline 236 (Schweizer et al., 2019). Fish embryos were exposed to concentrations between 1.69 and 1690.7 mg glyphosate/L in an unbuffered aqueous medium, as well as at pH 7, for 96 hours post fertilization (hpf). In unbuffered glyphosate medium LC50 (96 hpf) value was 98.4 mg a.s./L. The authors also reported EC10 for heart rates of 7.27 mg a.s./L and 96 hpf -EC10 and EC50 for hatching rate of 26.2 mg a.s./L and 37.9, respectively. The developmental delays was noted with a 24 hpf EC10 value of 21.3 mg a.s./L. Malformations were found in embryos of all glyphosate treatments but with rates below 20% (EC10 = 30.2 mg a.s./L). The results from this study are considered reliable with restrictions (Fertilisation rate of the batch of eggs not reported. no analytical verification).

Gaur H. et al., 2019, investigated effect on the hatching rate and mortality of zebrafish embryo. Zebrafish embryos treated with 50 and 100 mg/L glyphosate showed abnormalities like pericardial edema, yolk sac edema and tail bending in the treated embryos. Hatching was significantly delayed in zebrafish embryos exposed to glyphosate

at concentrations of 50 mg/L and above. Glyphosate significantly reduced the heartbeat in a time and concentration-dependent manner indicating cardiotoxicity. The results from this study are considered reliable with restrictions (no analytical verification).

In Uren Webster T. M. et al., 2014, 10 mg/L glyphosate reduced egg production but not fertilization rate in breeding colonies of zebrafish (Danio rerio), and increased early stage embryo mortalities and premature hatching. However, exposure during embryogenesis alone did not increase embryo mortality, suggesting that this effect was caused primarily by exposure during gametogenesis. No NOEC could be determined, then this study provides no endpoint usable for the risk assessment. The study authors claim that early stage mortality was not the result of direct toxicity of the chemical exposure on embryos. Their assumption is based on the fact that exposed embryos originating from a control population of untreated adults exposed at concentrations of up to 10 mg/L of Roundup and 10 mg/L glyphosate had no effect on embryo survival at <3.5 or 3.5−24 hpf. However RMS notes that the chosen glyphosate concentration of 10 mg/L is clearly above the NOEC based on mortality on zebrafish of 1 mg/L (Dias Correa Tavares, C.M., 2000 where mortality was of 26.7% at the tested concentration (nominal) of 10 mg/L). This study is relevant and reliable (for toxic effects of glyphosate, but not for investigation of potential for endocrine disruption).

Zhang S. et al., 2017, investigated the effects of glyphosate on early development of larval zebrafish via morphological, biomechanics, behavioral and physiological analyses. The following was stated:

NOEC for morphological alterations =10 mg/L (epiboly process and body length, eye and head area)

NOEC Surface tension of chorion < 1mg/L (not concentration dependant), the study author claims that it is not significant at concentrations below 1mg/L but the data are not shown in this study

NOEC hatching rate = 200mg/L (increase with concentration)

NOEC larvae abnormality = 10 mg/L

It is hypothetized by the study authors that the decreased surface tension of chorion and the increased locomotive activities may contribute to the hatching rates after glyphosate treatment. The results from this study are considered reliable with restrictions (accuracy and precision of the dynamic light scattering method are unknown).

2.9.2.3.2 Chronic toxicity to aquatic invertebrates

Several studies on chronic effects of glyphosate, glyphosate acid and glyphosate salts considered as valid are available. NOECs range from 12.5 mg a.e./L to 100 mg a.e./L.

Valid studies

The lowest endpoint was derived in a 21-day toxicity test performed under semi-static conditions according to OECD 202, Part II ([redacted] 1999). The lethal and sub lethal effects of glyphosate acid on *Daphnia magna* were evaluated by exposing *D. magna* to 12.5, 25, 50, 100, and 200 mg a.s./L nominal concentrations and a control group. The mean measured concentrations of glyphosate acid in the new test solutions ranged from 100 to 104% of the nominal values. The mean measured concentrations in the old test solutions ranged from 96 to 104% of the nominal values. Therefore, the results are based on nominal glyphosate acid concentrations. The overall 21-day NOEC for the reproduction of *D. magna* exposed to glyphosate acid was 12.5 mg a.s./L. The study is considered to be valid. All validity criteria according to the pertinent OECD guideline 211 were fulfilled.

In a further test, the effects of glyphosate to *D. magna* were evaluated in a 21-day reproduction test under semi-static conditions ([redacted] 1995d). There was no test substance related mortality of parental daphnids at any test concentration. Statistical analysis demonstrated significant reduction of reproductive capacity of *Daphnia magna* at 100 mg/L. The overall 21-day NOEC was 56 mg a.e./L based on nominal concentrations. Measured concentrations ranged from 104% to 118% of nominal test concentration throughout the test. All validity criteria according to the pertinent OECD guideline 211 were fulfilled.

[redacted] (1993e) evaluated the effects of glyphosate IPA salt on reproduction of *D. magna* in a semi-static test. The NOEC for reproduction rate was calculated to be 94 mg test item/L equivalent to 57.90 mg glyphosate IPA salt/L and 42.90 mg a.e./L (nominal), respectively. No analytical verification was made at this concentration but measurements were available for all other tested concentrations that show recovery in the range of nominals ±20% throughout the test except in two instances at the lowest concentration (with decrease of more than 20%). All validity criteria according to the current OECD guideline 211 were fulfilled.

A further 21-day toxicity test performed under semi-static conditions assessed the effects of glyphosate on *D. magna* exposed to 3.0, 9.4, 30, 94.9, and 300 mg a.e./L nominal concentrations ([redacted] 1990e). The mean measured concentrations of glyphosate in the test solutions ranged from 99.9 to 126% of nominal values. Measured values exceed the upper limit of nominals ± 20% only for the highest concentration at which 100% reduction was noted on reproduction. On the basis of the analytical data, the nominal concentrations were used for the calculation and reporting of all results. The 21-day EC10 for reproduction was 22.65 mg a.e./L based on nominal concentrations. All validity criteria according to the OECD guideline 211 were fulfilled.

[redacted] (1989b) evaluated the effects of glyphosate technical in a further 21-day semi-static test. Recoveries were ranging from 92.3, to 108.0% of nominal concentrations. Therefore, ecotoxicological endpoints were based on nominal concentrations of the test item. No effects of glyphosate technical on survival, reproduction and time to first brood of *D: magna* after 21-day exposure were observed in any test item treatment. The 21-day NOEC was determined to be 100 mg a.e./L. All validity criteria according to OECD guideline 211 were fulfilled.

In a non-GLP study, [redacted] (1982) assessed the effects of glyphosate on *D. magna* in a 21-day chronic test in flow-through conditions. The test was performed using nominal concentrations of 25, 50, 99, 199 and 397 mg test item/L. Reproduction significantly decreased at the three highest test item concentrations. In contrast to that, at the lowest test item concentration (26 mg/L) an increase of reproduction when compared to the control was observed. The NOEC was determined to be 41 mg a.e./L (arithmetic mean). All validity criteria according to the OECD guideline 211 were fulfilled.

Based on its fate characteristics, glyphosate and AMPA are considered as persistant in sediment and chronic exposure of the sediment dwellers is expected. According to EU Reg 283/2013 section 8.2.5.4 test using spiked sediment or at least analytics in sediment is required to set an endpoint. (data gap)

In relation with e-fate data gap, provide further information to assess the risk assessment for metabolite 1-oxo-AMPA for sediment dwelling organisms. For details, please refer to Volume 3 (AS) B.8 point B.8.2.2.5.

Supportive studies

Furthermore, a study testing the toxicity of glyphosate acid to the sediment dweller *Chironomus riparius* is available ([redacted] [redacted] 2020). In this sediment-water toxicity test using spiked water first-instar larvae of freshwater dipteran *C. riparius* were exposed to concentrations of 100 and 1000 mg a.e./L according to OECD guideline 219 for 28 days. A concentration-response relationship was not observed for emergence ratio and development rate after 28 days of exposure. A statistically significant inhibition compared to the control was not found up to and including the highest test concentration. Therefore, a 28-day NOEC of 1000 mg a.s./L was derived based on nominal test concentrations. One validity criterion according to OECD guideline 218 was not met. Several midges in the control emerged later than required in the guideline. Since total emergence in the control exceeded 90% of inserted animals, and since more than 89% of the emerged control midges had emerged by day 23, this is not considered to have any impact on the integrity of the study. The study is therefore considered valid. Based on its fate characteristics, glyphosate is considered as persistant in sediment and chronic exposure of the sediment dwellers is expected. According to EU Reg 283/2013 section 8.2.5.4 test using spiked sediment or at least analytics in sediment is required to set an endpoint. The absence of analytics in sediment do not allow deriving a robust endpoint, this study is considered informative only.

Avigliano L. et al., 2014, assessed the effects of sublethal concentrations of glyphosate on early juvenile of the crayfish Cherax quadricarinatus, in terms of growth rate, metabolic rate and energy reserves levels, to determine how glyphosate affects the activity level of key metabolic enzymes, such as pyruvate kinase and to determine the levels of both alanine and aspartate aminotransferase activities (ALAT and ASAT respectively) as indicative of tissue damage. The highest mortality value (33 %) was seen in animals exposed to 40 mg/L of glyphosate; A significant decrease in weight gain (35 % lower than control) was seen after the first month of exposure to 40 mg/L of glyphosate. Significant decrease in total protein content in both muscle, at 40 mg/L, and hepatopancreas, at both assayed concentrations. Besides, a significant decrease in total lipid content was observed in muscle. At the 10 mg/L exposure, muscle pyruvate kinase activities were significantly lower (while no differences were seen in the hepatopancreas. Both lipids and proteins are closely involved with the energy available for crustacean growth. This study states that glyphosate is able to reduce growth rates and protein and lipid reserves in chronically exposed (60 days, semi-static, concentrations were maintained) early juvenile crayfish at concentrations of 40 mg/L. Some effects (decrease in protein reserves in hepatopancreas and an apparent metabolic depression in muscle) were observed at 10 mg/L. Overall, RMS considers this study as relevant and reliable with restrictions.

Avigliano L. et al., 2018, exposed adult females of the estuarine crab Neohelice granulata during the 3-month pre-reproductive period (winter) to the herbicide glyphosate, at three different concentrations (0.02, 0.2, and 1 mg/L, as active ingredient). A decrease in the body weight gain on adult female crab was observed by effect of pure glyphosate, at all concentrations assayed (NOEC < 0.02 mg/L). It is likely due to treatment but does not appear concentration related. Concentrations were analytically verified but only graphs were presented. Concerning the potential impact of using wild-caught organisms, RMS then cannot discard the presence of other toxicants in the estuary from which these were caught. The results are reliable with restrictions.

Canosa I. S. et al., 2019, exposed males of the estuarine crab (Neohelice granulate) to pure glyphosate. The in vivo assays comprised the exposure for 30 d to 1 mg/L of the herbicide, until finally assessing weight gain, levels of energy reserves, sperm number per spermatophore, proportion of abnormal spermatophores, and sperm viability. Overall, decrease in weight gain and muscle protein levels and higher incidence of abnormal spermatophores may be attributed to glyphosate at the concentration of 1.27 mg/L. Concentrations were analytically verified. Concerning the potential impact of using wild-caught organisms, RMS then cannot discard the presence of other toxicants in the estuary from which these were caught. The study is considered reliable with restrictions (for effects on bodyweight gain, not reliable for endocrine properties). RMS however notes that only bodyweight gain is reported not bodyweight itself. So the magnitude of the effect is uncertain and potentially low.

In conclusion the most sensitive chronic endpoint of glyphosate towards aquatic invertebrates resulted in the study by [redacted] (1999) with a 21-day NOEC of 12.5 mg a.s./L (*Daphnia magna*).

2.9.2.3.3 Chronic toxicity to algae or aquatic plants

The results of all available studies are summarised and endpoints relevant to acute and chronic assessments are considered below.

Valid studies (aquatic algae)

The lowest derived effect value was determined in a 120-hour, static test assessing the toxicity of glyphosate acid on marine algae *Skeletonema costatum* ([redacted]. (1996a). The culture vessels were incubated at 20 ± 1 °C for 120 hours. The validity criteria according to the current OECD guideline 201 were met and this study is considered

valid. Recovery of test concentrations ranged from 94 to 106%. Therefore, endpoints are based on nominal concentrations. In a statistical re-calculation ([redacted] 2020a), the 72-hour NOE_rC was determined to be 5.6 mg a.s./L and the 72-hour E_rC_{10} was 1.87 mg a.s./L. Nominal 72-hour EC_{50} values for growth and yield were 13.5 and 8.99 mg a.s./L, respectively. The validity criteria according to the current guideline OECD Guideline 201 were met and this study is considered valid.

The effects of glyphosate isopropylamine (IPA) salt on *Pseudokirchneriella subcapitata* (currently named as *Raphidocelis subcapitata*) were evaluated in a 96-hour static toxicity test at nominal concentrations between 4.27 and 100 mg test item/L according to OECD 201 ([redacted] 2002). The initial algal cell concentration was 1 x 10^4 cells/mL. The 72-hour E_yC_{50} value for *P. subcapitata* exposed to glyphosate IPA salt was calculated to be 9.25 mg/L, equivalent to 6.85 mg a.e./L (mean measured). The 72-hour ErC50 was not considered reliable. The 72-hour NOE_rC for *P. subcapitata* exposed to glyphosate IPA salt was calculated to be 4.27 mg/L, equivalent to 2.21 mg a.e./L (mean measured). The derived 72-hour ErC_{10} was 8.16 mg/L, equivalent to 4.23 mg a.e./L. The 96-hour E_rC_{50} and E_yC_{50} value for *P. subcapitata* exposed to glyphosate IPA salt was calculated to be 45.7 and 14.7 mg/L, equivalent to 23.7 and 7.63 mg a.e./L (mean measured). The 96-hour NOE_rC for *P. subcapitata* exposed to glyphosate IPA salt was calculated to be 9.39mg/L, equivalent to 4.87 mg a.e./L (mean measured). The derived 96-hour ErC_{10} was 13.7 mg/L, equivalent to 7.11mg a.e./L. All validity criteria were met.

In another guideline study, the toxicity of glyphosate acid to the green alga *Selenastrum capricornutum* (currently known as *R. subcapitata*) was determined in a 120-hour, static test conducted at six nominal glyphosate acid concentrations (5.6, 10, 18, 32, 56, and 100 mg test item/L) and a control ([redacted] 1995). Each replicate test vessel was inoculated with a nominal cell density of 3 × 10^3 cells/mL. The mean measured glyphosate acid concentrations, measured at the start and at the end of the test, ranged from 100 to 111% of the nominal values. In a statistical re-evaluation ([redacted] 2020a), the nominal based 72-hour E_yC_{50} and E_rC_{50} were calculated to be 16.4 and 17.3 mg a.s./L. The 72-hour NOE_rC was determined to be 10.0 mg a.s./L (nominal). The 72-hour E_rC_{10} was 5.74 mg a.s./L (nominal). The validity criteria according to the current OECD Guideline 201 were met.

The effects of glyphosate technical on *P. subcapitata*, (currently named as *R. subcapitata*) were evaluated in a 7-day static toxicity test ([redacted] 1987a). *P. subcapitata* was exposed to five nominal concentrations of 10, 18, 32, 56 and 100 mg test item/L including a control. Recovery of test item concentrations ranged from 104 – 110%. A statistical re-evaluation [redacted] 2020c) derived nominal based 72-hour EC_{50} values for growth and yield of 20.1 and 12.1 mg a.e./L, respectively. 72-hour NOE_rC and E_rC_{10} were both determined to be < 10.0 mg a.e./L (nominal). The validity criteria according to the current guideline OECD Guideline 201 were met.

[redacted] (1987b) evaluated the effects of glyphosate technical on *Anabaena flos-aquae* in a 7day, static test. *A. flos-aquae* was exposed to five nominal concentrations of 10, 18, 32, 56 and 100 mg test item/L including a control. Recovery of mean measured concentrations ranged from 97 to 102%. Therefore, endpoints are based on nominal test concentrations. A statistical re-evaluation of [redacted] (2020d) calculated the 72-hour EC_{50} values for growth and yield to be 33.4 and 16.4 mg a.e./L. The derived 72-hour NOE_rC was 10 mg a.e./L and the E_rC_{10} was calculated to be 7.63 mg a.e./L. The validity criteria according to the current guideline OECD Guideline 201 were met. However, there is a data gap for 96 hour endpoints.

A 72-hour static toxicity test by [redacted] (1995b) evaluating the effects of glyphosate on *Raphidocelis subcapitata* in which the main test was performed with five concentration ranges, 10, 18, 32, 56 and 100 mg test item/L. Recovery of nominal concentrations ranged from 106 to 108% at test initiation and from 103 to 111% at test termination. The 72-hour NOE_rC and NOE_bC were determined to be 32 mg test item/L and 10 mg test item/L, respectively. 72-hour E_rC_{50} and E_bC_{50} were calculated to be 54 mg test item/L and 48 mg test item/L, respectively. 72-hour E_rC_{10} and E_bC_{10} were calculated to be 33 mg test item/L and 18 mg test item/L, respectively. The validity criteria according to the current guideline OECD Guideline 201 were met.

Furthermore, the 7-day EC_{50} for *N. pelliculosa* exposed to glyphosate technical was calculated to be 24.9 mg test item/L (mean measured), in a study performed by [redacted] (1987c). This study was previously considered invalid by applicant. Nevertheless, RMS checked the validity criteria and found that all validity criteria were met according to OECD 201 (2011) guideline studies. As only a 7d EC50 based on yield is available in the study report, 72h ECx (EC10, EC20 and EC50) based on yield and growth rate should be calculated (data gap).

Supportive studies (aquatic algae)

In addition to the presented studies which were considered valid for classification purposes, a number of supportive algal studies are available.

In a study by [redacted] (2000b), the toxicity of glyphosate technical to the green alga *Selenastrum capricornutum* was determined in a 96-hour, static testThis study is considered supportive only. The 72-h NOEC of both, growth and yield derived in a statistical re-evaluation by [redacted] (2020e) was 5.6 mg a.e./L (nominal). The 72-hour E_rC_{10} was determined to be 62.6 mg a.e./L. The 72-hour EC_{50} values on growth and yield were 469 and 75.9 mg a.e./L (nominal), respectively. The validity criteria according to the current guideline OECD 201 were met. Analytical work was performed only at test initiation, yet not throughout the test nor at test end, as required in current test guidelinesThis study is considered supportive only.

In the study by [redacted] (1995c), a 72-hour NOE_rC of 25 mg a.s./L was derived in a study assessing the toxicity of glyphosate acid on *D. subspicatus*. The study report is not available and validity cannot be checked. The study was therefore not assessed and its endpoint is not critical. This also applies to a further supporting study by Dengler (1994) testing the toxicity of glyphosate IPA salt on single cell green alga *Scenedesmus subspicatus.* The 72-hour NOE_rC and NOE_bC values were determined to be 24 and 4.8 mg a.s./L. 72-hour E_rC_{50} and E_bC_{50} values were 166 and 72.9 mg a.s./L, respectively. Data were provided in the Monograph 2001 and relied upon in the previous evaluation, RAR 2015.

Invalid studies (aquatic algae)

Furthermore, several studies considered as invalid are available and presented here as well for the sake of completeness.

The toxicity of glyphosate acid to the blue-green alga *Anabaena flos-aquae* was determined in a 120-hour, static test by [redacted] (1996b). The nominal based 72-hour NOE_bC and NOE_rC values were both 12 mg a.s./L, respectively. Nominal based 72-hour E_bC_{50} was determined to be 8.5 mg a.s/L and the 72-hour E_rC_{50} was 22 mg a.s./L. Raw data of the study is given in optical density. A satisfactory correlation between optical density and biomass cannot be made as the report does not provide a calibration curve. Therefore, this study is not considered reliable.

[redacted] (2003) determined the toxicity of glyphosate K-salt on *Raphidocelis subcapitata* in a 72-hour static toxicity test. The 72-hour NOEC was 30 mg test item/L. The 72-hour E_bC_{50} and E_rC_{50} were determined at 74 and 114 mg test item/L, respectively. The validity criteria according to the current OECD guideline 201 were not met. Therefore, this study is not considered valid.

In another invalid study by [redacted] (1993d), the 72-hour NOE_rC for *Desmodesmus subspicatus* and glyphosate IPA salt was determined to be 15.8 mg test item/L. The 72-hour E_rC_{50} was determined to be 241 mg/L and the derived 72-hour E_bC_{50} was 41.1 mg /L. The validity criteria according to the current OECD guideline 201 were not met in this study.

[redacted] (1990) derived a 96-hour LC_{50} value of 136 mg glyphosate/L in a study with *D. subspicatus* considered as not relevant according to various shortcomings (validity criteria could not be checked, no analytical measurements).

[redacted] (1990d) assessed the effects of glyphosate on *D. subspicatus* in a 96-hour static toxicity test. The 96-hour NOE_bC was 40 mg test item/L. The 72-hours E_bC_{50} was 326.9 mg/L, the 96-hours E_bC_{50} was 117.8 mg/L. The validity criteria according to the current OECD guideline 201 were not met. Hence, this study is not considered valid.

In a further invalid study, the toxicity of glyphosate acid to the freshwater diatom *Navicula pelliculosa* was determined in a 120-hour, static test ([redacted], 1996c). The NOE_rC after 72 hours of exposure was 18 mg test item/L. The NOE_bC was 3.2 mg test item/L. The 72-hour E_bC_{50} was 16 mg test item/L; the 72-hour E_rC_{50} was 17 mg test item/L. The test was not considered valid since the current guideline OECD 201 validity criteria were not met.

Furthermore, the 7-day EC_{50} for *Skeletonema costatum* exposed to glyphosate technical was calculated to be 0.64 mg test item/L (mean measured), in a study performed by [redacted] (1987d). The validity criteria according to the current OECD guideline 201 were not met. Therefore, the study is not valid.

Another non-GLP study ([redacted] 1978b) studied the effects of seven glyphosate-related test items on *S. costatum* in algal toxicity test. For the solid test items (Glyphosate, BN-78-44, and Glyphosate intermediate, BN-78-45), the EC_{50} values varied from 1.2 mg test item/L to 320 mg test item/L. For the liquid test items (Comp. #1, BN-78-46, Comp. #2, BN-78-47, Comp. #3A, BN-78-48, Comp. #4, BN-78-49 and Comp. 5A.), the EC_{50} values varied between 1% effluent to 19% effluent. Validity criteria, biomass and growth rates could not be checked as only

percentage changes in cell number and chlorophyll a compared to control were presented in the study report. In addition, the study is not GLP, analytical measurements of tested concentrations were not conducted and the exact composition of the 7 tested items is not clear, as no analytical certificate are available. Therefore, RMS considers this study not reliable for risk assessment.

Furthermore, [redacted] (1996) calculated the NOEC for biomass and growth rate both to be 1.0 mg test item/L. The 96-hour E_rC_{50} was determined to be 11.90 mg test item/L. The 96-hour E_bC_{50} for *Nitzschia palea* was 4.47 mg test item/L. The static toxicity test is not considered valid since the validity criteria according to the current OECD guideline 201 were also not met.

Several studies were cited but could not be assessed because the study report were missing ([redacted]. (1995c), [redacted] (1994), Report no.: 95-00554 (CA 8.2.6.1/012), [redacted] (1995c), Report no.: R481 (CA 8.2.6.1/008). For more details about this, please consider the information indicated in the Volume 3 CA B.9.

The most sensitive acute endpoint resulted in the study with the marine algae *Skeletonema costatum* by [redacted] (1996a) considering the updated evaluation by [redacted] (2020a) with a 72-hour E_rC_{50} of 13.5 mg a.s./L (nominal).

The most sensitive chronic endpoint resulted in the study with the marine algae *Skeletonema costatum* by [redacted] (1996a) considering the updated evaluation by [redacted] (2020a) with a 72-hour E_rC_{10} of 1.87 mg a.s./L (nominal).

Valid studies (aquatic plants)

Long-term effects of glyphosate on aquatic plants were tested in six experimental studies. The results of all available studies are presented below.

The effect of glyphosate IPA-salt on the growth of the duckweed *Lemna minor* was evaluated in a 7 day semi-static toxicity test at nominal concentrations of 2.92, 5.83, 11.7, 24.3, 48.6 and 97.2 mg test item/L, equivalent to 2.16, 4.32, 8.64, 18.0, 36.0 and 72.0 mg a.e./L ([redacted], 2002). The mean measured content of the IPA-salt ranged between 96 and 104% of nominal concentrations. According to the statistical re-analysis ([redacted] 2020f), the overall NOEC over a 7-day exposure period was 8.65 mg a.e./L for growth (frond number), for yield (dry weight and frond number) and for visual phyotoxic effects. The EC_{10} for growth based on frond number was 8.16 mg a.e./L (nominal). The EC_{10} for yield based on frond number was 7.8 mg a.e./L (nominal) and based on dry weight was 5.72 mg a.e./L (nominal). The 7-day E_rC_{50} was 30.3 mg a.e./L (nominal) and the 7-day E_yC_{50} was 16.5 mg a.e./L (nominal) based on frond numbers. The 7-day E_yC_{50} was 32.1 mg a.e./L (nominal) based on dry weight. A data gap is set for determination of ErCx values based on dry weight. This study is considered valid.

Similar results were obtained in a further guideline study conducted by [redacted] (1996d). In this study, the effects of glyphosate acid on growth of *L. gibba* were evaluated in a 14-day semi-static toxicity test. The test was performed with eight concentration levels, 0.75, 1.5, 3.0, 6.0, 12, 24, 48 and 96 mg a.s./L. The measured concentrations of glyphosate acid in the fresh media ranged from 90 – 108% of nominal and in the old media from 87 – 102% of nominal (overall mean measured: 93 – 100% of nominal). Results showed a significant inhibition of frond number growth of *L. gibba*. A statistical re-analysis of endpoints has been performed ([redacted] 2020h). The 7-day yield and growth rate based NOEC values were 6.0 and 12.0 mg a.s./L, respectively. The 7-day NOEC value for visual phytotoxic effects was 1.5 mg a.e./L. The E_rC_{10} (based on frond number) was 13.3 mg a.s./L. The 7-day EC_{50} values for yield and growth rate based on frond numbers were 24.0 and 36.0 mg a.s./L, respectively. The validity criteria according to the current OECD guideline 221 were met and the study is considered valid.

Another valid guideline study assessed the effects of glyphosate technical in a further 14 day static toxicity test with *L. gibba* ([redacted], 1987e). The test was performed with five concentrations ranging from 4.28 to 49.4 mg glyphosate/L (mean measured). Calculated endpoints are based on geometric mean measured concentrations. Statistical re-analysis of endpoints has been performed [redacted], 2020i). Based on frond number, the calculated EC_{10} value is 18.2 mg a.e./L for yield and 20.8 mg a.e./L for growth rate. NOEC values for yield and growth rate were both 16.6 mg a.e./L. The calculated EC_{50} value is 25.0 mg a.e./L for yield (frond number) and >49.4 mg a.e./L for growth rate (frond number). All validity criteria according to the OECD guideline 221 were fulfilled.

Invalid studies (aquatic plants)

The toxicity of glyphosate acid on growth of *Myriophyllum aquaticum* was evaluated in a 14-day static toxicity test, with subsequent 7-day recovery period ([redacted] 2012). Glyphosate acid significantly inhibited the fresh weight of *M. aquaticum* after 14 days at a nominal concentration of <5.0 mg glyphosate acid/L. Shoot length was inhibited at or above nominal concentrations of 5.0 mg glyphosate acid/L. The 14-d EC_{50} value for fresh weight inhibition was 12.3 mg glyphosate acid/L and for shoot length it was 78.7 mg glyphosate acid/L. *M. aquaticum* pre-exposed for 14 days to up to 50.0 mg glyphosate acid/L were able to recover in untreated culture medium after a 7-day recovery period. Lowest derived 7-day EC_{50} value was 23.4 mg glyphosate acid/L based on growth rate (fresh weight). Lowest derived chronic value was the growth rate based EC_{10} of 2.40 mg glyphosate acid/L. nevertheless study did not fulfill the validity criteria and could therefore not be considered to be valid.

In a further study, the effects of glyphosate IPA-salt on growth of *Lemna gibba* were evaluated in a 14-day semistatic toxicity test with five concentrations in the range between 6.25 and 100 mg test item/L ([redacted]-, 1999). Analytical recovery of the test item ranged from 78 to 113% from 4 to 7 days. Therefore, calculated endpoints are based on geometric mean concentrations. According to the statistical re-analysis ([redacted] 2020g), the overall NOEC to *L. gibba* over a 7-day exposure period was 14.7 mg a.e./L and the E_rC_{10} (based on frond number) was 12.8 mg a.e./L. The 7-day E_rC_{50} is 34.8 mg a.e./L. The validity criteria according to the current guideline OECD 221 were met but the analytical measurements were not conducted between day 0 and day 4 and between day 7 and day 11. Therefore, as the actual exposure is questionable, the study is considered not reliable.

Additionally, one literature article is available assessing the inhibitory activities of glyphosate on the aquatic macrophyte *Spirodela polyrhiza* (Yanhui *et al.*, 2015). The effects of glyphosate were tested in a semi-static exposure of 7 days at concentrations between 8.4 and 20.902 mg/L. The results showed that glyphosate had remarkable effects on the growth inhibition of *S. polyrhiza*, and the inhibitory rate increased with higher concentrations. The 168-hour EC_{50} value was determined to be 12.82 mg/L. This study was conducted according to guideline but not according to GLP. The test concentrations were not analytically verified and thus the exact exposure concentrations of the aquatic macrophyte are unknown. The study report is in Chinese and a translated version was not available to RMS. Validity criteria, biomass and growth rates could not be checked as no raw data is presented. Therefore, the study was considered as not reliable.

One invalid non-GLP study is documented in the dossier ([redacted], 1987f) since study report was not available.

The most sensitive acute endpoint for aquatic plants resulted in the study with *Lemna minor* by [redacted] (2002) with a 7-day E_rC_{50} of 30.3 mg a.s./L (nominal).

The most sensitive chronic endpoint for aquatic plants resulted in the study with *Lemna gibba* by [redacted] (1996) with a 7-day NOEC of 1.5 mg a.s./L (nominal) based on visual phytotoxic effects. Based on growth rates, the most sensitive chronic endpoint for aquatic plants resulted in the study with *Lemna minor* by [redacted] (2002) and [redacted] (2020f) with a 7-day E_rC_{10} of 8.16 mg a.s./L (nominal).

2.9.2.3.4 Chronic toxicity to other aquatic organisms

One study ([redacted] 2012b) is available testing the effects of glyphosate acid on amphibian metamorphosis of the African clawed frog (*Xenopus laevis*). The Amphibian Metamorphosis Assay was conducted under flow-through conditions and amphibian larvae were exposed to glyphosate acid at nominal concentrations of 0 (negative control), 0.16, 0.80, 4.0, 20, and 100 mg a.s./L. Arithmetic mean-measured concentrations were < 0.100 (<LOQ; control), 0.13, 0.79, 4.3, 20, and 90 mg a.s./L.

There were no treatment related effects on survival, stage, or normalized hind-limb length during the 21-day test. Histopathologic analysis showed no treatment related changes in the thyroid glands of *Xenopus laevis* tadpoles when compared to negative control animals. There was a slight increase in wet weight in the 100 mg a.s./L treatment group and in snout-to-vent length in the 4.0 and 100 mg a.s./L treatment groups at the end of the 21-day test, however, this difference in snout-vent length was not significant when normalized with hind-limb length. The study is considered valid and an overall 21-day NOEC of ≥100 mg a.s./L (arithmetic mean measured) was derived.

2.9.2.3.5 Chronic toxicity data for the metabolites of glyphosate

Table 2.9.2.3.5-1: Studies on chronic toxicity to aquatic organisms of the metabolites of glyphosate and the representative formulation MON 52276

Annex point	Study	Substance(s)	Test species	Study type	NOEC (mg a.e./L)	Status	Remark
CA 8.2.2.1/004	[redacted] 2011	AMPA	*Pimephales promelas*	Chronic, flow-through	12 (mm)	Valid	- (data gap : A statistical power analysis as presented in appendix 5 of the OECD 210 guideline)
CA 8.2.2.1/005	Rodrigues *et al.*, 2019*).*	AMPA	*Danio rerio* embryo	acute toxicity to zebrafish embryos	LC50 > 100 mg/L	reliable with restrictions	No analytical verification literature data
CA 8.2.5.1/007	[redacted] 2011	AMPA	*Daphnia magna*	21 d Reproduction semi-static	**Reproduction: 15 nom**	Valid	
CA 8.2.2.1/005 Literature data	Rodrigues *et al.*, 2019*).*	AMPA	*Danio rerio* embryo	acute toxicity to zebrafish embryos	96h-LC50 > 100 mg/L	supportive	no information on hatching rates in the treatment and control groups
CA 8.2.6.1/016 CA 8.2.6.1/017	[redacted] 1998 [redacted] 2020	AMPA	*Pseudokirchneriella subcapitata* *(Raphidocelis subcapitata)*	72 h algae inhibition	72h ErC50 = 191 mg AMPA/L (nom) 72h NOErC = 100 mg AMPA/L 72h ErC10 = 92.8 mg AMPA/L 72h ErC20 = 119 mg AMPA/L (nom) 72h EyC50 = 110 mg AMPA/L (nom) 72h NOEyC = 46 mg AMPA/L 72h EyC10 = 58.2 mg AMPA/L 72h EyC20 = 72.5 mg	valid	

Annex point	Study	Substance(s)	Test species	Study type	NOEC (mg a.e./L)	Status	Remark
					AMPA/L (nom)		
CA 8.2.6.1/018	[redacted] 1994	AMPA	*Scenedesmus subspicatus* *(Desmodesmus subspicatus)*	72 h algae inhibition	-	invalid	
CA 8.2.6.1/019 CA 8.2.6.1/020	[redacted] [redacted] 2011 [redacted] 2020	HMPA	*Pseudokirchneriella subcapitata* *(Raphidocelis subcapitata)*	72 h algae inhibition	72h ErC50 >120 mg HMPA/L (nom) 72h NOErC = 60 mg HMPA/L 72h ErC10 >120 mg HMPA/L 72h ErC20 >120 mg HMPA/L (nom) 72h EyC50 > 120 mg HMPA/L (nom) 72h NOEyC = 60 mg HMPA/L 72h EyC10 = 57.8 mg HMPA/L 72h EyC20 = 80.4 mg HMPA/L (nom)	valid	
CA 8.2.7/011	[redacted] 2012	AMPA	*Myriophyllum aquaticum*	14-d static	<u>Shoot length</u> 14d ErC50 > 94.6 mg AMPA/L (mm) 14d NOErC = 14.3 mg AMPA/L 14d ErC10 = 6.1 mg AMPA/L 14d ErC20 = 22.5 mg AMPA/L (mm) 14d EyC50 > 94.6 mg AMPA/L (mm) 14d NOEyC = 5.43 mg AMPA/L	valid	

Annex point	Study	Substance(s)	Test species	Study type	NOEC (mg a.e./L)	Status	Remark
					14d EyC10 = 1.3 mg AMPA/L 14d EyC20 = 5.8 mg AMPA/L (mm) <u>Shoot fresh weight</u> 14d ErC50 > 94.6 mg AMPA/L (mm) 14d NOErC = 14.3 mg AMPA/L 14d ErC10 = 24.2 mg AMPA/L 14d ErC20 = 39 mg AMPA/L (mm) 14d EyC50 = 70.8 mg AMPA/L (mm) 14d NOEyC = 14.3 mg AMPA/L 14d EyC10 = 19.7 mg AMPA/L 14d EyC20 = 30.6 mg AMPA/L (mm) <u>Shoot dry weight</u> 14d ErC50 = 72 mg AMPA/L (mm) 14d NOErC = 37.1 mg AMPA/L 14d ErC10 = 38.4 mg AMPA/L 14d ErC20 = 47.6 mg AMPA/L (mm) 14d EyC50 = 63.2 mg AMPA/L (mm)		

Annex point	Study	Substance(s)	Test species	Study type	NOEC (mg a.e./L)	Status	Remark
					14d NOEyC = 37.1 mg AMPA/L 14d EyC10 = 33.9 mg AMPA/L 14d EyC20 = 42 mg AMPA/L (mm) <u>Root length</u> 14d ErC50 > 94.6 mg AMPA/L (mm) 14d NOErC = 14.3 mg AMPA/L 14d ErC10 = 17 mg AMPA/L 14d ErC20 = 35.9 mg AMPA/L (mm) 14d EyC50 = 31.1 mg AMPA/L (mm) 14d NOEyC = 2.23 mg AMPA/L 14d EyC10 = 5.1 mg AMPA/L 14d EyC20 = 9.5 mg AMPA/L (mm)		
CA 8.2.7/012	[redacted] 2011	HMPA	*Lemna gibba*	7-d, semi-static	<u>Frond number/biomass dry weight</u> 7d ErC50 > 123 mg HMPA/L (nom) 7d NOECr = 123 mg HMPA/L 7d ErC10 > 123 mg HMPA/L 7d ErC20 > 123 mg HMPA/L (nom) 7d EyC50 > 123 mg HMPA/L (nom)	Valid	

Annex point	Study	Substance(s)	Test species	Study type	NOEC (mg a.e./L)	Status	Remark
					7d NOECy = 123 mg HMPA/L 7d EyC10 > 123 mg HMPA/L 7d EyC20 > 123 mg HMPA/L (nom)		

2.9.2.4 Comparison with the CLP criteria

2.9.2.4.1 Acute aquatic hazard

Table 72: Summary of information on acute aquatic toxicity relevant for classification

Method	Species	Test material	Results	Remarks	Reference
US EPA Guideline, FIFRA subdivision E, section 71-1 GLP	*Lepomis macrochirus*	Glyphosate acid (95.6% purity)	LC_{50} (96 h): >32 mg a.s./L (nom)	pH issue (pH outside the recommended range at all tested concentration. Endpoints set at the highest dose without mortality)	[redacted] (1995a), Report no.: [redacted]5553/B (CA 8.2.1/009)
EPA FIFRA, Subdivision E, Guideline 72-3 ASTM (1989) E724/9-85-012 (OPPTS 850.1055) GLP	*Crassostrea gigas*	Glyphosate acid (95.6% purity)	EC_{50} (48 h): 40 mg a.e./L (nom)	-	[redacted] (1996a), Report no.: AB0503/G (CA 8.2.4.2/003)
OECD Guideline No. 201 (1984) US EPA Guideline 540/09-82-020 (1982) GLP	*Skeletonema costatum*	Glyphosate acid (95.6% purity)	E_rC_{50} (72 h): 13.5 mg a.e./L (nom)	Statistical re-evaluation	[redacted] (1996a), Report no.: AB0503/I (CA 8.2.6.2/006) [redacted] (2020a), Report no.: 110054-007 (updated statistical evaluation) (CA 8.2.6.2/007)
OECD Guideline 221 GLP	*Lemna minor*	Glyphosate isopropylamine salt (97.1% purity)	Frond number 7d ErC50 = 30.3 mg a.e./L (nom)	Results based on statistical re-evaluation	[redacted] (2002), Report no.: CEMR-1873 (CA

				Data gap: ErCx values based on dry weight	8.2.7/001) [redacted] (2020f), Report no.: 110054-008 (updated statistical evaluation) (CA 8.2.7/002)

a.e.: acid equivalents, nom: nominal,

2.9.2.4.2 Long-term aquatic hazard (including bioaccumulation potential and degradation)

Table 73: Summary of information on long-term aquatic toxicity relevant for classification

Method	Species	Test material	Results	Remarks	Reference
Guideline 72-6	Bluegill Sunfish (*Lepomis macrochirus)*	^{14}C glyphosate (N-phosphonomethylglycine-methyl-14C, 99.2% purity)	No reliable BCF value. Indication of low bioaccumulation potential.	provide evidence that the potential for bioaccumulation of glyphosate is low	[redacted] (1989a), Report no.: [redacted]-9304 (CA 8.2.2.3/001) [redacted] (1989), Report no.: [redacted]-9303 (CA 8.2.2.3/002)
IBAMA 1990: Manual de testes para avaliacao da ecotoxicidade de agentes quimicos GLP	*Brachydanio rerio*	Glyphosate acid (954.9 g/kg acid equivalent)	NOEC (7 d): 1 mg a.s./L (nom)	-	[redacted] (2000c), Report No.: [redacted]-D62.16/99 (CA 8.2.2.1/002)
OECD Guideline 202, Part II, Reproduction Test (1984) GLP	*Daphnia magna*	Glyphosate acid (97.6% purity)	NOEC (21 d): 12.5 mg a.s./L (nom)	-	[redacted] (1999), Report no.: AF0497/B (CA 8.2.5.1/001)
OECD Guideline. 201 (1984) US EPA Guideline 540/09-82-020 (1982) GLP	*Skeletonema costatum*	Glyphosate acid (95.6% purity)	E_rC_{10} (72 h): 1.87 mg a.s./L (nom) NOErC (72 h): 5.6 mg a.s./L (nom)	-	[redacted] (1996a), Report no.: AB0503/I (CA 8.2.6.2/006) [redacted] (2020a), Report no.: 110054-007 (updated statistical evaluation) (CA

					8.2.6.2/007)
OECD Guideline 221 GLP	*Lemna minor*	Glyphosate isopropylamine salt (97.1% purity)	Frond number 7d NOErC = 8.65 mg a.e./L (nom) 7d ErC10 = 8.16 mg a.e./L (nom)	-	[redacted] (2002), Report no.: CEMR-1873 (CA 8.2.7/001) [redacted] (2020f), Report no.: 110054-008 (updated statistical evaluation) (CA 8.2.7/002)

a.e.: acid equivalents, am.: arithmetic mean measured, gm: geometric mean measured, nom: nominal
Note: The substance is considered as not rapidly degradable (see 2.8.2).

2.9.2.5 Conclusion on classification and labelling for environmental hazards

Ready biodegradability of glyphosate was investigated in 1 study and showed that it is not readily biodegradable under the conditions of the test. Glyphosate was also shown to be not inherently biodegradable under the conditions of 2 tests. In addition, results from hydrolysis and water/sediment studies show that glyphosate is not degraded in the aquatic environment to a level > 70 % within a 28-day period. As a consequence, glyphosate is considered not rapidly degradable (see 2.8.2 for more details).

No validated BCF value is available but the study submitted provide evidence that the potential for bioaccumulation of glyphosate is low. This result on low bioaccumulation potential is further supported by the low log P_{ow} values below the trigger value of 4. Therefore, glyphosate is not considered as bioaccumulative for classification purposes.

Acute aquatic toxicity data on glyphosate are available for fish, invertebrates, algae and aquatic plants. Aquatic algae are the most sensitive trophic level. The lowest reliable acute endpoint is the 72-hour EC_{50} of 13.5 mg a.e./L (nominal) for *Skeletonema costatum* ([redacted] 1996a). This value is >1 mg/L and thus, glyphosate does not fulfill the criterion to be classified and labelled for acute aquatic hazards according to the consolidated version of Regulation (EC) No 1272/2008. An M-Factor does not need to be derived.

Chronic aquatic toxicity data on glyphosate are available for fish, aquatic invertebrates, algae, and aquatic plants. The lowest reliable chronic effect concentration is considered to be the 7-day NOEC of 1 mg a.s./L (nominal) for *Brachydanio rerio* ([redacted] (2000c)). As the lowest NOEC/EC_{10} is ≤ 1 mg/L and the substance is considered as non rapidly degradable, glyphosate is classified as Aquatic chronic 2 and should be labelled H411 "Toxic to aquatic life with long lasting effects" according to the consolidated version of Regulation (EC) No 1272/2008. An M-Factor does not need to be derived.

2.9.3 Summary of effects on arthropods

Honeybees

Table 2.9.3-1: Endpoints and effect values of glyphosate relevant for the risk assessment for honey bees, bumble bees and solitary bees

Annex point	Study	Test species	Substance(s)	Study type	LD_{50} (µg a.e./bee)	NOED (µg a.e./bee)	Status	Remark
Acute toxicity								
CA 8.3.1.1.1/001	[redacted] 2003	*Apis mellifera L.*	Glyphosate K-salt	Acute oral	**>104**	-	Valid	-
CA 8.3.1.1.1/002	[redacted] 1998	*Apis mellifera L.*	Glyphosate acid	Acute oral	>182	182	Valid	-

CA 8.3.1.1.1/003	[redacted] 1996	*Apis mellifera L.*	Glyphosate	Acute oral	>40	-	Valid	-
CA 8.3.1.1.1/004	[redacted] 1995	*Apis mellifera L.*	Glyphosate acid	Acute oral	>200	-	Valid	-
CA 8.3.1.1.1/005	[redacted] 1995	*Apis mellifera L.*	Glyphosate	Acute oral	116.67	-	Valid	-
CA 8.3.1.1.1/006	[redacted] 1972	*Apis mellifera L.*	Glyphosate technical and IPA-salt	Acute oral	-	-	Invalid	Cf RMS comment in study summary
CA 8.3.1.1.1/007	[redacted] 2017a	*Bombus terrestris*	Glyphosate IPA-salt (in MON 0139)	Acute oral	>412	412	Valid	-
CA 8.3.1.1.2/001	[redacted] 2003	*Apis mellifera L.*	Glyphosate K-salt	Acute contact	**>100**	-	Valid	-
CA 8.3.1.1.2/002	[redacted] 2000	*Apis mellifera L.*	Glyphosate isopropylamine salt	Acute contact	>61.3 (IPA salt equivalent) *	-	Valid	-
CA 8.3.1.1.2/003	[redacted] 1998	*Apis mellifera L.*	Glyphosate acid	Acute contact	>103	-	Valid	-
CA 8.3.1.1.2/004	[redacted] 1996	*Apis mellifera L.*	Glyphosate	Acute contact	>20	-	Valid	-
CA 8.3.1.1.2/005	[redacted] 1995	*Apis mellifera L.*	Glyphosate acid	Acute contact	>200	-	Valid	-
CA 8.3.1.1.2/006	[redacted] 1995	*Apis mellifera L.*	Glyphosate	Acute contact	>100	-	Valid	-
CA 8.3.1.1.2/007	[redacted] 1972	*Apis mellifera L.*	Glyphosate technical and IPA-salt	Acute contact	-	-	Invalid	Cf RMS comment in study summary
CA 8.3.1.1.2/008	[redacted] 2017a	*Bombus terrestris*	Glyphosate IPA-salt	Acute contact	>461	461	Valid	-
CA 8.3.1.1.2/009	[redacted] 2017b	*Osmia bicornis*	Glyphosate IPA-salt	Acute contact	>461	461	Valid	-
CP 10.3.1.1.1/001	[redacted] 2001	*Apis mellifera*	MON 52276	Acute oral, 48 h	**> 77**	-	Valid	-
CP 10.3.1.1.2/001	[redacted] 2001	*Apis mellifera*	MON 52276	Acute contact, 48 h	**> 100**	-	Valid	-

Chronic toxicity

Annex point	Study	Test species	Substance(s)	Study type	LDD_{50} (µg a.e./bee/d)	NOEDD (µg a.e./bee/d)	Status	Remark
CA 8.3.1.2/001	[redacted] 2017	*Apis mellifera*	Glyphosate IPA-salt (in MON 0139)	Chronic, Adult 10 days	>179.9	**179.9**	Valid	-

Honey bee development and other honey bee life stages toxicity

Annex point	Study	Test species	Substance(s)	Study type	LD_{50} (µg a.e./larva)	NOED (µg a.e./larva)	Status	Remark
CA 8.3.1.3/001	[redacted] 2020	*Apis mellifera*	Glyphosate IPA-salt (in MON 0139)	Chronic larvae, 22-day	-	80 **ED10 = 75.6**	Valid	-

Sub-lethal toxicity

Annex point	Study	Test species	Substance(s)	Study type	LD_{50} (µg a.e./L)	NOAEL (µg a.e./L)	Status	Remark
CA 8.3.1.4/001	[redacted] 2012	*Apis mellifera*	Glyphosate IPA-salt (in MON 0139)	Bee brood feeding test. Field study	-	**301 mg/L (nominal), 266 mg/kg, (measured)** .	Valid	-

Other studies

Annex point	Study	Test species	Substance(s)	Study type	Magnitude of residues in mg a.e./kg	Status	Remark
CP 10.3.1.5/001	[redacted] 2011	*Apis mellifera*	MON 52276	Residues in honeybee colony - Phacelia semi-field application at 8 L product/ha (2.88 g a.e./ha) during flowering and in the presence of foraging bees	Total daily intake of glyphosate residues (via nectar + pollen) of: - 269.3 mg a.e. (based on day 1 maximum mean residues), - 141.8 mg a.e. (based on mean residues over days 1-3).	Valid	-

a.e.: acid equivalents
Endpoints in **bold** is used for risk assessment
* acid equivalent purity not provided

Arthropods other than honeybees

Studies on effects of the representative formulation MON 52276 on non-target arthropods to fulfill the data requirements according to EU Regulation No 284/2013 are presented in the following. The validity of all studies (newly submitted as well as already submitted) have been checked based on latest guidelines available at time of assessment. The table below summarised the reliable and supportive data on non target arthropods.

Table 2.9.3-2: Endpoints and effect values of representative formulation MON 52276 relevant for the risk assessment for non-target arthropods other than bees

Reference	Test item	Species	Test design	Status	Mortality LR_{50}	Effects on reproduction
Tier 1 – laboratory studies						
[redacted] 1995CP 10.3.2.1/001	MON 52276	*Aphidius rhopalosiphi*	Laboratory	Supportive	10 L MON 52276/ha (3.6 kg a.e/ha) = 100% mortality at 24 hrs.	No reproduction endpoints available.

Reference	Test item	Species	Test design	Status	Mortality LR_{50}	Effects on reproduction
[redacted] 1995 CP 10.3.2.1/002	MON 52276	*Typhlodromus pyri*	Laboratory	Supportive	10 L MON 52276/ha (3.6 kg a.e/ha) = 100% mortality at day 4.	No reproduction endpoints.
[redacted] 1995 CP 10.3.2.1/003	MON 52276	*Poecilus cupreus*	Laboratory	Valid	> 10 L/ha (3600 g a.e./ha)	-
[redacted], 1995 CP 10.3.2.1/004	MON 52276	*Pardosa sp.*	Laboratory	Valid	> 10 L/ha (3600 g a.e./ha)	-
Tier 2 – extended laboratory and aged residue						
[redacted] 1998 CP 10.3.2.2/003	MON 52276	*Typhlodromus pyri*	Extended laboratory	Supportive	Indicative of an effect on mortality at 6 L/ha (84%) and 12 L/ha (89%)	-
[redacted] 2010 CP 10.3.2.2/001	MON 52276	*Typhlodromus pyri*	Extended laboratory 2D	Valid	> 16.0 L/ha (5760 g a.e./ha)	**$ER_{50} \geq 12$ L/ha (4320 g a.e./ha) Reduction in no. of egg/female 44.9 % at 12 L/ha** NOER = 8 L/ha (2880 g a.e./ha)
[redacted], 1999 CP 10.3.2.2/005	MON 52276	*Aphidius rhopalosiphi*	Extended laboratory	Supportive	Effects on mortality: less than 50% expected up to 12 L/ha	No adverse effects on reproduction expected up to 12L/ha
[redacted] 2010 CP 10.3.2.2/004	MON 52276	*Aphidius rhopalosiphi*	Extended laboratory 3D	Valid	> 16.0 L/ha (5760 g a.e./ha)	**$ER_{50} > 16$ L/ha (5760 g a.e./ha)** NOER ≥ 16 L/ha (5760 g a.e./ha)

Reference	Test item	Species	Test design	Status	Mortality LR_{50}	Effects on reproduction
[redacted] 2010 CP 10.3.2.2/007	MON 52276	*Aleochara bilineata*	Extended laboratory	Valid	> 12.0 L/ha (4320 g a.e./ha)	ER_{50} > 12 L/ha (4320 g a.e./ha) NOER ≥ 12 L/ha (4320 g a.e./ha)
[redacted] 1999 CP 10.3.2.2/008	MON 52276	*Chrysoperla carnea*	Extended laboratory	Supportive	LR_{50}: 10.34 L MON 52276/ha (supportive)	No reliable endpoint could be set for reproduction.

a.e. glyphosate acid equivalents
Endpoints in **bold** are used for risk assessment

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

Chronic earthworm toxicity studies have been conducted with glyphosate, the main metabolite AMPA and the product MON 52276. Studies on other soil organisms are available with glyphosate and the main metabolite AMPA.

Table 2.9.4-1: Endpoints and effect values of glyphosate, metabolite AMPA and representative formulation MON 52276 relevant for the risk assessment for soil organisms

Reference	Test item	Species	Test design/ GLP	Status	NOEC
[redacted] 2009 CA 8.4.1/001	Glyphosate IPA-salt (in MON 0139)	*Eisenia fetida andrei*	Mixed into substrate 56 d, chronic 10% peat content	valid	473 mg a.e./kg dry soil
[redacted] 2000 CA 8.4.1/002	Glyphosate IPA salt (in MON 0139)	*Eisenia fetida*	Mixed into substrate 56 d, chronic 10% peat content	Supportive*	21.31 mg a.e./kg dry soil
[redacted] 2009 CA 8.4.2.1/002	Glyphosate IPA-salt (in MON 0139)	*Hypoaspis aculeifer*	Mixed into substrate 14 d, chronic 5% peat content	valid	473 mg a.e./kg dry soil
[redacted] 2010 CA 8.4.2.1/001	Glyphosate IPA-salt (in MON 0139)	*Folsomia candida*	Mixed into substrate 28 d, chronic 10% peat content	valid	587 mg a.e./kg dry soil
[redacted] 2000 CA 8.4.1/002	AMPA	*Eisenia fetida*	Mixed into substrate 56 d, chronic 10% peat content	Supportive**	28.12 mg met./kg dry soil
[redacted] 2003 CA 8.4.1/003	AMPA	*Eisenia fetida fetida*	Mixed into substrate	valid	131.9 mg met./kg dry soil

Reference	Test item	Species	Test design/ GLP	Status	NOEC
			56 d, chronic 10% peat content		
[redacted] 2002 CA 8.4.1/004	AMPA	*Eisenia fetida fetida*	Mixed into substrate 56 d, chronic 10% peat content	Supportive***	19.7 mg met./kg dry soil
[redacted] 2010 CA 8.4.2.1/004	AMPA	*Hypoaspis aculeifer*	Mixed into substrate 14 d, chronic 5% peat content	valid	320 mg met./kg dry soil
[redacted] 2010 CA 8.4.2.1/003	AMPA	*Folsomia candida*	Mixed into substrate 28 d, chronic 5% peat content	valid	315 mg met./kg dry soil
[redacted] 2020 CP 10.4.1.1/001	MON 52276	*Eisenia fetida*	Mixed into substrate 56 d, chronic 10% peat content	Valid	38 mg a.e./kg dry soil
[redacted] 2020 CP 10.4.2.1/002	MON 52276	*Hypoaspis aculeifer*	Mixed into substrate 14 d, chronic 5% peat content	Valid	1802 mg a.e./kg dry soil
[redacted]2020 CP 10.4.2.1/001	MON 52276	*Folsomia candida*	Mixed into substrate 28 d, chronic 5% peat content	Valid	1802 mg a.e./kg dry soil

a.e. glyphosate acid equivalents
*not in line with latest guideline (assimilation to limit-test possible but will have required higher number of replicates)
**not in line with latest guideline (assimilation to limit-test as 2 concentrations instead of 5 will require 8 replicates instead of 4)
***design not in line with latest guideline. One validaity criteria not met (CV<30%, actual 38%) (assimilation to limit-test possible but will have required higher number of replicates)

2.9.5 Summary of effects on soil nitrogen transformation

Chronic earthworm toxicity studies have been conducted with glyphosate, the main metabolite AMPA and the product MON 52276. Studies on other soil organisms are available with glyphosate and the main metabolite AMPA.

Table 2.9.5-1: Endpoints and effect values of glyphosate, metabolite AMPA and representative formulation MON 52276 relevant for the risk assessment for soil microflora

Reference	Test item	Species	Test design	Effect
[redacted] 2014 CA 8.5/001	Glyphosate acid	N-mineralisation	28 d, aerobic	**< 25% effect at Day 28 at 33.1 mg/kg dry soil ***
[redacted] 2010 CA 8.5/004	AMPA	N-mineralisation	28 d, aerobic	**< 25% effect at Day 28 at 160 mg/kg dry soil** (supportive**)
[redacted] 2012 CP 10.5/001	MON 52276	N- mineralisation, 28 d	28 d, aerobic	< 25% effect at Day 28 at 28.8 mg a.e./kg dry soil

a.e. glyphosate acid equivalents
* Data gap: No nitrate was measured at day 7 in none of the treatments including control. The study do not report any malfunction nor comments the absence of nitrate at this time point. The applicant is requested to provide clarification on this point (see study summary)
** Data gap: applicant to submit soil nitrogen transformation rate expressed in mg nitrate/kg dry weight soil/day between each measurement day (see study summary)

2.9.6 Summary of effects on terrestrial non-target higher plants

Studies considering the toxicity of glyphosate to terrestrial non-target plants were assessed for their validity to current and relevant guidelines for MON 52276. Endpoints of studies for the representative formulation MON 52276 considered in the risk assessment is presented in the table below.

Table 2.9.6-1: Studies on toxicity of representative formulation to terrestrial non-target higher plants

Annex point	Study	Study type	Test species	Substance (s)	Status	ER_{50} (g a.e./ha)	NOER (g a.e./ha)	Remark
CA 8.6.2/001	[redacted] 1994	Vegetative vigour, 21d	Soybean, Lettuce, Radish, Tomato, Cucumber, Cabbage, Oat, Ryegrass, Corn, Onion	Glyphosate	Valid	145.7 (tomato, dry weight)		ER50 is provisional Datagap set for ECx values for phytotoxicity
CA 8.6.2/002	[redacted] 1994		Onion, Field corn, Oat, Wheat, Soybean, Radish, Cucumber, Sunflower, Tomato, Carrot	Glyphosate	Invalid			already invalid in RAR 2015
CP 10.6.2/001	[redacted], 2019	Seedling emergence, 21d	*Cucumis sativus* *Brassica napus* *Raphanus sativus* *Glycine max* *Helianthus annuus* *Lycopersicon esculentum* *Zea mays* *Triticum aestivum* *Avena sativa* *Allium cepa*	MON 52276	Valid	> 3610 (all tested species and all parameters)	≥ 3610 (all tested species and all parameters)	-
CP 10.6.2/002	[redacted] 2013	Vegetative vigour, 21d	*Zea mays* *Avena sativa* *Allium cepa* *Triticum aestivum* *Cucumis sativus* *Brassica napus* *Raphanus sativus* *Glycine max* *Helianthus annuus* *Lycopersicon esulentum*	MON 52276	supportive	28.4 (cucumber, shoot length)	< 20 (cucumber: shoot length, shoot weight; sunflower, tomato: shoot weight)	potential under estimation of effects
CP 10.6.2/003	[redacted] 2005	Vegetative vigour, 21d	*Beta vulgaris* *Raphanus rapistrum* *Lepidium sativum*	MON 52276	Invalid	-	-	already invalid in RAR 2015

Annex point	Study	Study type	Test species	Substance (s)	Status	ER_{50} (g a.e./ha)	NOER (g a.e./ha)	Remark
			Pisum sativum *Lolium perenne* *Triticum aestivum*					
CP 10.6.2/004	[redacted] 2012	Comparison of Post-Emergence Phytotoxicity	*Echinochloa crus-galli* *Xanthium strumarium* *Zea mays* *Digitaria ischaemum* *Setaria veridis* *Chenopodium album* *Ipomoea sp.* *Panicum miliaceum* *Oryza sativa* *Polygonum pensylvanicum* *Sorghum bicolor* *Glycine max* *Beta vulgaris* *Abutilon theophrasti* *Triticum aestivum* *Polygonum convolvulus*	MON 52276 and AMPA	Supportive	-	-	Full evaluation of study not feasible
CP 10.6.2/005	[redacted] 2021	Vegetative Vigour test, 21d	*Zea mays* *Avena sativa* *Allium cepa* *Triticum aestivum* *Cucumis sativus* *Brassica napus* *Raphanus sativus* *Glycine max* *Helianthus annuus* *Lycopersicon esulentum*	MON 52276	Valid but result of cucumber unreliable	69.87 (shoot fresh weight of *Lycopersicon esculentum* (tomato)	15.7 (shoot fresh weight of *Glycine max* (soybean) and shoot height of *Brassicus napus* (oilseed rape).	Results for cucumber are not reliable.

2.9.7 Summary of effects on other terrestrial organisms (flora and fauna)

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2.9.8 Summary of effects on biological methods for sewage treatment

Table 2.9.8-1: Effects on biological methods for sewage treatment

Reference (Data owner)	Test item	Species	Test design/ GLP	EC_{50} (mg a.e./L)	NOEC (mg a.e./L)
[redacted] 1990 CA 8.8/002	Glyphosate technical	Activated sludge bacteria	Oxygen consumption of activate sludge over 3 h	> 100	100

2.9.9 Summary of product exposure and risk assessment

2.9.9.1 Summary of risk assessment for birds and other terrestrial vertebrates

A risk assessment for birds and mammals was conducted according to the Guidance Document on Risk Assessment for Birds and Mammals (EFSA 2009/1438). The following use scenarios were covered by the assessment.

GAP number and summary of use	Application rate considered (28 day interval unless otherwise stated)									
	1 × 540 g/ha	1 × 720 g/ha	1 × 1080 g/ha	2 × 720 g/ha	1 × 1440 g/ha	3 × 720 g/ha	1 × 1800 g/ha	2 × 1080 g/ha[1]	2 × 1440 g/ha	2 × 1800 g/ha (90 days apart)
Uses 1a-c: Applied to weeds; pre-sowing, pre-planting, pre emergence of field crops.		X	X		X					
Uses 2 a-c: Applied to weeds; post-harvest, pre-sowing, pre-planting of field crops.		X	X	X	X	X		X		
Use 3 a-b: Applied to cereal volunteers; post-harvest, pre-sowing, pre-planting of field crops.	X									
Use 4 a-c: Applied to weeds (post emergence) below trees in orchards.		X	X	X	X	X		X	X	
Use 5 a-c: Applied to weeds (post emergence) below vines in vineyards		X	X	X	X	X		X	X	
Use 6 a-b: Applied to weeds (post emergence) in field crops BBCH < 20		X	X							
Use 7 a-b: Applied to weeds (post emergence) around railroad tracks							X			X
Use 8 and 9: Applied to invasive species (post emergence) in agricultural and non-agricultural areas							X			
Uses 10 a-c: Applied to couch grass; post-harvest, pre-sowing, pre-planting of field crops		X	X							

X = this use is covered by the application rate indicated.
1 Due to the long spray interval of 28 days this use covers also the following possible application pattern: 2 × 1080 g a.e./ha plus 1 × 720 g a.e./ha (28 day interval between each application).

Birds

The acute risk to birds were concluded to be low for all use scenarios based on the screening step according to EFSA (2009), while additional consideration was needed for some scenarios in the long-term risk assessment. From the first tier calculations, the long-term risk to birds was concluded to be low for all representative uses of glyphosate. The RMS considers that no higher tier refinement is needed for birds.

Mammals

The first tier calculations based the acute risk scenarios for mammals using the geomean acute oral LD50, resulted in no need for further refinements for any of the representative uses of glyphosate. For the long term risk, further considerations were needed for the vole scenario at all representative uses.

Higher tier

To address the identified risks to mammals, the applicant provided a refined risk assessment. Refinements were based on substance specific residue decline data to derive more realistic fTWA and MAF values in plant material. Based on this assessment, low risk was concluded for all representative use scenarios except for the use of 1.8 kg a.s./ha on railway tracks and control of invasive species, where a risk was still identified for 'voles'. This refinement is considered acceptable by the RMS, provided that some further information related to the kinetic evaluation of the residue decline data is presented by the applicant.

The applicant provided further justifications to demonstrate low risk for voles at the use on railway tracks and at control of invasive species. It was proposed that due to the spray applications targeted to a specific area (on and around railways, and on plant or stand of plants, respectively), the long term risk to mammals can be considered as low for these representative uses.

Overall, the RMS concludes that further information is needed to confirm a low long-term risk to wild mammals for all representative uses of glyphosate.

Amphibians and reptiles

No specific risk assessment has been provided for amphibians and/or reptiles. From the previous evaluation for renewal of glyphosate, it was concluded that possible risk for amphibians would be sufficiently covered by the risk assessment for aquatic organisms. According to the applicant, the new literature search did not reveal any adverse data that would change the conclusion from the previous evaluation. However, based the RMS' evaluation of the new literature data, effects on amphibians cannot be excluded even from low glyphosate exposure levels. Hence, the aquatic risk assessment may not be sufficiently protective for amphibians and therefore it is proposed that further consideration is needed. It is acknowledged that there is no agreed EU guidance on how to carry out the risk assessment for these groups, however, some useful advice and recommendations are presented in the EFSA opinion from 2018: Scientific Opinion on the state of the science on pesticide risk assessment for amphibians and reptiles - - 2018 - EFSA Journal - Wiley Online Library.

Further consideration is also needed on possible risk to reptiles following direct overspray in the field.

2.9.9.2 Summary of risk assessment for aquatic organisms

PECsw/sed calculations provided by the applicant are not considered acceptable (see e-fate section). In order to provide a 1st informative estimation of PECsw for the peer review, STEP 1-2 PECsw were recalculated by RMS for the worst-case application pattern. In addition, endpoints used for risk assessment below are temporary since several data gaps were identified by RMS in studies for aquatic organisms. Therefore, these endpoints and PEC/RAC ratios may change after further information is submitted.

Moreover the risk assessment presented is considered as not finalized for both algae and aquatic plants. Indeed for algae it can not be confirmed that the risk assessment based on active substance data is protective as the toxicity of the product is not known. For aquatic plants, the test design of the Lemna studies (mix in media) is considered not appropriate for a contact herbicide (see above). There is a need to have results for emergent macrophytes available with a different exposure design (overspray) Moreover a test with Myriophyllum is required with the active substance. Therefore, a data gap is set for aquatic plants. In addition, as glyphosate is persistant in sediment, RMS considered that a test with a rooted macrophytes is necessary to finalise the risk assessment of aquatic plants.

Moreover based on their fate characteristics, glyphosate and AMPA are considered as persistant in sediment and chronic exposure of the sediment dwellers is expected. According to EU Reg 283/2013 section 8.2.5.4 test using spiked sediment or at least analytics in sediment is required to set an endpoint. However according to the current EFSA guidance on aquatic organisms (2013) and the EFSA opinion on sediment organisms (2015), sediment toxicity studies are triggered when the water–sediment study indicates that > 10 % of the applied radioactivity is present in the sediment at or after day 14 and the outcome of a chronic Daphnia test (or another comparable study with insects) results in an EC10 (or NOEC) < 0.1 mg/L. Since the lowest chronic Daphnia endpoint is greater than 0.1 mg/L, this study is not considered necessary for risk assessment purpose. However for compliance with the EU Reg 283/2013,

further information to assess the effects of glyphosate and AMPA on sediment dwelling organisms is required (data gap).

In relation with e-fate data gap, provide further information to assess the risk assessment for metabolite 1-oxo-AMPA for sediment dwelling organisms. For details, please refer to Volume 3 (AS) B.8 point B.8.2.2.5.

In the following tables, the ratios between predicted environmental concentrations of glyphosate in surface water (PEC_{SW}) and regulatory acceptable concentrations (RAC) for aquatic organisms are given per intended use (as described in below) for each FOCUS scenario and for each organism group.

Table 2.9.9.2-1: $FOCUS_{sw}$ step 1-2 – PEC/RACs for glyphosate – field uses at 2 x 1440 g a.s./ha

		fish acute	**fish chronic**	**Aquatic invertebrates**	**Aquatic invertebrates prolonged**	**Algae**	**Higher plant**
		Lepomis macrochirus	*Brachydanio rerio*	*Crassostrea gigas*	*Daphnia magna*	*Skeletonema costatum*	*Myriophyllum aquaticum*
		LC_{50}	NOEC	EC_{50}	NOEC	ErC_{50}	ErC_{50}
		32000 µg/L	1000 µg/L	40000 µg/L	12500 µg/L	13500 µg/L	10330 µg/L
AF		100	10	100	10	10	10
RAC (µg/L)		320	100	400	1250	1350	1033
Scenario	**PEC global max (µg L)**						
FOCUS Step 1							
	167.72	0.52	**1.68**	0.42	0.13	0.12	0.16
FOCUS Step 2							
North Europe	69.95	0.22	0.70	0.17	0.06	0.05	0.07
South Europe	56.86	0.18	0.57	0.14	0.05	0.04	0.06

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**

Table 2.9.9.2-2: FOCUS$_{sw}$ step 1-2 - TERs for AMPA – field uses at 2 x 1440 g a.s./ha

		fish acute	**fish chronic**	**Aquatic invertebrates**	**Aquatic invertebrates prolonged**	**Algae**	**Higher plant**
		Oncorhynchus mykiss	*Pimephales promelas*	*Daphnia magna*	*Daphnia magna*	*Pseudokirchneriella subcapitata*	*Myriophyllum aquaticum*
		LC_{50}	NOEC	EC_{50}	NOEC	ErC_{50}	ErC_{50}
		100000 µg/L	12000 µg/L	100000 µg/L	15000 µg/L	191000 µg/L	72000 µg/L
AF		100	10	100	10	10	10
RAC (µg/L)		1000	1200	1000	1500	19100	7200
Scenario	**PEC global max (µg L)**						
FOCUS Step 1							
	111.02	0.11	0.09	0.11	0.07	0.01	0.02
FOCUS Step 2							
North Europe	52.47	0.05	0.04	0.05	0.03	0.003	0.01

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**

Table 2.9.9.2-3: FOCUS$_{sw}$ step 1-2 – PEC/RACs for HMPA – field uses at 2 x 1440 g a.s./ha

		Aquatic invertebrates	**Algae**	**Higher plant**
		Daphnia magna	*Pseudokirchneriella subcapitata*	*Lemna gibba*
		EC_{50}	ErC_{50}	EC_{50}
		> 100000 µg/L	> 120000 µg/L	> 123000 µg/L
		100	10	10
		> 1000	> 12000	> 12300
Scenario	**PEC global max (µg L)**			
FOCUS Step 1				
	58.06	0.06	0.005	0.005
FOCUS Step 2				
North Europe	52.47	0.05	0.004	0.004

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**

Table 2.9.9.2-4: PEC/RACs for glyphosate – railways at 1 x 3600 g a.s./ha

		fish acute	**fish chronic**	**Aquatic invertebrates**	**Aquatic invertebrates prolonged**	**Algae**	**Higher plant**
		Lepomis macrochirus	*Brachydanio rerio*	*Crassostrea gigas*	*Daphnia magna*	*Skeletonema costatum*	*Myriophyllum aquaticum*
		LC_{50}	NOEC	EC_{50}	NOEC	ErC_{50}	ErC_{50}
		32000 µg/L	1000 µg/L	40000 µg/L	12500 µg/L	13500 µg/L	10330 µg/L
AF		100	10	100	10	10	10
RAC (µg/L)		320	100	400	1250	1350	1033
Scenario	**PEC global max (µg L)**						
Railway ditch	9.458	0.03	0.09	0.02	0.01	0.01	0.01

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**

Table 2.9.9.2-5: PEC/RACs for AMPA – railways at 1 x 3600 g a.s./ha

		fish acute	**fish chronic**	**Aquatic invertebrates**	**Aquatic invertebrates prolonged**	**Algae**	**Higher plant**
		Oncorhynchus mykiss	*Pimephales promelas*	*Daphnia magna*	*Daphnia magna*	*Pseudokirchneriella subcapitata*	*Myriophyllum aquaticum*
		LC_{50}	NOEC	EC_{50}	NOEC	ErC_{50}	ErC_{50}
		100000 µg/L	12000 µg/L	100000 µg/L	15000 µg/L	191000 µg/L	72000 µg/L
AF		100	10	100	10	10	10
RAC (µg/L)		1000	1200	1000	1500	19100	7200
Scenario	**PEC global max (µg L)**						
Railway ditch	6.210	0.01	0.01	0.006	0.004	0.0003	0.001

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**

Table 2.9.9.2-6: PEC/RACs for HMPA – railways at 1 x 3600 g a.s./ha

		Aquatic invertebrates	Algae	Higher plant
		Daphnia magna	*Pseudokirchneriella subcapitata*	*Lemna gibba*
		EC_{50}	ErC_{50}	EC_{50}
		> 100000 µg/L	> 120000 µg/L	> 123000 µg/L
AF		100	10	10
RAC (µg/L)		> 1000	> 12000	> 12300
Scenario	**PEC global max (µg L)**			
Railway ditch	0.627	> 0.001	> 0.0001	> 0.0001

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**

A summary of the risk assessment regarding aquatic biodiversity and indirect effects through trophic interaction resulted from uses of glyphosate is presented under Volume 3 CP B.9.14.

A summary of the risk assessment regarding aquatic biodiversity via indirect effects and trophic interactions resulted from uses of glyphosate is presented under point 2.9.9.8.

2.9.9.3 Summary of risk assessment for arthropods

2.9.9.3.1 Summary of risk assessment for bees

Risk assessment according to SANCO/10329/2002 rev 2 final

The hazard quotients for oral and contact exposure of honey bees are based on the recommended field use rates and are presented in the table below.

Table 2.9.9.3.1-1: Assessment of the risk of glyphosate for honey bees due to the use of MON 52276

Intended use	All uses (Uses: 1a-10c)		
Active substance	Glyphosate		
Use pattern	1-2 x 1800 g a.e./ha, 1-2 x 1440 g a.e./ha, 1-3 x 1080 g a.e./ha, 1-3 x 720 g a.e./ha, 1 x 540 g a.e./ha		
Test design	**LD_{50} (lab.) (µg a.e./bee)**	**Single max. application rate (g a.e./ha)**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	>77	1800	< 23.4
		1440	< 18.7
		1080	< 14.0
		720	< 9.4
		540	< 7.0
Contact toxicity	>100	1800	<18.0
		1440	<14.4
		1080	<11.0
		720	<7.2
		540	<5.4

Q_{HO}, Q_{HC}: Hazard quotients for oral and contact exposure.

According to the risk assessment conducted according to SANCO/10329/2002 rev 2 final , the oral and contact hazard quotients (QHO, QHC) are below the trigger value of 50. An acceptable risk to honey bees is concluded for all intended use patterns.

Further considerations regarding the chronic risk to bees

The applicant provided a chronic risk assessment based results of [redacted] (2011, Vol.3 CP 10.3.1.5/001). RMS presented thereafter the risk assessment as proposed by the applicant with correction of the residues in nectar and pollen that RMS has recalculated (please refer to the study summary of [redacted] (2011, Vol.3 CP 10.3.1.5/001) above.

[redacted] (2011, Vol.3 CP 10.3.1.5/001) provides measurements of the levels of exposure in nectar and honey following an application at 2.88 kg a.e./ha, which exceeds the maximum single application rate of the proposed uses in the GAP. Residues in nectar samples taken from forager bees at various time points after application were up to 62.6 mg a.e./kg (based on RMS recalculation). Residues in pollen samples taken from the pollen trap (higher than from pollen taken from foragers) at various times after application were up to 1148 mg a.e./kg (based on RMS estimation). Using this information, a risk assessment may be conducted in line with the recommendations of Reg (EU) No 283/2013 section 8(10) which states: "Pending the validation and adoption of new studies and of a new risk assessment scheme, existing protocols shall be used to address the acute and chronic risk to bees, including those on colony survival and development, and the identification and measurement of relevant sub-lethal effects in the risk assessment". Furthermore, under section 8.3.1. Effects on bees of the same Regulation it states that: "[…] risk assessment shall be based on a comparison of the relevant endpoint with those residue concentrations. If this

comparison indicates that an exposure to toxic levels cannot be excluded, effects shall be investigated with higher tier tests."

A comparison can be made between the chronic and larval endpoint based on concentration in test diets and the maximum concentrations of glyphosate measured in nectar and pollen. In the chronic adult study the NOEC and NOEDD values (10 days) were 10000 mg a.e./kg feeding solution and 179.9 µg a.e./bee/day, respectively. As forager bees consume a diet which is virtually 100% nectar this endpoint can be compared to the maximum measured residues in nectar of 62.6 mg a.e./kg demonstrating a margin of safety of 16.

In the larval toxicity study the EC10 and ED10 values (over the larval development period) were 477 mg a.e./kg diet and 75.6 µg a.e./larva. Because larvae consume a mix of nectar and pollen it is necessary to consider the proportion of nectar and pollen in the diet and the contribution towards the exposure concentration. According to [redacted] (2015) a single larva consumes 59.4 mg sugar and 5.4 mg pollen over 5 days. Assuming the nectar is foraged from treated weeds with a sugar content of 30% (w/w) this means that the larval diet consists of 198 mg nectar and 5.4 mg of pollen, i.e. a ratio of 0.973:0.027 (nectar:pollen). As the maximum concentration in nectar was 62.6 mg a.e./kg and in pollen 1148 mg a.e./kg the diet would have a concentration of:

Nectar: 0.973 x 62.6 mg a.e./kg = 60.9 mg a.e./kg + Pollen: 0.027 x 1148 mg a.e./kg = 31 mg a.e./kg diet

Concentration of glyphosate in the larval diet = 91.9 mg a.e./kg (based on nectar and pollen)

Comparing the larval endpoint to the maximum measured residues in the larval diet of 91.9 mg a.e./kg a margin of safety of 5.2 is calculated. Note: This is considered a worst-case estimate of exposure as honey bee larvae are fed with royal jelly for the first two days of their development period.

Overall, a margin of safety between 16 and 5.2 is demonstrated for chronic exposure to adult honey bees and honey bee larvae. This approach indicates that the risk to honey bees is acceptable.

Risk assessment according to the EFSA GD on the Risk Assessment on Bees (2013)

In addition, the risk assessment for honey bees is performed in accordance with the recommendations of the "Guidance on the risk assessment of plant protection products on bees (Apis mellifera, Bombus spp. and solitary bees)" (EFSA Journal 2013;11(7):3295 doi: 10.2903/j.efsa.2013.3295, July 04, 2014).

Regarding the risk due to exposure to metabolites of glyphosate, the residue section concluded that the metabolism studies for rotational crops are not sufficient to predict the residue level as they do not cover the maximum PEC soil of AMPA. Therefore, a data gap has been set for residue level in rotational crops (see Volume 1, point 2.7.7). Therefore the conclusions proposed by the applicant cannot be confirmed. In relation to the data gap set for rotational crops in the residue section, further consideration of the relevance of metabolites for bees will have to be provided.

Table 2.9.9.3.1-2: Screening assessment of the risk of glyphosate for honey bees due to the use of MON 52276

Intended use	All uses (Uses: 1a-10c)
Application method	downward spraying
Active substance	Glyphosate
Use pattern	1-2 x 1800 g a.e./ha, 1-2 x 1440 g a.e./ha, 1-3 x 1080 g a.e./ha, 1-3 x 720 g a.e./ha, 1 x 540 g a.e./ha

Type design	**LD_{50} (µg a.e./bee)**	**Max. single application rate (g a.e./ha)**	**$HQ_{contact}$ criterion**	**Trigger**
Adult acute contact toxicity	>100	1800	<18.0	42
		1440	<14.4	
		1080	<10.8	
		720	<7.2	
		540	<5.4	

Type design	**Endpoint**	**Max. single application rate (kg a.e./ha)**	**E_f × SV**	**ETR**	**Trigger**
Adult acute oral toxicity	LD_{50} = 77 µg a.e./bee	1.80	7.6	0.18	≤ 0.2
		1.44		0.14	
		1.08		0.11	
		0.72		0.07	
		0.54		0.05	
Adult chronic oral toxicity	LDD_{50} > 179.9 µg a.e./bee/day	1.80	7.6	**<0.076**	≤ 0.03
		1.44		**<0.06**	
		1.08		**<0.04**	
		0.72		**<0.0304**	
		0.54		<0.023	
Larval toxicity	ED10 = 75.6 µg a.e./larva	1.80	4.4	0.10	≤ 0.2
		1.44		0.08	
		1.08		0.06	
		0.72		0.04	
		0.54		0.03	

Ef: exposure factor; SV: shortcut value; $HQ_{contact}$: Hazard quotient for contact exposure; ETR: Exposure toxicity ratio; ETR values shown in **bold** breach the relevant trigger.

The exposure toxicity ratio (ETR) for adult chronic toxicity is above the respective trigger value for application rates of 720 g a.e./ha, 1080 g a.e./ha, 1440 g a.e./ha and 1800 g a.e./ha. Therefore, a Tier 1 risk assessment is required for

these use patterns. An acceptable risk is indicated at the screening step for the use rate of 540 g a.e./ha.

First Tier risk assessment for adult chronic oral exposure

Table 2.9.9.3.1-3: First-tier assessment (oral exposure) of the risk for honey bees due to the use of MON 52276 in orchard crops and vines at 1440 g a.e./ha

Intended use	Orchard crops, vines (Uses: 4a, 5a)
Application method	downward spraying
Crop Category	under crop application[1]
Active substance	Glyphosate
Use pattern	1-2 x 1440 g a.e./ha[2]

Test design	Endpoint (lab.)	Scenario	BBCH	E_f	SV	ETR	Trigger
Adult chronic oral toxicity	LDD_{50} > 179.9 µg a.e./bee/day	Weeds	weed <10	1	0.27	<0.01	0.03
			weed ≥10	1	2.9	<0.02	
		field margin	weed <10	0.0092	2.9	<0.01	
			weed ≥10	0.0092	2.9	<0.01	
		adjacent crop	weed <10	0.0033	5.8	<0.01	
			weed ≥10	0.0033	5.8	<0.01	
		next crop	weed <10	1	0.54	<0.01	
			weed ≥10	1	0.54	<0.01	

Ef: exposure factor; SV: shortcut value; ETR: exposure toxicity ratio.
[1] Crop category chosen according to the recommendations of the EFSA GD on the Risk Assessment on Bees (2013) and the EFSA Screening Step and 1st Tier Calculator
[2] Max. single application rate of 1440 g a.e./ha considered for risk calculation

Table 2.9.9.3.1-4: First-tier assessment (oral exposure) of the risk for honey bees due to the use of MON 52276 in orchard crops and vines at 1080 g a.e./ha

Intended use	Orchard crops, vines (Uses: 4a, 4b, 5a, 5b)
Application method	downward spraying
Crop category	under crop application[1]
Active substance	Glyphosate
Use pattern	1-3 x 1080 g a.e./ha

Test design	Endpoint (lab.)	Scenario	BBCH	E_f	SV	ETR	Trigger
Adult chronic oral toxicity	LDD_{50} > 179.9 µg a.e./bee/day	Weeds	weed <10	1	0.27	<0.001	0.03
			weed ≥10	1	2.9	<0.013	
		field margin	weed <10	0.0092	2.9	<0.001	
			weed ≥10	0.0092	2.9	<0.001	
		adjacent crop	weed <10	0.0033	5.8	<0.001	
			weed ≥10	0.0033	5.8	<0.001	
		next crop	weed <10	1	0.54	<0.002	
			weed ≥10	1	0.54	<0.002	

Ef: exposure factor; SV: shortcut value; ETR: exposure toxicity ratio.
[1] Crop category chosen according to the recommendations of the EFSA GD on the Risk Assessment on Bees (2013) and the EFSA Screening Step and 1st Tier Calculator
[2] Max. single application rate of 1080 g a.e./ha considered for risk calculation

Table 2.9.9.3.1-5: First-tier assessment (oral exposure) of the risk for honey bees due to the use of MON 52276 in orchard crops and vines at 720 g a.e./ha

Intended use	Orchard crops, vines (Uses: 4b, 4c, 5b, 5c)
Application method	downward spraying
Crop Category	under crop application[1]
Active substance	Glyphosate
Use pattern	1-3 x 720 g a.e./ha[2]

Test design	Endpoint (lab.)	Scenario	BBCH	E_f	SV	ETR	Trigger
Adult chronic oral toxicity	LDD_{50} > 179.9 µg a.e./bee/day	Weeds	weed <10	1	0.27	<0.001	0.03
			weed ≥10	1	2.9	<0.008	
		field margin	weed <10	0.0092	2.9	<0.001	
			weed ≥10	0.0092	2.9	<0.001	
		adjacent crop	weed <10	0.0033	5.8	<0.001	
			weed ≥10	0.0033	5.8	<0.001	
		next crop	weed <10	1	0.54	<0.002	
			weed ≥10	1	0.54	<0.002	

Ef: exposure factor; SV: shortcut value; ETR: exposure toxicity ratio.
[1] Crop category chosen according to the recommendations of the EFSA GD on the Risk Assessment on Bees (2013) and the EFSA Screening Step and 1st Tier Calculator
[2] Max. single application rate of 720 g a.e./ha considered for risk calculation

Table 2.9.9.3.1-6: First-tier assessment (oral exposure) of the risk for honey bees due to the use of MON 52276 – railroad tracks at 1800 g a.e./ha

Intended use	Railroad tracks (Uses: 7a, 7b)
Application method	downward spraying
Crop Category	under crop application[1]
Active substance	Glyphosate
Use pattern	1-2 x 1800 g a.e./ha[2]

Test design	Endpoint (lab.)	Scenario	BBCH	E_f	SV	ETR	Trigger
Adult chronic oral toxicity	LDD_{50} > 179.9 µg a.e./bee/day	Weeds	weed <10	1	0.27	<0.002	0.03
			weed ≥10	1	2.9	<0.021	
		field margin	weed <10	0.0092	2.9	<0.001	
			weed ≥10	0.0092	2.9	<0.001	
		adjacent crop	weed <10	0.0033	5.8	<0.001	
			weed ≥10	0.0033	5.8	<0.001	
		next crop	weed <10	1	0.54	<0.004	
			weed ≥10	1	0.54	<0.004	

Ef: exposure factor; SV: shortcut value; ETR: exposure toxicity ratio.
[1] As no definite scenario for railroad tracks is provided by the EFSA GD on the Risk Assessment on Bees (2013) and the EFSA Screening Step and 1st Tier Calculator, the under crop application scenario was considered to address uses on railroad tracks
[2] Max. single application rate of 1800 g a.e./ha considered for risk calculation

Table 2.9.9.3.1-7: First-tier assessment (oral exposure) of the risk for honey bees due to the use of MON 52276 – invasive plant species in agricultural and non-agricultural areas at 1800 g a.e./ha

Intended use	invasive plant species in agricultural and non-agricultural areas (Uses: 8, 9)
Application method	downward spraying
Crop Category	under crop application[1]
Active substance	Glyphosate
Use pattern	1 x 1800 g a.e./ha

Test design	Endpoint (lab.)	Scenario	BBCH	E_f	SV	ETR	Trigger
Adult chronic oral toxicity	LDD_{50} > 179.9 µg a.e./bee/day	Weeds	weed <10	1	0.27	<0.002	0.03
			weed >10	1	2.9	<0.021	
		field margin	weed <10	0.0092	2.9	<0.001	
			weed >10	0.0092	2.9	<0.001	
		adjacent crop	weed <10	0.0033	5.8	<0.001	
			weed >10	0.0033	5.8	<0.001	
		next crop	weed <10	1	0.54	<0.004	
			weed >10	1	0.54	<0.004	

Ef: exposure factor; SV: shortcut value; ETR: exposure toxicity ratio.
[1] As no definite scenario for invasive weeds is provided by the EFSA GD on the Risk Assessment on Bees (2013) and the EFSA Screening Step and 1st Tier Calculator, under crop application: giant hogweed (*Heracleum* spp.) and Japanese knotweed (*Reynoutria japonica*)

Table 2.9.9.3.1-8: First-tier assessment (oral exposure) of the risk for honey bees due to the use of MON 52276 – pre-sowing, pre-planting and post-harvest uses at 1440 g a.e./ha

Intended use	Root & tuber vegetables, Bulb vegetables, Fruiting vegetables, Brassica, Leafy vegetables, Stem vegetables, Sugar beet (Uses: 1a, 2a)
Application method	downward spraying
Crop category	bare soil application – crop attractive for pollen and nectar[1]
Active substance	Glyphosate
Use pattern	1-2 x 1440 g a.e./ha[2]

Test design	Endpoint (lab.)	Scenario	BBCH	E_f	SV	ETR	Trigger
Adult chronic oral toxicity	LDD_{50} > 179.9 µg a.e./bee/day	treated crop	<10	1	0.54	<0.003	0.03
		Weeds	<10	1	0.27	<0.002	
		field margin	<10	0.0092	2.9	<0.001	
		adjacent crop	<10	0.0033	5.8	<0.001	
		next crop	<10	1	0.54	<0.003	

Ef: exposure factor; SV: shortcut value; ETR: exposure toxicity ratio.
[1] Crop category chosen according to the recommendations of the EFSA GD on the Risk Assessment on Bees (2013) and the EFSA Screening Step and 1st Tier Calculator
[2] Max. single application rate of 1440 g a.e./ha considered for risk calculation

Table 2.9.9.3.1-9: First-tier assessment (oral exposure) of the risk for honey bees due to the use of MON 52276 - pre-sowing, pre-planting and post-harvest uses at 1080 g a.e./ha

Intended use	Root & tuber vegetables, Bulb vegetables, Fruiting vegetables, Brassica, Leafy vegetables, Stem vegetables, Sugar beet, Legume vegetables (Uses: 1b, 2a, 2b, 2c, 6a, 10a)
Application method	downward spraying
Crop category	bare soil application – crop attractive for pollen and nectar[1]
Active substance	Glyphosate
Use pattern	1-3 x 1080 g a.e./ha[2]

Test design	Endpoint (lab.)	Scenario	BBCH	E_f	SV	ETR	Trigger
Adult chronic oral toxicity	LDD_{50} > 179.9 µg a.e./bee/day	treated crop	<10	1	0.54	<0.002	0.03
		Weeds	<10	1	0.27	<0.001	
		field margin	<10	0.0092	2.9	<0.001	
		adjacent crop	<10	0.0033	5.8	<0.001	
		next crop	<10	1	0.54	<0.002	

Ef: exposure factor; SV: shortcut value; ETR: exposure toxicity ratio.
[1] Crop category chosen according to the recommendations of the EFSA GD on the Risk Assessment on Bees (2013) and the EFSA Screening Step and 1st Tier Calculator
[2] Max. single application rate of 1080 g a.e./ha considered for risk calculation

Table 2.9.9.3.1-10: First-tier assessment (oral exposure) of the risk for honey bees due to the use of MON 52276 - pre-sowing, pre-planting and post-harvest uses at 720 g a.e./ha

Intended use	Root & tuber vegetables, Bulb vegetables, Fruiting vegetables, Brassica, Leafy vegetables, Stem vegetables, Sugar beet, Legume vegetables (Uses: 1c, 2b, 6b, 10b, 10c)
Application method	downward spraying
Crop category	bare soil application – crop attractive for pollen and nectar[1]
Active substance	Glyphosate
Use pattern	1-3 x 720 g a.e./ha[2]

Test design	Endpoint (lab.)	Scenario	BBCH	E_f	SV	ETR	Trigger
Adult chronic oral toxicity	LDD_{50} > 179.9 µg a.e./bee/day	treated crop	<10	1	0.54	<0.002	0.03
		Weeds	<10	1	0.27	<0.001	
		field margin	<10	0.0092	2.9	<0.001	
		adjacent crop	<10	0.0033	5.8	<0.001	
		next crop	<10	1	0.54	<0.002	

Ef: exposure factor; SV: shortcut value; ETR: exposure toxicity ratio.
[1] Crop category in the first tier oral assessment according to the EFSA GD on the Risk Assessment on Bees (2013)
[2] Max. single application rate of 720 g a.e./ha considered for risk calculation

Table 2.9.9.3.1-11: First-tier assessment (oral exposure) of the risk for honey bees due to the use of MON 52276 – fruiting vegetables

Intended use	Fruiting vegetables, (Uses: 1, 2, 3, 6, 10)
Application method	downward spraying
Crop category	fruiting vegetables 1, fruiting vegetables 2[1]
Active substance	Glyphosate
Use pattern	1-2 x 1440 g a.e./ha[2]

Test design	Endpoint (lab.)	Scenario	BBCH	E_f	SV	ETR	Trigger
Fruiting vegetables 1							
Adult chronic oral toxicity	LDD_{50} > 179.9 µg a.e./bee/day	treated crop	< 10	1	0.54	0.003	0.03
			10 - 49[3]	1	5.8	**0.033**	
			≥ 70	1	0	0.000	
		Weeds	< 10	1	2.9	0.017	
			10 - 49[3]	1	2.9	0.017	
			≥ 70	0.3	2.9	0.005	
		field margin	< 10	0.0092	2.9	0.000	
			10 - 49[3]	0.0092	2.9	0.000	
			≥ 70	0.0092	2.9	0.000	
		adjacent crop	< 10	0.0033	5.8	0.000	
			10 - 49[3]	0.0033	5.8	0.000	
			≥ 70	0.0033	5.8	0.000	
		next crop	< 10	1	0.54	0.003	

Intended use	Fruiting vegetables, (Uses: 1, 2, 3, 6, 10)
Application method	downward spraying
Crop category	fruiting vegetables 1, fruiting vegetables 2[1]
Active substance	Glyphosate
Use pattern	1-2 x 1440 g a.e./ha[2]

Test design	Endpoint (lab.)	Scenario	BBCH	E_f	SV	ETR	Trigger
			10 - 49[3]	1	0.54	0.003	
			≥ 70	1	0.54	0.003	
Fruiting vegetables 2							
Adult chronic oral toxicity	LDD_{50} > 179.9 µg a.e./bee/day	treated crop	< 10	1	0.012	0.000	0.03
			10 - 49[3]	1	0.92	0.005	
			≥ 70	1	0	0.000	
		Weeds	< 10	1	2.9	0.017	
			10 - 49[3]	1	2.9	0.017	
			≥ 70	0.3	2.9	0.005	
		field margin	< 10	0.0092	2.9	0.000	
			10 - 49[3]	0.0092	2.9	0.000	
			≥ 70	0.0092	2.9	0.000	
		adjacent crop	< 10	0.0033	5.8	0.000	
			10 - 49[3]	0.0033	5.8	0.000	
			≥ 70	0.0033	5.8	0.000	
		next crop	< 10	1	0.54	0.003	
			10 - 49[3]	1	0.54	0.003	
			≥ 70	1	0.54	0.003	

Ef: exposure factor; SV: shortcut value; ETR: exposure toxicity ratio.

[1] Crop category chosen according to the recommendations of the EFSA GD on the Risk Assessment on Bees (2013) and the EFSA Screening Step and 1st Tier Calculator

[2] Max. single application rate of 1440 g a.e./ha considered for risk calculation as it covers lower rates.

[3] Scenario only relevant for uses 6a and b for which the highest intended application rate is 1.08 kg a.s./ka.

Table 2.9.9.3.1-12: First-tier assessment (oral exposure) of the risk for honey bees due to the use of MON 52276 - root vegetables

Intended use	Root vegetables (Uses: 1, 2, 3, 6, 10)
Application method	downward spraying
Crop category	Root vegetables[1]
Active substance	Glyphosate
Use pattern	1-3 x 1440 g a.e./ha[2]

Test design	Endpoint (lab.)	Scenario	BBCH	Ef	SV	ETR	Trigger
Adult chronic oral toxicity	LDD_{50} > 179.9 µg a.e./bee/day	treated crop	< 10	1	0.54	0.003	0.03
			10 - 39[3]	1	5.8	**0.033**	
			≥ 70	1	0	0.000	
		Weeds	< 10	1	2.9	0.017	
			10 - 39[3]	1	2.9	0.017	
			≥ 70	0.3	2.9	0.005	
		field margin	< 10	0.0092	2.9	0.000	
			10 - 39[3]	0.0092	2.9	0.000	
			≥ 70	0.0092	2.9	0.000	
		adjacent crop	< 10	0.0033	5.8	0.000	
			10 - 39[3]	0.0033	5.8	0.000	
			≥ 70	0.0033	5.8	0.000	
		next crop	< 10	1	0.54	0.003	
			10 - 39[3]	1	0.54	0.003	
			≥ 70	1	0.54	0.003	

Ef: exposure factor; SV: shortcut value; ETR: exposure toxicity ratio.

[1] Crop category chosen according to the recommendations of the EFSA GD on the Risk Assessment on Bees (2013) and the EFSA Screening Step and 1st Tier Calculator, e.g. fruiting vegetables 2 = tomatoes, eggplants

[2] Max. single application rate of 1080 g a.e./ha considered for risk calculation as it covers lower rates.

[3] Scenario only relevant for uses 6a and b for which the highest intended application rate is 1.08 kg a.s./ka.

Table 2.9.9.3.1-13: First-tier assessment (oral exposure) of the risk for honey bees due to the use of MON 52276 –tuber vegetables

Intended use	Tuber vegetables (Uses: 1, 2, 3, 6, 10)
Application method	downward spraying
Crop category	potatoes[1]
Active substance	Glyphosate
Use pattern	1-3 x 1440 g a.e./ha[2]

Test design	Endpoint (lab.)	Scenario	BBCH	Ef	SV	ETR	Trigger
Adult chronic oral toxicity	LDD_{50} > 179.9 µg a.e./bee/day	treated crop	< 10	1	0.012	0.000	0.03
			10 - 39[3]	1	0.92	0.005	
			≥ 70	1	0	0.000	
		Weeds	< 10	1	2.9	0.017	
			10 - 39[3]	1	2.9	0.017	
			≥ 70	0.3	2.9	0.005	
		field margin	< 10	0.0092	2.9	0.000	
			10 - 39[3]	0.0092	2.9	0.000	
			≥ 70	0.0092	2.9	0.000	
		adjacent crop	< 10	0.0033	5.8	0.000	
			10 - 39[3]	0.0033	5.8	0.000	
			≥ 70	0.0033	5.8	0.000	
		next crop	< 10	1	0.54	0.003	
			10 - 39[3]	1	0.54	0.003	
			≥ 70	1	0.54	0.003	

Ef: exposure factor; SV: shortcut value; ETR: exposure toxicity ratio.

[1] Crop category chosen according to the recommendations of the EFSA GD on the Risk Assessment on Bees (2013) and the EFSA Screening Step and 1st Tier Calculator, e.g. fruiting vegetables 2 = tomatoes, eggplants

[2] Max. single application rate of 1440 g a.e./ha considered for risk calculation as it covers lower rates.

[3] Scenario only relevant for uses 6a and b for which the highest intended application rate is 1.08 kg a.s./ka.

Table 2.9.9.3.1-14: First-tier assessment (oral exposure) of the risk for honey bees due to the use of MON 52276 – Bulb vegetables

Intended use	Bulb vegetables (Uses: 1, 2, 3, 6, 10)
Application method	downward spraying
Crop category	bulb vegetables[1]
Active substance	Glyphosate
Use pattern	1-2 x 1440 g a.e./ha[2]

Test design	Endpoint (lab.)	Scenario	BBCH	Ef	SV	ETR	Trigger
Adult chronic oral toxicity	LDD_{50} > 179.9 µg a.e./bee/day	treated crop	< 10	1	0.54	0.003	0.03
			10 - 39[3]	1	5.8	**0.033**	
			≥ 70	1	0	0.000	
		Weeds	< 10	1	2.9	0.017	
			10 - 39[3]	1	2.9	0.017	
			≥ 70	0.6	2.9	0.010	
		field margin	< 10	0.0092	2.9	0.000	
			10 - 39[3]	0.0092	2.9	0.000	
			≥ 70	0.0092	2.9	0.000	
		adjacent crop	< 10	0.0033	5.8	0.000	
			10 - 39[3]	0.0033	5.8	0.000	
			≥ 70	0.0033	5.8	0.000	
		next crop	< 10	1	0.54	0.003	
			10 - 39[3]	1	0.54	0.003	
			≥ 70	1	0.54	0.003	

Ef: exposure factor; SV: shortcut value; ETR: exposure toxicity ratio.
[1] Crop category chosen according to the recommendations of the EFSA GD on the Risk Assessment on Bees (2013) and the EFSA Screening Step and 1st Tier Calculator,
[2] Max. single application rate of 1440 g a.e./ha considered for risk calculation as it covers lower rates.
[3] Scenario only relevant for uses 6a and b for which the highest intended application rate is 1.08 kg a.s./ka.

Table 2.9.9.3.1-15: First-tier assessment (oral exposure) of the risk for honey bees due to the use of MON 52276 - Brassica, leafy and stem vegetables

Intended use	Brassica, leafy vegetables, stem vegetables (Uses: 1, 2, 3, 6, 10)
Application method	downward spraying
Crop category	leafy vegetables, lettuce[1]
Active substance	Glyphosate
Use pattern	1-3 x 1440 g a.e./ha[2]

Test design	Endpoint (lab.)	Scenario	BBCH	Ef	SV	ETR	Trigger
Leafy vegetables							
Adult chronic oral toxicity	LDD_{50} > 179.9 µg a.e./bee/day	treated crop	< 10	1	0.54	0.003	0.03
			10 - 49[3]	1	5.8	**0.033**	

Intended use	Brassica, leafy vegetables, stem vegetables (Uses: 1, 2, 3, 6, 10)
Application method	downward spraying
Crop category	leafy vegetables, lettuce[1]
Active substance	Glyphosate
Use pattern	1-3 x 1440 g a.e./ha[2]

Test design	Endpoint (lab.)	Scenario	BBCH	Ef	SV	ETR	Trigger
			≥ 70	1	0	0.000	
		Weeds	< 10	1	2.9	0.017	
			10 - 49[3]	1	2.9	0.017	
			≥ 70	0.3	2.9	0.005	
		field margin	< 10	0.0092	2.9	0.000	
			10 - 49[3]	0.0092	2.9	0.000	
			≥ 70	0.0092	2.9	0.000	
		adjacent crop	< 10	0.0033	5.8	0.000	
			10 - 49[3]	0.0033	5.8	0.000	
			≥ 70	0.0033	5.8	0.000	
		next crop	< 10	1	0.54	0.003	
			10 - 49[3]	1	0.54	0.003	
			≥ 70	1	0.54	0.003	
Lettuce							
Adult chronic oral toxicity	LDD_{50} > 179.9 µg a.e./bee/day	treated crop	< 10	1	0.012	0.000	0.03
			10 - 49[3]	1	0.92	0.005	
			≥ 70	1	0	0.000	
		Weeds	< 10	1	2.9	0.017	
			10 - 49[3]	1	2.9	0.017	
			≥ 70	0.3	2.9	0.005	
		field margin	< 10	0.0092	2.9	0.000	
			10 - 49[3]	0.0092	2.9	0.000	
			≥ 70	0.0092	2.9	0.000	
		adjacent crop	< 10	0.0033	5.8	0.000	
			10 - 49[3]	0.0033	5.8	0.000	
			≥ 70	0.0033	5.8	0.000	
		next crop	< 10	1	0.54	0.003	
			10 – 49[3]	1	0.54	0.003	
			≥ 70	1	0.54	0.003	

Ef: exposure factor; SV: shortcut value; ETR: exposure toxicity ratio.

[1] Crop category chosen according to the recommendations of the EFSA GD on the Risk Assessment on Bees (2013) and the EFSA Screening Step and 1st Tier Calculator,

[2] Max. single application rate of 1440 g a.e./ha considered for risk calculation as it covers lower rates.

[3] Scenario only relevant for uses 6a and b for which the highest intended application rate is 1.08 kg a.s./ka.

Table 2.9.9.3.1-16: First-tier assessment (oral exposure) of the risk for honey bees due to the use of MON 52276 - Sugar beet

Intended use	Sugar beet (Uses: 1, 2, 3, 10)
Application method	downward spraying
Crop category	sugar beet[1]
Active substance	Glyphosate
Use pattern	1-3 x 1440 g a.e./ha[2]

Test design	Endpoint (lab.)	Scenario	BBCH	Ef	SV	ETR	Trigger
Adult chronic oral toxicity	LDD_{50} > 179.9 µg a.e./bee/day	treated crop	< 10	1	0.54	0.003	0.03
			≥ 70	1	0	0.000	
		Weeds	< 10	1	2.9	0.017	
			≥ 70	0.25	2.9	0.004	
		field margin	< 10	0.0092	2.9	0.000	
			≥ 70	0.0092	2.9	0.000	
		adjacent crop	< 10	0.0033	5.8	0.000	
			≥ 70	0.0033	5.8	0.000	
		next crop	< 10	1	0.54	0.003	
			≥ 70	1	0.54	0.003	

Ef: exposure factor; SV: shortcut value; ETR: exposure toxicity ratio.
[1] Crop category chosen according to the recommendations of the EFSA GD on the Risk Assessment on Bees (2013) and the EFSA Screening Step and 1st Tier Calculator,
[2] Max. single application rate of 1440 g a.e./ha considered for risk calculation as it covers lower rates.

Table 2.9.9.3.1-17: First-tier assessment (oral exposure) of the risk for honey bees due to the use of MON 52276 - legume vegetables

Intended use	Legume vegetables (Uses: 1, 2, 3, 6, 10)
Application method	downward spraying
Crop category	pulses[1]
Active substance	Glyphosate
Use pattern	1-2 x 1440 g a.e./ha[2]

Test design	Endpoint (lab.)	Scenario	BBCH	Ef	SV	ETR	Trigger
Adult chronic oral toxicity	LDD_{50} > 179.9 µg a.e./bee/day	treated crop	< 10	1	0.54	0.003	0.03
			10 49[3]	1	5.8	**0.033**	
			≥ 70	1	0	0.000	
		Weeds	< 10	1	2.9	0.017	
			10 - 49[3]	1	2.9	0.017	
			≥ 70	0.3	2.9	0.005	
		field margin	< 10	0.0092	2.9	0.000	
			10 - 49[3]	0.0092	2.9	0.000	
			≥ 70	0.0092	2.9	0.000	
		adjacent crop	< 10	0.0033	5.8	0.000	
			10 - 49[3]	0.0033	5.8	0.000	
			≥ 70	0.0033	5.8	0.000	
		next crop	< 10	1	0.54	0.003	
			10 - 49[3]	1	0.54	0.003	
			≥ 70	1	0.54	0.003	

Ef: exposure factor; SV: shortcut value; ETR: exposure toxicity ratio.
[1] Crop category chosen according to the recommendations of the EFSA GD on the Risk Assessment on Bees (2013) and the EFSA Screening Step and 1st Tier Calculator,
[2] Max. single application rate of 1440 g a.e./ha considered for risk calculation as it covers lower rates.
[3] Scenario only relevant for uses 6a and b for which the highest intended application rate is 1.08 kg a.s./ka.

Table 2.9.9.3.1-18: First-tier assessment (oral exposure) of the risk for honey bees due to the use of MON 52276 on fruiting, root, bulb and leafy vegetables and pulses for "treated crop" scenario at all application rates for uses 6a and 6b

Crop	Fruiting vegetables 1, Root vegetables, Bulb vegetables, Leafy vegetables, Pulses (uses 6a and 6b)
Application method	downward spraying
Active substance	Glyphosate
Toxicity value	LDD_{50} > 179.9 µg a.e./bee/day

Scenario	BBCH stage	Max. single application rate (kg a.e./ha)	E_f	SV	ETR	Trigger
Treated crop	BBCH 10-39 or BBCH 10-49	1.08	1	5.8	<0.025	0.03
		0.72	1	5.8	<0.017	

All exposure toxicity ratios (ETRs) for adult chronic toxicity are below the respective trigger value, indicating an

acceptable risk to honey bees following application of MON 52276 for all intended uses.

Overall, an acceptable risk to honey bees has been demonstrated in the risk assessment above for all uses according to proposed GAP.

In addition, a honey bee brood feeding test ([redacted], 2012, KCA 8.3.1.4/001) was conducted to evaluate the potential risk to honey bee brood when they are directly exposed to glyphosate (tested as IPA salt). This study provides further information regarding the chronic risk to honey bees and honey bee brood. The dose levels of the test item were based on the residues characterised in the glasshouse study ([redacted], 2011, CP 10.3.1.5/001). The highest dose was of 1 L syrup at 301 mg a.e./L. This dose covers the total intake of glyphosate residues (via nectar + pollen) measured in the glasshouse study i.e. 269.3 mg a.e. (based on day 1 maximum mean residues, and covering all application rates intended), Mortality of adult honey bees as well as honey bee brood was assessed over a period of 7 days. Overall, no treatment related effects were observed. The NOAEL for adult mortality and brood development was the highest dose tested; 301 mg a.e./L nominal (equivalent to 266 mg/kg, measured concentration).

Table 2.9.9.3.1-19: Assessment of the risk for bees due to the use of MON 52276 considering exposure to contaminated water

Intended use	All uses (Uses: 1a-10c)
Application method	downward spraying
Active substance	Glyphosate
Use pattern	2 x 1440 g a.e./ha (worst-case identified for PECsw see B.9.4)
Water solubility	100000 mg/L (see Volume 1, [redacted] (2020a), KCA 2.5/001)
PEC_{sw}	worst case Step 2 of 69.95 µg/L
PEC_{puddle}	worst case Step 2 of 65.47 µg/L

Surface water[1] (provisional)				
Test design	**Endpoint (lab.)**	**water consumption (µl)**	**ETR[1]**	**Trigger**
Acute	77 µg a.e./bee	11.4	0.00	0.2
Chronic	>179.9 µg a.e./bee/day	11.4	0.000	0.03
Larvae	75.6 µg a.e./larva	111	0.00	0.2
Puddle water[1,2] (provisional)				
Test design	**Endpoint (lab.)**	**water consumption (µl)**	**ETR[2]**	**Trigger**
Acute	77 µg a.e./bee	11.4	0.00	0.2
Chronic	>179.9 µg a.e./bee/day	11.4	0.000	0.03
Larvae	75.6 µg a.e./larva	111	0.00	0.2
Guttation water				
Test design	**Endpoint (lab.)**	**water consumption (µl)**	**ETR**	**Trigger**
Acute	77 µg a.e./bee	11.4	**14.8**	0.2
Chronic	>179.9 µg a.e./bee/day	11.4	**<3.3**	0.03
Larvae	75.6 µg a.e./larva	111	**105.7**	0.2

ETR: exposure toxicity ratio.
Values shown in **bold** breach the relevant trigger.

ETR exceeds the trigger values for guttation water scenario. RMS considers that the occurrence of glyphosate secretion via guttation should be limited. Guttation events that may be observed in some crops are expected less important on weed communities as not all species produce guttation water. Besides these weeds will be present at different growth stages, RMS therefore believes the availability of guttation water at the time of spray should be limited. Taking into account that root pressure and cell turgor are required for a plant to produce guttation fluid, in the case of glyphosate, a chronic exposure is unlikely (plants wilt soon after treatment). Overall, it seems unlikely

that guttation water will represent a significant (major) source of water in more than 10% of cases (i.e. hives at the edge of the fields).
Also considering the absence of effects from the bee brood feeding test ([redacted] 2012), RMS considers that potential for adverse effects on colonies via guttation water is unlikely.

Risk assessment for bumble bees

The risk assessment for the proposed uses of MON 52276 and the effects on bumble bees is provided below.

Table 2.9.9.3.1-20: Screening assessment of the risk of glyphosate for bumble bees due to the use of MON 52276

Intended use	All uses (Uses: 1a to 10c)				
Application method	downward spraying				
Active substance	Glyphosate				
Use pattern	1-2 x 1800 g a.e./ha, 1-2 x 1440 g a.e./ha, 1-3 x 1080 g a.e./ha, 1-3 x 720 g a.e./ha, 1 x 540 g a.e./ha				
Type design	**LD_{50} (µg a.e./bee)**	**Max. single application rate (g a.e./ha)**		**$HQ_{contact}$ criterion**	**Trigger**
Acute contact toxicity	>461	1800		<3.9	**7**
		1440		<3.1	
		1080		<2.3	
		720		<1.6	
		540		<1.2	
Type design	**LD_{50} (µg a.e./bee)**	**Max. single application rate (kg a.e./ha)**	**E_f × SV**	**ETR**	**Trigger**
Acute oral toxicity	>412	1.80	11.2	**<0.05**	**0.036**
		1.44		**<0.04**	
		1.08		<0.03	
		0.72		<0.02	
		0.54		<0.01	

Ef: exposure factor; SV: shortcut value; $HQ_{contact}$: Hazard quotient for contact exposure; ETR: Exposure toxicity ratio; ETR values shown in **bold** breach the relevant trigger.

The exposure toxicity ratio (ETR) for acute oral toxicity is above the respective trigger value for the application rates of 1440 g a.e./ha and 1800 g a.e./ha. Therefore, Tier 1 risk assessment is required for these use patterns. No risk is indicated at the screening step for the use rate of 540 g a.e./ha, 720 g a.e./ha and 1080 g a.e./ha.

First Tier risk assessment for acute oral exposure of bumble bees

Table 2.9.9.3.1-21: First-tier assessment (oral exposure) of the risk for bumble bees due to the use of MON 52276 in orchard crops and vines at 1440 g a.e./ha

Intended use	Orchard crops, vines (Uses: 4a, 5a)
Application method	downward spraying
Crop Category	under crop application[1]
Active substance	Glyphosate
Use pattern	1-2 x 1440 g a.e./ha[2]

Test design	Endpoint (lab.)	Scenario	BBCH	E_f	SV	ETR	Trigger
Acute oral toxicity	LD_{50} > 412 µg a.e./bee	weeds	weed <10	1	0.46	<0.01	0.036
			weed ≥10	1	6.5	<0.023	
		field margin	weed <10	0.0092	6.5	<0.01	
			weed ≥10	0.0092	6.5	<0.01	
		adjacent crop	weed <10	0.0033	11.2	<0.01	
			weed ≥10	0.0033	11.2	<0.01	
		next crop	weed <10	1	0.9	<0.01	
			weed ≥10	1	0.9	<0.01	

Ef: exposure factor; SV: shortcut value; ETR: exposure toxicity ratio.
[1] Crop category chosen according to the recommendations of the EFSA GD on the Risk Assessment on Bees (2013) and the EFSA Screening Step and 1st Tier Calculator
[2] Max. single application rate of 1440 g a.e./ha considered for risk calculation

Table 2.9.9.3.1-22: First-tier assessment (oral exposure) of the risk for bumble bees due to the use of MON 52276 – railroad tracks at 1800 g a.e./ha

Intended use	Railroad tracks (Uses: 7a, 7b)
Application method	downward spraying
Crop Category	under crop application[1]
Active substance	Glyphosate
Use pattern	1-2 x 1800 g a.e./ha[2]

Test design	Endpoint (lab.)	Scenario	BBCH	E_f	SV	ETR	Trigger
Acute oral toxicity	LD_{50} > 412 µg a.e./bee	weeds	weed <10	1	0.46	<0.002	0.036
			weed ≥10	1	6.5	<0.028	
		field margin	weed <10	0.0092	6.5	<0.001	
			weed ≥10	0.0092	6.5	<0.001	
		adjacent crop	weed <10	0.0033	11.2	<0.001	
			weed ≥10	0.0033	11.2	<0.001	
		next crop	weed <10	1	0.9	<0.004	
			weed ≥10	1	0.9	<0.004	

Ef: exposure factor; SV: shortcut value; ETR: exposure toxicity ratio.
[1] As no definite scenario for railroad tracks is provided by the EFSA GD on the Risk Assessment on Bees (2013) and the EFSA Screening Step and 1st Tier Calculator, the under crop application was considered to address uses on railroad tracks
[2] Max. single application rate of 1800 g a.e./ha considered for risk calculation

Table 2.9.9.3.1-23: First-tier assessment (oral exposure) of the risk for bumble bees due to the use of MON 52276 – invasive plant species in agricultural and non-agricultural areas at 1800 g a.e./ha

Intended use	invasive plant species in agricultural and non-agricultural areas (Uses: 8, 9)
Application method	downward spraying
Crop Category	under crop application [1]
Active substance	Glyphosate
Use pattern	1 x 1800 g a.e./ha[2]

Test design	Endpoint (lab.)	Scenario	BBCH	E_f	SV	ETR	Trigger
Acute oral toxicity	LD_{50} > 412 µg a.e./bee	weeds	weed <10	1	0.46	<0.002	0.036
			weed >10	1	6.5	<0.028	
		field margin	weed <10	0.0092	6.5	<0.001	
			weed >10	0.0092	6.5	<0.001	
		adjacent crop	weed <10	0.0033	11.2	<0.001	
			weed >10	0.0033	11.2	<0.001	
		next crop	weed <10	1	0.9	<0.004	
			weed >10	1	0.9	<0.004	

Ef: exposure factor; SV: shortcut value; ETR: exposure toxicity ratio.
[1] As no definite scenario for invasive weeds is provided by the EFSA GD on the Risk Assessment on Bees (2013) and the EFSA Screening Step and 1st Tier Calculator, under crop application: giant hogweed (*Heracleum* spp.), Japanese knotweed (*Reynoutria japonica*)
[2] Max. single application rate of 1800 g a.e./ha considered for risk calculation

Table 2.9.9.3.1-24: First-tier assessment (oral exposure) of the risk for bumble bees due to the use of MON 52276 –pre-sowing, pre-planting and post-harvest uses at 1440 g a.e./ha

Intended use	Root & tuber vegetables, Bulb vegetables, Fruiting vegetables, Brassica, Leafy vegetables, Stem vegetables, Sugar beet (Uses: 1a, 2a)
Application method	downward spraying
Crop category	bare soil application – crop attractive for pollen and nectar[1]
Active substance	Glyphosate
Use pattern	1-2 x 1440 g a.e./ha[2]

Test design	Endpoint (lab.)	Scenario	BBCH	E_f	SV	ETR	Trigger
Acute oral toxicity	LD_{50} > 412 µg a.e./bee	treated crop	<10	1	0.9	<0.004	0.036
		weeds	<10	1	0.46	<0.002	
		field margin	<10	0.0092	6.5	<0.001	
		adjacent crop	<10	0.0033	11.2	<0.001	
		next crop	<10	1	0.9	<0.004	

Ef: exposure factor; SV: shortcut value; ETR: exposure toxicity ratio.
[1] Crop category chosen according to the recommendations of the EFSA GD on the Risk Assessment on Bees (2013) and the EFSA Screening Step and 1st Tier Calculator
[2] Max. single application rate of 1440 g a.e./ha considered for risk calculation as it covers lower application rates.

Table 2.9.9.3.1-25: First-tier assessment (oral exposure) of the risk for bumble bees due to the use of MON 52276 – fruiting vegetables

Intended use	Fruiting vegetables, (Uses: 1, 2, 3, 6, 10)
Application method	downward spraying
Crop category	fruiting vegetables 1, fruiting vegetables 2[1]
Active substance	Glyphosate
Use pattern	1-2 x 1440 g a.e./ha[2]

Test design	Endpoint (lab.)	Scenario	BBCH	E_f	SV	ETR	Trigger
Fruiting vegetables 1							
Acute oral toxicity	LD_{50} > 412 µg a.e./bee	treated crop	< 10	1	0.9	0.0031	0.036
			10 - 49[3]	1	11.2	**0.0391**	
			≥ 70	1	0	0.0000	
		Weeds	< 10	1	6.5	0.0227	
			10 - 49[3]	1	6.5	0.0227	
			≥ 70	0.3	6.5	0.0068	
		field margin	< 10	0.0092	6.5	0.0002	
			10 - 49[3]	0.0092	6.5	0.0002	
			≥ 70	0.0092	6.5	0.0002	
		adjacent crop	< 10	0.0033	11.2	0.0001	
			10 - 49[3]	0.0033	11.2	0.0001	
			≥ 70	0.0033	11.2	0.0001	
		next crop	< 10	1	0.9	0.0031	

Intended use	Fruiting vegetables, (Uses: 1, 2, 3, 6, 10)
Application method	downward spraying
Crop category	fruiting vegetables 1, fruiting vegetables 2[1]
Active substance	Glyphosate
Use pattern	1-2 x 1440 g a.e./ha[2]

Test design	Endpoint (lab.)	Scenario	BBCH	E_f	SV	ETR	Trigger
			10 - 49[3]	1	0.9	0.0031	
			≥ 70	1	0.9	0.0031	
Fruiting vegetables 2							
Acute oral toxicity	LD_{50} > 412 µg a.e./bee	treated crop	< 10	1	0.03	0.0001	0.036
			10 - 49[3]	1	2.3	0.0080	
			≥ 70	1	0	0.0000	
		Weeds	< 10	1	6.5	0.0227	
			10 - 49[3]	1	6.5	0.0227	
			≥ 70	0.3	6.5	0.0068	
		field margin	< 10	0.0092	6.5	0.0002	
			10 - 49[3]	0.0092	6.5	0.0002	
			≥ 70	0.0092	6.5	0.0002	
		adjacent crop	< 10	0.0033	11.2	0.0001	
			10 - 49[3]	0.0033	11.2	0.0001	
			≥ 70	0.0033	11.2	0.0001	
		next crop	< 10	1	0.9	0.0031	
			10 - 49[3]	1	0.9	0.0031	
			≥ 70	1	0.9	0.0031	

Ef: exposure factor; SV: shortcut value; ETR: exposure toxicity ratio.

[1] Crop category chosen according to the recommendations of the EFSA GD on the Risk Assessment on Bees (2013) and the EFSA Screening Step and 1st Tier Calculator,

[2] Max. single application rate of 1440 g a.e./ha considered for risk calculation as it covers lower rates.

[3] Scenario only relevant for uses 6a and b for which the highest intended application rate is 1.08 kg a.s./ka.

Table 2.9.9.3.1-26: First-tier assessment (oral exposure) of the risk for bumble bees due to the use of MON 52276 –rootvegetables

Intended use	Root vegetables (Uses: 1, 2, 3, 6, 10)
Application method	downward spraying
Crop category	Root vegetables[1]
Active substance	Glyphosate
Use pattern	1-3 x 1440 g a.e./ha[2]

Test design	Endpoint (lab.)	Scenario	BBCH	Ef	SV	ETR	Trigger
Acute oral toxicity	LD_{50} > 412 µg a.e./bee	treated crop	< 10	1	0.9	0.0031	0.036
			10 - 39[3]	1	11.2	**0.0391**	
			≥ 70	1	0	0.0000	
		Weeds	< 10	1	6.5	0.0227	
			10 - 39[3]	1	6.5	0.0227	
			≥ 70	0.3	6.5	0.0068	
		field margin	< 10	0.0092	6.5	0.0002	
			10 - 39[3]	0.0092	6.5	0.0002	
			≥ 70	0.0092	6.5	0.0002	
		adjacent crop	< 10	0.0033	11.2	0.0001	
			10 - 39[3]	0.0033	11.2	0.0001	
			≥ 70	0.0033	11.2	0.0001	
		next crop	< 10	1	0.9	0.0031	
			10 - 39[3]	1	0.9	0.0031	
			≥ 70	1	0.9	0.0031	

Ef: exposure factor; SV: shortcut value; ETR: exposure toxicity ratio.
[1] Crop category chosen according to the recommendations of the EFSA GD on the Risk Assessment on Bees (2013) and the EFSA Screening Step and 1st Tier Calculator,
[2] Max. single application rate of 1440 g a.e./ha considered for risk calculation as it covers lower rates.
[3] Scenario only relevant for uses 6a and b for which the highest intended application rate is 1.08 kg a.s./ka.

Table 2.9.9.3.1-27: First-tier assessment (oral exposure) of the risk for bumble bees due to the use of MON 52276 - tuber vegetables

Intended use	Tuber vegetables (Uses: 1, 2, 3, 6, 10)
Application method	downward spraying
Crop category	potatoes[1]
Active substance	Glyphosate
Use pattern	1-3 x 1440 g a.e./ha[2]

Test design	Endpoint (lab.)	Scenario	BBCH	Ef	SV	ETR	Trigger
Acute oral toxicity	LD_{50} > 412 µg a.e./bee	treated crop	< 10	1	0.03	0.0001	0.036
			10 - 39[3]	1	2.3	0.0080	
			≥ 70	1	0	0.0000	
		Weeds	< 10	1	6.5	0.0227	

Intended use	Tuber vegetables (Uses: 1, 2, 3, 6, 10)
Application method	downward spraying
Crop category	potatoes[1]
Active substance	Glyphosate
Use pattern	1-3 x 1440 g a.e./ha[2]

Test design	Endpoint (lab.)	Scenario	BBCH	Ef	SV	ETR	Trigger
			10 - 39[3]	1	6.5	0.0227	
			≥ 70	0.3	6.5	0.0068	
		field margin	< 10	0.0092	6.5	0.0002	
			10 - 39[3]	0.0092	6.5	0.0002	
			≥ 70	0.0092	6.5	0.0002	
		adjacent crop	< 10	0.0033	11.2	0.0001	
			10 - 39[3]	0.0033	11.2	0.0001	
			≥ 70	0.0033	11.2	0.0001	
		next crop	< 10	1	0.9	0.0031	
			10 - 39[3]	1	0.9	0.0031	
			≥ 70	1	0.9	0.0031	

Ef: exposure factor; SV: shortcut value; ETR: exposure toxicity ratio.

[1] Crop category chosen according to the recommendations of the EFSA GD on the Risk Assessment on Bees (2013) and the EFSA Screening Step and 1st Tier Calculator

[2] Max. single application rate of 1440 g a.e./ha considered for risk calculation as it covers lower rates.

[3] Scenario only relevant for uses 6a and b for which the highest intended application rate is 1.08 kg a.s./ka.

Table 2.9.9.3.1-28: First-tier assessment (oral exposure) of the risk for bumble bees due to the use of MON 52276 - Bulb vegetables

Intended use	Bulb vegetables (Uses: 1, 2, 3, 6, 10)
Application method	downward spraying
Crop category	bulb vegetables[1]
Active substance	Glyphosate
Use pattern	1-2 x 1440 g a.e./ha[2]

Test design	Endpoint (lab.)	Scenario	BBCH	Ef	SV	ETR	Trigger
Acute oral toxicity	LD_{50} > 412 µg a.e./bee	treated crop	< 10	1	0.9	0.0031	0.036
			10 - 39[3]	1	11.2	0.0391	
			≥ 70	1	0	0.0000	
		Weeds	< 10	1	6.5	0.0227	
			10 - 39[3]	1	6.5	0.0227	
			≥ 70	0.6	6.5	0.0136	
		field margin	< 10	0.0092	6.5	0.0002	
			10 - 39[3]	0.0092	6.5	0.0002	
			≥ 70	0.0092	6.5	0.0002	
		adjacent crop	< 10	0.0033	11.2	0.0001	
			10 - 39[3]	0.0033	11.2	0.0001	
			≥ 70	0.0033	11.2	0.0001	
		next crop	< 10	1	0.9	0.0031	
			10 - 39[3]	1	0.9	0.0031	
			≥ 70	1	0.9	0.0031	

Ef: exposure factor; SV: shortcut value; ETR: exposure toxicity ratio.
[1] Crop category chosen according to the recommendations of the EFSA GD on the Risk Assessment on Bees (2013) and the EFSA Screening Step and 1st Tier Calculator
[2] Max. single application rate of 1440 g a.e./ha considered for risk calculation as it covers lower rates.
[3] Scenario only relevant for uses 6a and b for which the highest intended application rate is 1.08 kg a.s./ka.

Table 2.9.9.3.1-29: First-tier assessment (oral exposure) of the risk for bumble bees due to the use of MON 52276 - Brassica, leafy and stem vegetables

Intended use	Brassica, leafy vegetables, stem vegetables (Uses: 1, 2, 3, 6, 10)
Application method	downward spraying
Crop category	leafy vegetables, lettuce[1]
Active substance	Glyphosate
Use pattern	1-3 x 1440 g a.e./ha[2]

Test design	Endpoint (lab.)	Scenario	BBCH	Ef	SV	ETR	Trigger
Leafy vegetables							
Acute oral toxicity	LD_{50} > 412 µg a.e./bee	treated crop	< 10	1	0.9	0.0031	0.036
			10 - 49[3]	1	11.2	**0.0391**	

Intended use	Brassica, leafy vegetables, stem vegetables (Uses: 1, 2, 3, 6, 10)
Application method	downward spraying
Crop category	leafy vegetables, lettuce[1]
Active substance	Glyphosate
Use pattern	1-3 x 1440 g a.e./ha[2]

Test design	Endpoint (lab.)	Scenario	BBCH	Ef	SV	ETR	Trigger
			≥ 70	1	0	0.0000	
		Weeds	< 10	1	6.5	0.0227	
			10 - 49[3]	1	6.5	0.0227	
			≥ 70	0.3	6.5	0.0068	
		field margin	< 10	0.0092	6.5	0.0002	
			10 - 49	0.0092	6.5	0.0002	
			≥ 70	0.0092	6.5	0.0002	
		adjacent crop	< 10	0.0033	11.2	0.0001	
			10 - 49[3]	0.0033	11.2	0.0001	
			≥ 70	0.0033	11.2	0.0001	
		next crop	< 10	1	0.9	0.0031	
			10 - 49[3]	1	0.9	0.0031	
			≥ 70	1	0.9	0.0031	
Lettuce							
Acute oral toxicity	LD_{50} > 412 µg a.e./bee	treated crop	< 10	1	0.03	0.0001	0.036
			10 - 49[3]	1	2.3	0.0080	
			≥ 70	1	0	0.0000	
		Weeds	< 10	1	6.5	0.0227	
			10 - 49[3]	1	6.5	0.0227	
			≥ 70	0.3	6.5	0.0068	
		field margin	< 10	0.0092	6.5	0.0002	
			10 - 49[3]	0.0092	6.5	0.0002	
			≥ 70	0.0092	6.5	0.0002	
		adjacent crop	< 10	0.0033	11.2	0.0001	
			10 - 49[3]	0.0033	11.2	0.0001	
			≥ 70	0.0033	11.2	0.0001	
		next crop	< 10	1	0.9	0.0031	
			10 – 49[3]	1	0.9	0.0031	
			≥ 70	1	0.9	0.0031	

Ef: exposure factor; SV: shortcut value; ETR: exposure toxicity ratio.

[1] Crop category chosen according to the recommendations of the EFSA GD on the Risk Assessment on Bees (2013) and the EFSA Screening Step and 1st Tier Calculator,

[2] Max. single application rate of 1440 g a.e./ha considered for risk calculation as it covers lower rates.

[3] Scenario only relevant for uses 6a and b for which the highest intended application rate is 1.08 kg a.s./ka.

Table 2.9.9.3.1-30: First-tier assessment (oral exposure) of the risk for bumble bees due to the use of MON 52276 - Sugar beet

Intended use	Sugar beet (Uses: 1, 2, 3, 10)
Application method	downward spraying
Crop category	sugar beet[1]
Active substance	Glyphosate
Use pattern	1-3 x 1440 g a.e./ha[2]

Test design	Endpoint (lab.)	Scenario	BBCH	Ef	SV	ETR	Trigger
Acute oral toxicity	LD_{50} > 412 µg a.e./bee	treated crop	< 10	1	0.9	0.0031	0.036
			≥ 70	1	0	0.0000	
		Weeds	< 10	1	6.5	0.0227	
			≥ 70	0.25	6.5	0.0057	
		field margin	< 10	0.0092	6.5	0.0002	
			≥ 70	0.0092	6.5	0.0002	
		adjacent crop	< 10	0.0033	11.2	0.0001	
			≥ 70	0.0033	11.2	0.0001	
		next crop	< 10	1	0.9	0.0031	
			≥ 70	1	0.9	0.0031	

Ef: exposure factor; SV: shortcut value; ETR: exposure toxicity ratio.
[1] Crop category chosen according to the recommendations of the EFSA GD on the Risk Assessment on Bees (2013) and the EFSA Screening Step and 1st Tier Calculator,
[2] Max. single application rate of 1440 g a.e./ha considered for risk calculation as it covers lower rates.

Table 2.9.9.3.1-31: First-tier assessment (oral exposure) of the risk for bumble bees due to the use of MON 52276 - legume vegetables

Intended use	Legume vegetables (Uses: 1, 2, 3, 6, 10)
Application method	downward spraying
Crop category	pulses[1]
Active substance	Glyphosate
Use pattern	1-2 x 1440 g a.e./ha[2]

Test design	Endpoint (lab.)	Scenario	BBCH	Ef	SV	ETR	Trigger
Acute oral toxicity	LD_{50} > 412 µg a.e./bee	treated crop	< 10	1	0.9	0.0031	0.03
			10 49[3]	1	11.2	**0.0391**	
			≥ 70	1	0	0.0000	
		Weeds	< 10	1	6.5	0.0227	
			10 - 49[3]	1	6.5	0.0227	
			≥ 70	0.3	6.5	0.0068	
		field margin	< 10	0.0092	6.5	0.0002	
			10 - 49[3]	0.0092	6.5	0.0002	
			≥ 70	0.0092	6.5	0.0002	

Intended use	Legume vegetables (Uses: 1, 2, 3, 6, 10)
Application method	downward spraying
Crop category	pulses[1]
Active substance	Glyphosate
Use pattern	1-2 x 1440 g a.e./ha[2]

Test design	Endpoint (lab.)	Scenario	BBCH	Ef	SV	ETR	Trigger
		adjacent crop	< 10	0.0033	11.2	0.0001	
			10 - 49[3]	0.0033	11.2	0.0001	
			≥ 70	0.0033	11.2	0.0001	
		next crop	< 10	1	0.9	0.0031	
			10 - 49[3]	1	0.9	0.0031	
			≥ 70	1	0.9	0.0031	

Ef: exposure factor; SV: shortcut value; ETR: exposure toxicity ratio.
[1] Crop category chosen according to the recommendations of the EFSA GD on the Risk Assessment on Bees (2013) and the EFSA Screening Step and 1st Tier Calculator,
[2] Max. single application rate of 1440 g a.e./ha considered for risk calculation as it covers lower rates.
[3] Scenario only relevant for uses 6a and b for which the highest intended application rate is 1.08 kg a.s./ka.

All exposure toxicity ratios (ETRs) for acute oral toxicity are below the respective trigger value, except for the "treated crop" scenario at BBCH 10-49 or BBCH 10-39 for fruiting vegetables, root vegetables, bulb vegetables, leafy vegetables and legume vegetables at the highest intended rate of 1440 g a.e./ha. Nevertheless, these scenarios are only relevant for uses 6a and b for which the highest intended application rate is 1080 g a.s./ha. As this application rate presented an acceptable risk at screening step, an acceptable risk to bumble bees following application of MON 52276 can be concluded for all uses.

Risk assessment for solitary bees

The risk assessment for the proposed uses of MON 52276 and the effects on solitary bees is provided below.

Table 2.9.9.3.1-32: Screening assessment of the risk of glyphosate for solitary bees due to the use of MON 52276

Intended use	All uses (Uses: 1a-10c)			
Application method	downward spraying			
Active substance	Glyphosate			
Use pattern	1-2 x 1800 g a.e./ha, 1-2 x 1440 g a.e./ha, 1-3 x 1080 g a.e./ha, 1-3 x 720 g a.e./ha, 1 x 540 g a.e./ha			
Type design	**LD_{50} (µg a.e./bee)**	**Max. single application rate (g a.e./ha)**	**$HQ_{contact}$ criterion**	**Trigger**
Adult acute contact toxicity	>461	1800	<3.9	8
		1440	<3.1	
		1080	<2.3	
		720	<1.6	
		540	<1.2	

$HQ_{contact}$: Hazard quotient for contact exposure

The hazard quotients (HQ) for acute contact toxicity are below the respective trigger value for the application rates of 540 g a.e./ha, 720 g a.e./ha, 1080 g a.e./ha, 1440 g a.e./ha and 1800 g a.e./ha. Therefore, no Tier 1 risk assessment is required.

A summary of the risk assessment regarding bees biodiversity via indirect effects and trophic interactions resulted from uses of glyphosate is presented under point 2.9.9.8.

2.9.9.3.2 Summary of risk assessment for non-target arthropods

The risk assessment for non-target arthropods was performed according to the "Guidance Document on Terrestrial Ecotoxicology" (SANCO/10329/2002 rev.2 (final), October 17, 2002), and the guidance document ESCORT 2.

RMS considered a risk envelop approach[28] by presenting a risk assessment for the uses leading to the worst case exposure estimate. As a worst-case, a MAF factor of 2 is used in order to cover all intended intervals between applications for all uses. Considering this, the risk assessment was conducted for the uses around railroad tracks (2 times 1800 g glyphosate/ha). The risk assessment presented covers all other intended uses.

[28] SANCO (2011) Guidance document on the preparation and submission of dossiers for plant protection products according to the "risk envelope approach" SANCO/11244/2011 rev. 5, 14 March 2011

In-field risk assessment

Intended use	All uses
Active substance/product	Glyphosate/ MON52276
Application rate (g/ha)	2 × 1800 (90 d)
MAF	2 (foliar and/or soil))

Crop scenario	**Test species Tier I**	**LR_{50} (lab.) (g/ha)**	**$PER_{in\text{-}field}$ (g/ha)**	**$HQ_{in\text{-}field}$ criterion: HQ ≤ 2**
All uses	*Poecilus cupreus*	> 3600	3600	< 1
	Pardosa sp.	> 3600		< 1
Crop scenario	**Test species Tier II**	**LR_{50}/ER_{50} (ext. lab.) (g/ha)**	**$PER_{in\text{-}field}$ (g/ha)**	**$PER_{in\text{-}field}$ below rate with ≤ 50 % effect?**
All uses	*T. pyri*	>4320	3600	Yes
	A. rhopalosiphi	>5760		Yes
	Aleochara bilineata	>4320		Yes

a.e. glyphosate acid equivalents
PER: Predicted environmental rate

Off-field risk assessment

Intended use				All uses			
Active substance/product				Glyphosate/MON52276			
Application rate (g a.e./ha)				1800			
MAF				2 (foliar and/or soil)			
Drift rate (%)				2.38 (1 m)			
vdf				10 (Tier I) / 10 (Tier II, 2D test design) or 1 (Tier II, 3D test design)			
Crop scenario	**Test species Tier I**	**LR_{50} (lab.) (g/ha)**	**MAF (foliar/soil)**	**VDF**	**Correction factor**	**Corr. $PER_{off-field}$ (g/ha)**	**$HQ_{off-field}$ criterion: HQ ≤ 2**
All uses	*Poecilus cupreus* (2D)	> 3600	2	1**	10	428.4	0.024
	Pardosa sp. (2D)	> 3600		1**	10		0.024
Crop scenario	**Test species Tier II**	**LR_{50}/ER_{50} (ext. lab.) (g/ha)**	**MAF (foliar/soil)**	**VDF**	**Correction factor**	**$PER_{off-field}$ (g/ha)**	**$PER_{off-field}$ below rate with ≤ 50 % effect?**
All uses	*T. pyri* (2D)	>4320	2	5*	5	85.68	yes
	A. rhopalosiphi (3D)	>5760		1	5	428.4	yes
	Aleochara bilineata (2D)	>4320		1**	5	428.4	yes

a.e. glyphosate acid equivalents
PER: Predicted environmental rate, vdf: vegetation distribution factor; CF: correction factor
* as recommended in the Outcome of the Pesticides Peer Review Meeting on general recurring issues in ecotoxicology (EFSA Supporting publication 2019:EN-1673)
**A VDF of 1 has been considered since these species are considered to be soil-dwelling arthropods.

An acceptable risk can be expected for non-target arthropods other than bees from the proposed uses of MON 52276 considering in-field or off-field habitats of field crops, orchards, vineyards, railroad tracks and agricultural/non-agricultural areas for the control of invasive species.

A summary of the risk assessment regarding non-target arthropods biodiversity via indirect effects and trophic interactions resulted from uses of glyphosate is presented under point 2.9.9.8.

2.9.9.4 Summary of risk assessment for non-target soil meso- and macrofauna

The risk assessment is performed in accordance with the "Guidance Document on Terrestrial Ecotoxicology" (SANCO/10329/2002 rev.2 (final), October 17, 2002).
RMS considered a risk envelop approach[29] by presenting a risk assessment for the uses leading to the worst case PECsoil and thus covering all intended uses.

[29] SANCO (2011) Guidance document on the preparation and submission of dossiers for plant protection products according to the "risk envelope approach" SANCO/11244/2011 rev. 5, 14 March 2011

2.9.9.4.1 Summary of risk assessment for earthworms

Chronic effects on earthworms			
Intended use	All uses		
Product/active substance	**NOEC (mg/kg dw)**	**$PEC_{soil, accu}$ (mg/kg)**	**TER_{lt}***
Glyphosate	473	5.123	92.3
AMPA	131.9	6.845	19.3
MON 52276	38	5.123	7.4

* TER: toxicity to exposure ratio = Endpoint / PEC_{soil} given in mg glyphosate acid equivalents/kg dw.

An acceptable risk can be expected for earthworms from the proposed uses of MON 52276 on field crops, orchards, vineyards, railroad tracks and agricultural/non-agricultural areas for the control of invasive species.

2.9.9.4.2 Summary of risk assessment for other soil meso- and macrofauna

Intended use	All uses		
Chronic effects on *Hypoaspis aculeifer*			
Product/active substance	**NOEC (mg/kg dw)**	**$PEC_{soil, accu}$ (mg/kg)**	**TER_{lt}***
Glyphosate	473	5.123	92.3
AMPA	320	6.845	46.7
MON52276	1802	5.123	351.7
Chronic effects on *Folsomia candida*			
Product/active substance	**NOEC (mg/kg dw)**	**$PEC_{soil. accu}$ (mg/kg)**	**TER_{lt}***
Glyphosate	587	5.123	114.6
AMPA	315	6.845	46.0
MON52276	1802	5.123	351.7

* TER: toxicity to exposure ratio = Endpoint / PEC_{soil} given in mg glyphosate acid equivalents/kg dw.

An acceptable risk can be expected for soil macroorganisms other than earthworms from the proposed uses of MON 52276 MON 52276 on field crops, orchards, vineyards, railroad tracks and agricultural/non-agricultural areas for the control of invasive species.

A summary of the risk assessment regarding soil meso- and macrofauna biodiversity via indirect effects and trophic interactions n resulted from uses of glyphosate is presented under point 2.9.9.8.

2.9.9.5 Summary of risk assessment for soil nitrogen transformation

The risk assessment is performed in accordance with the "Guidance Document on Terrestrial Ecotoxicology" (SANCO/10329/2002 rev.2 (final), October 17, 2002).
RMS considered a risk envelop approach[30] by presenting a risk assessment for the uses leading to the worst case PECsoil and thus covering all intended uses.

Nitrogen transformation			
Intended use	All uses		
Product/active substance	**Max. conc. with effects ≤ 25% (mg/kg)**	**$PEC_{soil, accu}$ (mg/kg)**	**Risk acceptable?**
Glyphosate	≥ 33.1	5.123	yes
AMPA	≥ 160	6.845	yes

Risk for nitrogen transformation is considered acceptable for the intended uses of MON 52276.

A summary of the risk assessment regarding soil microorganisms biodiversity via indirect effects and trophic interactions resulted from uses of glyphosate is presented under point 2.9.9.8.

2.9.9.6 Summary of risk assessment for terrestrial non-target higher plants

The risk assessment is based on the "Guidance Document on Terrestrial Ecotoxicology", (SANCO/10329/2002 rev.2 final, 2002). It is restricted to off-field situations, as non-target plants are non-crop plants located outside the treated area.

RMS considered a risk envelop approach by presenting a risk assessment for the dose rates leading to the worst case PER (predicted environmental rates) for each type of use and thus covering all other intended rates for these uses.
The risk assessment for non-target terrestrial plants is grouped as follows:

- in field crops: assessed for rates of 3 x 720 g a.e./ha and 1 x 1440 g a.e./ha, covering GAP uses 1 a-c, 2 a-c, 3 a-b, 6 a-b, 10 a-c.
- in orchards/vineyards: assessed for rates of 3 x 720 g a.e./ha and 2 x 1440 g a.e./ha covering GAP uses 4 a-c, 5 a-c.
- around railroad tracks : assessed for rate of 2 x 1800 g a.e./ha covering GAP uses 7 a-b.
- in agricultural and non-agricultural areas to control invasive species: assessed for rate of 2 x 1800 g a.e./ha covering GAP uses 8 and 9.

[30] SANCO (2011) Guidance document on the preparation and submission of dossiers for plant protection products according to the "risk envelope approach" SANCO/11244/2011 rev. 5, 14 March 2011

Field Crops

Table 2.9.9.6-1: Deterministic assessment of the risk for non-target plants due to the use of MON 52276 – field crops considering downward ground directed spray

Crop scenario	Appl. Rate [g a.e./ha]	ER_{50} [g a.e./ha]	Drift [%]	PER [2] [g a.e./ha]	TER (criterion: TER ≥5)
Field Crops – GAP uses 1 a-c, 2 a-c, 3 a-b, 6 a-b, & 10 a-c					
Vegetative vigour					
All uses considering downward ground directed spray	3 x 720	28.4	2.77	19.9	**1.42**
	1 x 1440			39.9	**0.71**
Seedling emergence					
All uses considering downward ground directed spray	3 x 720	>3610	2.77	19.9	>181
	1 x 1440			39.9	>90.5

PER: Predicted environmental rate; TER: toxicity to exposure ratio. TER values shown in **bold** fall below the relevant trigger.
[2] PER (g a.e./ha) based on drift rate (%) of 2.77% at 1 m from the application area considering downward ground directed spray

Orchards

Table 2.9.9.6-2: Deterministic assessment of the risk for non-target plants due to the use of MON 52276 – orchards considering downward ground directed spray

Crop scenario	Appl. Rate [g a.e./ha]	ER_{50} [g a.e./ha]	Drift [%]	PER [2] [g a.e./ha]	TER (criterion: TER ≥5)
Orchards / vineyards – GAP uses 4 a-c & 5 a-c					
Vegetative vigour					
All uses considering downward ground directed spray	3 x 720	28.4	2.77	19.9	**1.42**
	2 x 1440			39.9	**0.71**
Seedling emergence					
All uses considering downward ground directed spray	3 x 720	>3610	2.77	19.9	>181
	2 x 1440			39.9	>90.5

PER: Predicted environmental rate; TER: toxicity to exposure ratio. TER values shown in **bold** fall below the relevant trigger.
[2] PER (g a.e./ha) based on drift rate (%) of 2.77% at 1 m from the application area considering downward ground directed spray

Railroad tracks

Table 2.9.9.6-3: Deterministic assessment of the risk for non-target plants due to the use of MON 52276 – railroad tracks considering downward ground directed spray

Crop scenario	Appl. Rate [g a.e./ha]	ER_{50} [g a.e./ha]	Drift [%]	PER [2] [g a.e./ha]	TER (criterion: TER ≥5)
Railroad tracks GAP uses 7 a-b					
Vegetative vigour					
All uses considering downward ground directed spray	2 x 1800	28.4	2.77	49.86	**0.57**
Seedling emergence					
All uses considering downward ground directed spray	2 x 1800	>3610	2.77	49.86	7>2.4

PER: Predicted environmental rate; TER: toxicity to exposure ratio. TER values shown in **bold** fall below the relevant trigger.
[2] PER (g a.e./ha) based on drift rate (%) of 2.77% at 1 m from the application area considering downward ground directed spray

Agricultural and non-agricultural area – Invasive species

Table 2.9.9.6-4: Deterministic assessment of the risk for non-target plants due to the use of MON 52276 – Agricultural and non-agricultural area – Invasive species considering downward ground directed spray

Crop scenario	Appl. Rate [g a.e./ha]	ER_{50} [g a.e./ha]	Drift [%]	PER [2] [g a.e./ha]	TER (criterion: TER ≥5)
Agricultural and non-agricultural area – Invasive species – uses 8 & 9					
Vegetative vigour					
All uses considering downward ground directed spray	1 x 1800	28.4	2.77	49.86	**0.57**
Seedling emergence					
All uses considering downward ground directed spray	1 x 1800	>3610	2.77	49.86	>72.4

PER: Predicted environmental rate; TER: toxicity to exposure ratio. TER values shown in **bold** fall below the relevant trigger.
[2] PER (g a.e./ha) based on drift rate (%) of 2.77% at 1 m from the application area considering downward ground directed spray

Field Crops

Table 2.9.9.6-5: Deterministic assessment of the risk for non-target plants due to the use of MON 52276 – field crops considering downward ground directed spray

Crop scenario	Appl. Rate [g a.e./ha]	ER_{50} [g a.e./ha]	Drift [%]	PER [2] [g a.e./ha]	TER (criterion: TER ≥5)
Field Crops – GAP uses 1 a-c, 2 a-c, 3 a-b, 6 a-b, & 10 a-c					
Vegetative vigour					
All uses considering downward ground directed spray	3 x 720	28.4	0.57 – at 5 m	4.10	6.92
	1 x 1440			8.21	**3.46**
All uses considering downward ground directed spray	1 x 1440		0.29 – at 10 m	4.18	6.80

PER: Predicted environmental rate; TER: toxicity to exposure ratio. TER values shown in **bold** fall below the relevant trigger.
[2] PER (g a.e./ha) based on drift rate (%) of 0.57% at 5 m and 0.29% at 10 m from the application area considering downward ground directed spray

Orchards

Table 2.9.9.6-6: Deterministic assessment of the risk for non-target plants due to the use of MON 52276 – orchards considering downward ground directed spray

Crop scenario	Appl. Rate [g a.e./ha]	ER_{50} [g a.e./ha]	Drift [%]	PER [2] [g a.e./ha]	TER (criterion: TER ≥5)
Orchards / vineyards – GAP uses 4 a-c & 5 a-c					
Vegetative vigour					
All uses considering downward ground directed spray	3 x 720	28.4	0.57 – at 5 m	4.10	6.92
	2 x 1440			8.21	**3.46**
All uses considering downward ground directed spray	2 x 1440		0.29 – at 10 m	4.18	6.80

PER: Predicted environmental rate; TER: toxicity to exposure ratio. TER values shown in **bold** fall below the relevant trigger.
[2] PER (g a.e./ha) based on drift rate (%) of 0.57% at 5 m and 0.29% at 10 m from the application area considering downward ground directed spray

Railroad tracks

Table 2.9.9.6-7: Deterministic assessment of the risk for non-target plants due to the use of MON 52276 – railroad tracks considering downward ground directed spray

Crop scenario	Appl. Rate [g a.e./ha]	ER_{50} [g a.e./ha]	Drift [%]	PER [2] [g a.e./ha]	TER (criterion: TER ≥5)
Railroad tracks – use 7 a-c					
Vegetative vigour					
All uses considering downward ground directed spray	2 x 1800	28.4	0.57 – at 5 m	10.26	**2.77**
			0.29 – at 10 m	5.22	5.44

PER: Predicted environmental rate; TER: toxicity to exposure ratio. TER values shown in **bold** fall below the relevant trigger.
[2] PER (g a.e./ha) based on drift rate (%) of 0.57% at 5 m and 0.29% at 10 m from the application area considering downward ground directed spray

Agricultural and non-agricultural area – Invasive species

Table 2.9.9.6-8: Deterministic assessment of the risk for non-target plants due to the use of MON 52276 – Agricultural and non-agricultural area – Invasive species considering downward ground directed spray

Crop scenario	Appl. Rate [g a.e./ha]	ER_{50} [g a.e./ha]	Drift [%]	PER [2] [g a.e./ha]	TER (criterion: TER ≥5)
Agricultural and non-agricultural area – Invasive species – uses 8 & 9					
Vegetative vigour					
All uses considering downward ground directed spray	1 x 1800	28.4	0.57 – at 5 m	10.26	**2.77**
			0.29 – at 10 m	5.22	5.44

PER: Predicted environmental rate; TER: toxicity to exposure ratio. TER values shown in **bold** fall below the relevant trigger.
[2] PER (g a.e./ha) based on drift rate (%) of 0.57% at 5 m and 0.29% at 10 m from the application area considering downward ground directed spray

Field crops

Table 2.9.9.6-9: Deterministic risk assessment for non-target terrestrial plants due to the use of MON52276 in field crops (3 x 720 g a.e./ha) considering risk mitigation (in-field no-spray buffer zones and drift-reducing nozzles)

Intended use		Field crops		
Application rate (g a.e./ha)		3 x 720		
MAF		1.0		
Buffer strip (m)	**Drift rate (%)**	**$PER_{off-field}$ 50 % drift red. (g a.e./ha)**	**$PER_{off-field}$ 75 % drift red. (g a.e./ha)**	**$PER_{off-field}$ 90 % drift red. (g a.e./ha)**
1	2.77	9.97	4.99	-
5	0.57	2.05	-	-
10	0.29	-	-	-
Toxicity value		**TER**		
ER_{50} = 28.4 g a.e./ha		**criterion: TER ≥ 5**		
1		**2.85**	5.70	-
5		13.84	-	-
10		-	-	-

Table 2.9.9.6-10: Deterministic risk assessment for non-target terrestrial plants due to the use of MON52276 in field crops (1 x 1440 g a.e./ha) considering risk mitigation (in-field no-spray buffer zones, and drift-reducing nozzles)

Intended use		Field crops		
Application rate (g a.e./ha)		1 x 1440		
MAF		1.0		
Buffer strip (m)	**Drift rate (%)**	**$PER_{off-field}$ 50 % drift red. (g a.e./ha)**	**$PER_{off-field}$ 75 % drift red. (g a.e./ha)**	**$PER_{off-field}$ 90 % drift red. (g a.e./ha)**
1	2.77	19.94	9.97	3.99
5	0.57	4.10	2.05	0.82
10	0.29	2.09	1.04	0.42
Toxicity value		**TER**		
ER_{50} = 28.4 g a.e./ha		**criterion: TER ≥ 5**		
1		**1.42**	**2.85**	7.12
5		6.92	13.84	34.60
10		13.60	27.20	68.01

Orchards

Table 2.9.9.6-11: Deterministic risk assessment for non-target terrestrial plants due to the use of MON52276 in orchards (3 x 720 g a.e./ha) considering risk mitigation (in-field no-spray buffer zones and drift-reducing nozzles)

Intended use		Orchards		
Application rate (g a.e./ha)		3 x 720		
MAF		1.0		
Buffer strip (m)	**Drift rate (%)**	**$PER_{off-field}$ 50 % drift red. (g a.e./ha)**	**$PER_{off-field}$ 75 % drift red. (g a.e./ha)**	**$PER_{off-field}$ 90 % drift red. (g a.e./ha)**
1	2.77	9.97	4.99	-
5	0.57	2.05	-	-
10	0.29	-	-	-
Toxicity value		**TER**		
ER_{50} = 28.4 g a.e./ha		**criterion: TER ≥ 5**		
1		**2.86**	5.72	-
5		13.89	-	-
10		-	-	-

Table 2.9.9.6-12: Deterministic risk assessment for non-target terrestrial plants due to the use of MON52276 in orchards (2 x 1440 g a.e./ha) considering risk mitigation (in-field no-spray buffer zones, and drift-reducing nozzles)

Intended use		Orchards		
Application rate (g a.e./ha)		2 x 1440		
MAF		1.0		
Buffer strip (m)	**Drift rate (%)**	**$PER_{off-field}$ 50 % drift red. (g a.e./ha)**	**$PER_{off-field}$ 75 % drift red. (g a.e./ha)**	**$PER_{off-field}$ 90 % drift red. (g a.e./ha)**
1	2.77	19.94	9.97	3.99
5	0.57	4.10	2.05	0.82
10	0.29	2.09	1.04	0.42
Toxicity value		**TER**		
ER_{50} = 28.4 g a.e./ha		**criterion: TER ≥ 5**		
1		**1.42**	**2.85**	7.12
5		6.92	13.84	34.60
10		13.60	27.20	68.01

Railroad tracks

Table 2.9.9.6-13: Deterministic risk assessment for non-target terrestrial plants due to the use of MON52276 on railroad tracks (2 x 1800 g a.e./ha) considering risk mitigation (in-field no-spray buffer zones, and drift-reducing nozzles)

Intended use		Railroad tracks		
Application rate (g a.e./ha)		2 x 1800		
MAF		1.0		
Buffer strip (m)	**Drift rate (%)**	**$PER_{off-field}$ 50 % drift red. (g a.e./ha)**	**$PER_{off-field}$ 75 % drift red. (g a.e./ha)**	**$PER_{off-field}$ 90 % drift red. (g a.e./ha)**
1	2.77	24.93	12.47	4.99
5	0.57	5.13	2.57	1.03
10	0.29	2.61	1.31	0.52
Toxicity value		**TER**		
ER_{50} = 28.4 g a.e./ha		**criterion: TER ≥ 5**		
1		**1.14**	**2.28**	5.70
5		5.54	11.07	27.68
10		10.88	21.76	54.41

Agricultural and non-agricultural area – Invasive species

Table 2.9.9.6-14: Deterministic risk assessment for non-target terrestrial plants due to the use of MON52276 in agricultural and non-agricultural area – invasive species (1 x 1800 g a.e./ha) considering risk mitigation (in-field no-spray buffer zones, and drift-reducing nozzles)

Intended use		Agricultural and non-agricultural area – Invasive species		
Application rate (g a.e./ha)		1 x 1800		
MAF		1.0		
Buffer strip (m)	**Drift rate (%)**	**$PER_{off-field}$ 50 % drift red. (g a.e./ha)**	**$PER_{off-field}$ 75 % drift red. (g a.e./ha)**	**$PER_{off-field}$ 90 % drift red. (g a.e./ha)**
1	2.77	24.93	12.47	4.99
5	0.57	5.13	2.57	1.03
10	0.29	2.61	1.31	0.52
Toxicity value		**TER**		
ER_{50} = 28.4 g a.e./ha		**criterion: TER ≥ 5**		
1		**1.14**	**2.28**	5.70
5		5.54	11.07	27.68
10		10.88	21.76	54.41

Conclusion

The risk to non target plants can be considered acceptable when risk mitigations to protect non target terrestrial plants at the edge of the field are implemented. The risk mitigations are reported in the table below.

Table 2.9.9.6-15: Risk mitigation measures for terrestrial non-target plants

GAP number and summary of use	Application rate considered (28 day internal unless otherwise stated)									
	1 × 540 g/ha	**1 × 720 g/ha**	**2 × 720 g/ha**	**3 × 720 g/ha**	**1 × 1080 g/ha**	**2 × 1080 g/ha[A]**	**1 × 1440 g/ha**	**2 × 1440 g/ha**	**1 × 1800 g/ha**	**2 × 1800 g/ha (90 days apart)**
Uses 1a-c: Applied to weeds; pre-sowing, pre-planting, pre-emergence of **field crops.**		5m BS Or 75% drift-reducting nozzles			10m BS Or 5m BS and 50% drift-reducting nozzles Or 90% drift-reducting nozzles		10m BS Or 5m BS and 50% drift-reducting nozzles Or 90% drift-reducting nozzles			
Uses 2 a-c: Applied to weeds; post-harvest, pre-sowing, pre-planting of **field crops.**		5m BS Or 75% drift-reducting nozzles			10m BS Or 5m BS and 50% drift-reducting nozzles Or 90% drift-reducting nozzles		10m BS Or 5m BS and 50% drift-reducting nozzles Or 90% drift-reducting nozzles			
Use 3 a-b: Applied to cereal volunteers; post-harvest, pre-sowing, pre-planting of **field crops.**	5m BS Or 75% drift-reducting nozzles									
Use 6 a-b: Applied to weeds (post-emergence) in **field crops BBCH < 20**		5m BS Or 75% drift-reducting nozzles			10m BS Or 5m BS and 50% drift-reducting nozzles Or 90% drift-reducting nozzles					
Uses 10 a-c: Applied to couch grass; post-harvest, pre-sowing,		5m BS Or 75% drift-reducting nozzles			10m BS Or 5m BS and 50% drift-					

GAP number and summary of use		Application rate considered (28 day internal unless otherwise stated)								
	1 × 540 g/ha	**1 × 720 g/ha**	**2 × 720 g/ha**	**3 × 720 g/ha**	**1 × 1080 g/ha**	**2 × 1080 g/ha[A]**	**1 × 1440 g/ha**	**2 × 1440 g/ha**	**1 × 1800 g/ha**	**2 × 1800 g/ha (90 days apart)**
pre-planting of **field crops**					reducting nozzles Or 90% drift-reducting nozzles					
Use 4 a-c: Applied to weeds (post-emergence) below trees in **orchards.**		5m BS Or 75% drift-reducting nozzles			10m BS Or 5m BS and 50% drift-reducting nozzles Or 90% drift-reducting nozzles					
Use 5 a-c: Applied to weeds (post-emergence) below vines in **vineyards**		5m BS Or 75% drift-reducting nozzles			10m BS Or 5m BS and 50% drift-reducting nozzles Or 90% drift-reducting nozzles					
Use 7 a-b: Applied to weeds (post-emergence) around **railroad tracks**									10m BS Or 5m BS and 50% drift-reducting nozzles Or 90% drift-reducting nozzles	
Use 8 and 9: Applied to invasive species (post-emergence) in **agricultural and non-agricultural areas**									10m BS Or 5m BS and 50% drift-reducting nozzles Or 90% drift-reducting nozzles	

BS = Untreated buffer strip

[A] Due to the long spray interval of 28 days this use covers also the following possible application pattern: 2 × 1080 g a.s./ha plus 1 x 720 g a.s./ha (28 day interval between each application)

A summary of the risk assessment regarding non-target terrestrial plants biodiversity via indirect effects and trophic interactions resulted from uses of glyphosate is presented under point 2.9.9.8.

2.9.9.7 Summary of risk assessment for biological methods for sewage treatment

No risk for biological methods for sewage treatment is expected.

2.9.9.8 Summary of assessment of risk to biodiversity via indirect effects and trophic interactions

The regulation (EU) 2017/2324 related to the approval of glyphosate stated that "Member States shall pay particular attention (…) to the risk to diversity and abundance of non-target terrestrial arthropods and vertebrates via trophic

interactions". Currently, there is no validated tools nor methodology for a European harmonized risk assessment of biodiversity and consideration of indirect effects via trophic interactions available.
RMS recommended to the applicant to have a broader consideration of potential effects on non target organisms by exploring the current state of the art in order to identify potential new data/information or new approach or tools that may help to provide some quantitative information to address this specific concern[31]. RMS also advises to use monitoring data to address the point. Furthermore, given the magnitude of use of glyphosate based herbicides, glyphosate is frequently observed in the environment. Even if biodiversity is not affected by glyphosate alone, its effects on biodiversity should be addressed. Indeed, a loss of vegetation/plant biodiversity following the application of plant protection products may affect the entire food web. It could affect the presence of adequate habitats for arthropods, as well as for birds and mammals. Moreover the presence of appropriate range of plants as food sources is vital to the survival of foliage eating arthropods, birds and mammals, as well as nectar and pollen sources for bees.

The applicant has provided a report entitled "Glyphosate: Indirect effects via trophic interaction - A Practical Approach to Biodiversity Assessment" [redacted] 2020, CA 8.8/001). This report presents the applicant's approach for assessing the risk to biodiversity by informing on potential indirect effects and trophic interactions. This report also provides information for risk managers aiming to provide additional risk mitigations options to protect aquatic and terrestrial biodiversity. It should be noted that some of the published papers cited in this report have not been provided, nor summarized in the re-assessment dossier nor in the previous RAR. Some of these papers may be of interest (they are listed in throughout the text).
In summary, the applicant made an attempt to assess biodiversity via an assessment of indirect effects and trophic interactions. RMS noted that even if indirect effects and trophic interactions are linked to biodiversity, there is much more to consider to protect biodiversity and the providing ecosystem services in Europe in adequacy with the various EU and national legislations. The approach of the applicant mainly focus on definition of Specific Protection Goals that are taken into account in the existing guidance documents. The EFSA guidance on specific protection goals (2016)[32] aims to make general protection goals operational for use in environment risk assessment and take into account biodiversity and ecosystem services. The method to define SPG follow "three sequential steps: (1) the identification of relevant ecosystem services; (2) the identification of service providing units (SPUs) for these ecosystem services; and (3) the specification of options for the level/parameters of protection of the SPUs using five interrelated dimensions" (ecological entity, attribute to protect, magnitude of effects, temporal scale and spatial scale of the biologically tolerable effects). Definition of SPG require a dialogue between risk assessors and risk managers. RMS considered that it is the most suitable approach available to assess biodiversity in the context of regulatory risk assessment.

For transparency, summaries of assessments and conclusions as proposed by the applicant were reported thereafter in grey boxes. They are followed by conclusion of RMS.

Aquatic organisms - Biodiversity Assessment via indirect effects via and trophic interactions

Summary by the applicant:
The following table assessment illustrates that ecological function of aquatic organisms in off-field / off-target areas / edge of field surface water, will be sufficiently maintained to achieve the specific protection goals (SPGs) for the aquatic organisms according to the protection goals as defined in the EFSA guidance (2016), that sustains habitat and food resources for other organisms whilst achieving low to negligible acute and chronic effects on aquatic plants and animals.

Table [...]: The relationship between the Specific Protection Goal, assessment endpoints and measurement endpoints for aquatic systems (wetlands, rivers and lakes) exposed by runoff and/or spray drift

Specific Protection Goal[1]	Assessment Endpoints	Measurement Endpoints	Glyphosate Study Types[2]

[31] Minutes pre-submission meeting GTF2-RMS Fate & Behaviour, Ecotoxicology, Endocrine Disruption of 17/10/2019
[32] EFSA Scientific Committee, 2016. Guidance to develop specific protection goals options for environmental risk assessment at EFSA, in relation to biodiversity and ecosystem services. EFSA Journal 2016;14(6):4499, 50 pp. doi:10.2903/j.efsa.2016.4499

Negligible acute and long-term effects to aquatic plant and animal populations from direct and indirect effects through trophic interactions.	Survival, growth and reproduction of aquatic populations	Acute and chronic toxicity to aquatic plants and animals and bioaccumulation	Algal Vascular plants Acute Daphnia Daphnia life-cycle Chironomid emergence Acute fish Fish ELS Fish repro screening * Fish Full Life-cycle* Amphibian metamorphosis * Fish bioconcentration
Biodiversity Assessment for Aquatic Ecosystems Based on the specific protection goal, inclusion of a 1 m buffer between the application area and the adjacent surface water body, for applications of MON 52276 made according to the representative GAP, is considered protective of both direct and indirect effects on biodiversity in aquatic ecosystems through trophic interactions.			

[1] By accepting no population-level effects on representative sensitive populations in edge-of-field surface waters, these populations will be protected and propagation of effects to the community-, ecosystem- and landscape-level will not occur (Option 1: EFSA aquatic guidance, 2013).
[2] Acute and chronic aquatic studies for aquatic plants and animals are presented in the ecotoxicology section. Endpoints for AMPA are similar to endpoints for the same studies with glyphosate.
* Note these studies were performed to assess the potential for impacts to the endocrine pathways. No effects to the four endocrine pathways can be concluded based on the results of these studies and a weight of evidence evaluation (USEPA, 2015, EFSA, 2017)

Conclusion to the biodiversity assessment for aquatic organisms;
The current direct effects aquatic risk assessment […]shows that inclusion of a one-meter buffer between the applied area and the edge-of-field surface water for glyphosate applications is considered protective of both direct effects and indirect effects through trophic interactions on aquatic biodiversity for the intended uses.

As a conservative approach for finalizing the aquatic biodiversity assessment, the lower tier assessment option known as the Ecological Threshold Option (ETO) from the EFSA's tiered guidance for aquatic risk assessments (EFSA (2013). This option aims at ensuring that negligible effects only, may occur in aquatic populations (transient effects followed by recovery are not accepted with this option). Both direct and indirect effects on the food chain are covered within this option. When applied to the representative sensitive populations in edge-of-field surface water, this option allows to conclude that aquatic populations will be protected, and that propagation of effects to the community-, ecosystem-, and landscape level will not occur.

The indirect effects via trophic interaction, a biodiversity assessment with further consideration of relevant and reliable literature, concerning aquatic organisms are discussed and presented.

Comment and conclusion of RMS for aquatic biodiversity assessment
The approach followed in the EFSA guidance for aquatic organisms (2013)[33] for the definition of specific protection goals is in line with EFSA guidance on specific protection goals (2016). As such it is the most suitable guidance document that allow to consider biodiversity and ecosystem services for aquatic organisms. The tiered approach developed aimed to protect populations of aquatic organisms by defining Regulatory Acceptable Concentration (RAC) based on two options: "(1) The ecological threshold option (ETO), accepting negligible population effects only, and (2) the ecological recovery option (ERO), accepting some population-level effects if ecological recovery takes place within an acceptable time period".
The assessment of glyphosate is performed using RAC values based on the ecological threshold option (ETO). An overview of the SPG as defined in the aquatic guidance is presented here.

[33] EFSA PPR Panel (EFSA Panel on Plant Protection Products and their Residues), 2013. Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters. EFSA Journal 2013;11(7):3290, 268 pp. doi:10.2903/j.efsa.2013.3290.

Table 13: Overview of proposed specific protection goals for the ecological threshold option

Organism group	Ecological entity	Attribute	Magnitude	Time
Algae	Population	Abundance/biomass	Negligible effect	Not applicable
Aquatic plants	Population	Survival/growth Abundance/biomass		
Aquatic invertebrates	Population	Abundance/biomass		
Vertebrates	Individual	Survival		
	Population	Abundance/biomass		
Aquatic microbes	Functional group	Processes (e.g. litter break down)	RA will not be developed since tier 1 data requirements are not defined	

From this approach, when considering the magnitude of effects as negligible for each ecological entity of each of the aquatic organisms, the risk assessment should be protective of both direct effects as well as indirect effects including trophic interaction among the aquatic food chain. This approach assumes that the current assessment factors used for assessing direct effects are protective enough to cover indirect effects.

The aquatic risk assessment of glyphosate is based on ecological threshold option. As such, the approach used could be considered appropriate to protect both direct effects as well as indirect effects including trophic interaction among the aquatic food chain in the sense of the EFSA aquatic guidance document (2013). However, given the data provided by the applicant and their assessment by RMS for glyphosate, it could not be considered that all indirect effects and food web interactions are addressed given that not all food sources are considered. For example, valid studies to assess the effects on sediment-dwelling organisms or rooted macrophytes of glyphosate that has a potential to partition in sediment are missing. Additionally, information on impact on decomposition processes in aquatic systems, or effects on the biofilm (algae, fungi and bacteria-matrix) would need to be considered. Further information on the effect to the aquatic community could also contribute to assess risk to biodiversity via indirect effects and trophic interactions.

Monitoring data confirmed that glyphosate and AMPA are frequently detected ([redacted] 2012, [redacted] 2020), with detection above the limit of quantification (>LOQ) occurring in ~40% of samples for glyphosate and ~64% for AMPA. Exceedances of regulatory acceptable concentration (RAC) and Environmental Quality Standard (EQS) are limited, with over 99% compliance reported for these different triggers for both glyphosate and AMPA.

Baker et al., 2016 studied the the effects of Roundup WeatherMax, alone or in combination with nutrient additions, on the changes in the phytoplankton and zooplankton communities in wetland. The purpose of the glyphosate application directly targeting the macrophyte community was to maximize the possibility of indirect impacts of glyphosate herbicides on the invertebrate or amphibian communities in wetland through direct effects to the plant community. This consistent amount of herbicide applied directly to the plant community on the treated sides of all wetlands was much higher than the dose received through the different treatment concentrations applied directly to the water's surface. However the study is of limited relevance as the exposure of emergent macrophytes (directly sprayed) was considerably higher than expected from a contamination via run-off/drift and may have resulted in indirect effect on phytoplankton and zooplankton communities.
Baker et al.,2014 focussed on the emergence of Chironomidae (Diptera) before and after herbicide-induced damage to macrophytes. There were no direct effects of treatment on the structure of the Chironomidae community or on the overall emergence rates. However, after macrophyte cover declined as a result of herbicide application, there were statistically significant increases in emergence in all but the highest herbicide treatment, which had also received no nutrients. There was a negative relationship between chironomid abundance and macrophyte cover on the treated sides of wetlands. Here again, the information are relevant for for aquatic uses where emergent macrophytes are directly sprayed with glyphosate-based products.
From the same experiments as above, Mudge J. F. et al., 2019 (see Appendix to Volume 3 (AS) B.9 on general literature data), assessed how different concentrations of glyphosate-based herbicides affect wetland plant communities over two years of herbicide application (alone and in combination with agricultural fertilizers) and two subsequent years without herbicide (or fertilizer) application. The study is of limited relevance as the exposure of emergent macrophytes (directly sprayed) was considerably higher than expected from a contamination via run-off/drift.
RMS notes that Edge et al. (2020) included an investigation of indirect effect on abundance of benthic invertebrates. From the same experiments as above, (Baker et al, 2014, 2016 and Mudge J. F. et al., 2019), indirect effects on the relative abundance of predatory benthic invertebrates (and the abundance of Wood Frog larvae) arose from the direct effects of the herbicide on macrophyte cover.
These indirect effects were in the opposite direction to the direct effects of the herbicide, resulting in a compensatory effect and no overall change. The study is of limited relevance as the exposure of emergent macrophytes (directly sprayed) was considerably higher than expected from a contamination via run-off/drift.

Edge et al. (2014) and Edge et al. (2020) are based on a common dataset that shows both an increase and a decrease in biodiversity metrics (e.g., increased green frog larval abundance at most treatments with glyphosate, and decreased wood frog larval survival at high glyphosate concentrations combined with nutrient enrichment during the first year of the study). The increase in green frog abundance was suggested to be due do dead plant material (from glyphosate treatment adjacent to the wetland) which provided an improved habitat for oviposition. The authors pointed out that the increased abundance of green frog is of concern, since this species is larger and capable of completely removing other frog species from wetlands by predation of egg masses. This is an illustrative example of an indirect effect on one species due to change in habitat, resulting in a subsequent effect on another species via trophic interactions.

Bees - Biodiversity Assessment via indirect effects via and trophic interactions

Summary by the applicant:
In the following table, the specific protection goals relevant to bees / pollinators are presented with the relationship between the specific protection goals (SPGs), the direct effects study types, assessment and measurement endpoints. The assessment endpoint is an explicit expression of an environmental entity and the specific property of that entity to be protected. Measurement endpoints relates directly to the effects study endpoints. A conclusion that a given data requirement has been satisfied, requires that an acceptable level of risk has been achieved (i.e. there is a protective margin of exposure or through a weight of evidence).

Table [...]: The relationship between Specific Protection Goals, assessment and measurement endpoints for bees from contact and dietary exposure

Specific Protection Goals	Assessment Endpoints	Measurement Endpoints	Study Types
No significant effect on honeybee colony survival and development.	Population size and stability of managed bees	Adult and larval survival and larval emergence	Adult honeybee acute Adult Bumble bee acute Adult solitary bee acute Adult honeybee chronic Larval honeybee emergence Honeybee semi-field brood study
Pollination services and production of hive products	Population size and stability of native and commercially managed bees and quantity and quality of honeybee hive products.	Adult and larval survival and larval emergence	
Bee Biodiversity	Species richness and abundance	Adult and larval survival and larval emergence	

Bee Biodiversity Assessment
The direct effects assessment demonstrates negligible acute and chronic risk to adult and larval bees and is protective of effects at the population level. Indirect effects to bee populations from in-crop weed control is unlikely because in-crop flowering weeds are not a significant resource for nectar and honey and the off-crop NTTP community will be protected by in-crop no spray zones. Taken together, impacts on bee biodiversity from the intended uses of glyphosate and following the required risk mitigation measures, impacts to bee biodiversity are unlikely.

Conclusion on the biodiversity assessment for bees – indirect effects via trophic interactions
Glyphosate is a critical tool to enable conservation tillage systems, which can greatly improve water quality in agroecosystems by reducing sediment and nutrient run-off. Negligible risk of direct effects to bee biodiversity is supported by measures of glyphosate residues in honey from monitoring programs. Indirect effects from in-crop weed control is unlikely to impact bee populations because in-crop flowering weeds are not a significant resource for nectar, pollen and honey. In addition, the off-crop NTTP community will be protected by in-crop no-spray zones as a required mitigation. Taken together, impacts on bee biodiversity from the intended uses of glyphosate and as necessary following the required risk mitigation measures, impacts to bee biodiversity are unlikely.

Comment and conclusion of RMS for bees biodiversity assessment
The approach followed in the EFSA guidance for bees (2013)[34] for the definition of specific protection goals is in line with EFSA guidance on specific protection goals (2016). As such it is the most suitable guidance document that allow to consider biodiversity and ecosystem services for bees.
Some references informed on the abundance of weeds in agricultural landscape (e.g. Last et al, 2019). The results from these efficacy trials may actually indicate the weeds are present and relevant in more than 10% of cases. RMS believes that weed relevance (in term of food supply) may depend on crops, tillage practices and timing of

[34] European Food Safety Authority, 2013. EFSA Guidance Document on the risk assessment of plant protection products on bees (Apis mellifera, Bombus spp. and solitary bees). EFSA Journal 2013;11(7):3295, 268 pp., doi:10.2903/j.efsa.2013.3295

applications. The dataset available in Last et al, 2019 may be of use to define these specific conditions/crops. The dataset available in Last et al, 2019 may be of use to define these specific conditions/crops in order to establish exposure scenario to assess both direct and indirect effects related to the availability of the food sources.
However in agricultural landscapes, weeds may be the only permanent source of food. Removing the weeds at early development stage may deprive bees of the only source of food normally available later on.
The applicant considered that indirect effects from in-crop weed control is unlikely to impact bee populations because in-crop flowering weeds are not a significant resource for nectar and pollen. In addition, the applicant considered that risk mitigation to protect off-field non-target terrestrial plants will benefit to bees and therefore no impact on bee biodiversity is concluded.
However indirect effects following reduction of floral resources that could follow application of herbicides such as glyphosate are not taken into account. RMS considered that reduction of floral resources and its impact on bees is difficult to handle in a risk assessment approach based on local scale (field). It requires the development of tools that allow assessment at landscape level. Thus, risk managers may consider the need of further mitigation measures to protect biodiversity in agricultural landscape from the effects of plant protection products. Please refer to "Additional mitigation measures to protect biodiversity" thereafter.

Arthropods other than Bees - Biodiversity Assessment via indirect effects via and trophic interactions

Summary by the applicant:
The following table assessment illustrates that ecological function of beneficial NTAs both in-field / in crop and off-field / off-crop (off-target) will be sufficiently maintained to achieve the SPG for the non-target arthropods according to the protection goals as defined in the ESCORT 2 and 3, that sustains a food resource for other animals, primarily birds and mammals.

Table [...]: The relationship between Specific Protection Goals and associated assessment and measurement endpoints for non-target arthropods (NTAs)

Specific Protection Goals[1]	Assessment Endpoints	Measurement Endpoints	Glyphosate Study Types
In-field Maintenance of ecological function of beneficial NTAs (i.e., pest control by parasitoids and predators), food source for wildlife, and effects not exceed the ability to recover.	Tier 1, at the maximum use rate (MUR) achieve an assessment factor of ≥ 2 with mortality Tier 2, at the MUR no significant mortality and < 50% effect on reproduction.	Survival (LR_{50}) and if appropriate, assess reproduction effects	Primary: *Typhlodromus pyri* (predatory mite) and *Aphidius rhopalosiphi* (parasitic wasp) Secondary: *O. laevigatus, C. carnea, C. septempunctata A. bilineata*
Off-field [1] Maintenance of NTA biodiversity and the ability to support in-field recovery			
NTA Biodiversity Assessment Following ESCORT3 risk assessment guidance there is low to negligible risk of unacceptable direct and indirect effects to NTA communities for the representative formulation. Risk mitigation measures required for protecting the off-crop NTTP community (e.g., in-field buffers) will be protective of off-crop NTA biodiversity. However, if additional risk mitigation measures are determined to be required, to mitigate indirect effects resulting from in-crop weed control on NTA communities, options to be considered by risk assessors and risk managers within Member States are presented in the Biodiversity Assessment Report ([...]) A summary of main points and options is provided in [...] "FURTHER INFORMATION TO BE SUBMITTED" in the sub-chapter "The benefit and contribution of Glyphosate to biodiversity (nature conservation and habitats) and to European environmental policy goals".			

[1]The off-crop area is defined as the area in-field that is not the crop. For NTA RA, the off-crop area is a default 1 meter distance between the last sprayed row of the crop and the edge of the in-field area.

Conclusion to the biodiversity assessment for arthropods other than bees;
Following ESCORT3 risk assessment guidance there is negligible risk of unacceptable direct and indirect effects to NTA communities for the representative formulation. Risk mitigation measures required for protecting the off-crop NTTP community (e.g., in-field buffers) will be protective of off-crop NTA biodiversity. The existing SPG for the in-crop assessment has been designed to only allow for up to a transient 50% effect

on the NTA community and it allows for in-crop recovery to minimize the likelihood of indirect effects to birds and mammals through trophic interactions. The SPG for the off-crop assessment is protective of biodiversity based on spray-drift mitigations developed to protect the NTTP community.

The indirect effects via trophic interaction, a biodiversity assessment with further consideration of relevant and reliable literature, concerning pollinators and arthropods other than bees are discussed and presented.

Comment and conclusion of RMS for non-target arthropods biodiversity

The approach used by the applicant consisted of defining specific protection goal considering some of the recommendations of EFSA scientific opinion on Non Target Arthropods (2015)[35]. Focus was made, for in-field, on the maintenance of ecological function of beneficial NTAs (i.e., pest control by parasitoids and predators), food source for wildlife, and effects not exceed the ability to recover and, for off-field, on maintenance of NTA biodiversity and the ability to support in-field recovery. The threshold of 50% effects was used for direct effects.

RMS considers that the current standard risk assessment and protection goals address the direct effect only and have not been defined to address specifically indirect effects. The indirect effect, due to the loss of habitat or food resources, is not considered to be addressed.

From the literature review of the previous RAR various effects depending on formulations where reported: Glyphosate containing products can be harmfull towards egg stages of *Trichogramma*, whereas at other parasitoid stages the same product was harmless. Sublethal effects of glyphosate were assessed in the laboratory on prey consumption, web building, fecundity, fertility and developmental time of progeny of a web weaver spider (*Alpaida veniliae*) in Argentina (Benamu et al., 2010) and on wolf spiders in north America (Evans et al., 2010). The authors concluded that the exposure to glyphosate containing products affects the behavior of the animals and their capacity to grow and persist in agroecosystems. In contrast, short term exposures (2h and one-day residues) of spiders and carabid beetles, respectively *Pardosa agricola* and *Poecilus cupreus*, did not affect mating or avoidance of the arthropods, but (only) slightly slower movement (Michalkova et al., 2009). RAR 2015 further stated that these effects together with the indirect effects of herbicide treatment on the vegetation of their habitat might have implications for the success of survival and reproduction.

RMS further consider that there is a need to investigate the impact of loss of habitats fro non-target arthropods. For this purpose it is requested to the applicant to consider the results of Pleasants et al (2012) on the effect of glyphosate on populations of the monarch butterfly due to habitat loss (data gap).

Guiseppe KFL et al (2006) reviewed articles related to ecological effects of the herbicide glyphosate used in forested landscapes. Among these papers, some stated that homopteran densities were lower in herbicide-treated plots compared with brush-saw-treated plots and non-treated control plots. It was hypothesized that indirect effects of herbicide treatment altered the nutritional quality of tree and shrub species (as homoptera feed on either phloem or xylem). Also indirect effects of herbicides on communities of herbivorous arthropods, in most cases, were hypothesized to be a result of reduced floral resources and the effect that this reduction would have on arthropods that require them during at least one phase of their life cycle. Studies are referenced that stated that herbicides have indirect effects on beneficial wasp and bees. These studies present correlative relationships that suggest that decreases in flowering plants in agricultural fields results in decreases in the abundance of wasps and bees and often concomitant increases in the density of insect pests. It is hypothesized that reliance of no-till agriculture on pesticides may have fewer off-farm environmental impacts than conventional tillage, but the sublethal and long-term effects of pesticides on animal populations using no-till fields are not well understood and must be considered. The authors also hypothesized that maintaining uncultivated areas in the field and between narrow crop rows may establish an equilibrium between predator and prey populations as they noted the absence of serious pest related problems during the study.

The review of Sullivan TP, Sullivan DS. 2003 concluded that the diversity of terrestrial invertebrates in glyphosate-treated areas is variable. Abundance and diversity of invertebrates in a given treated area is principally a function of the degree of vegetation control and changes in vegetation structure.

Garcia Ruiz E. et al., 2018 investigated the relationship between weed management and the beneficial predatory arthropods in a glyphosate-tolerant (GT) cotton crop. Glyphosate (applied post-emergence) in this three-year farm-scale study resulted in a shift in weed species composition, suggests a positive correlation between weed density and the diversity of carabids and interspecific competition may occur between predatory groups. This study is considered relevant for biodiversity and indirect effect issues. However its relevance is limited as it focusses on post-emergence glyphosate applications and results were compared to an other herbicide treatment only. So the differences observed in the study are very likely the consequence of the different timing of application. Besides RMS noted that insecticides were applied (at sowing). Herbicides treatments (other than glyphosate) were also

[35] EFSA Scientific Opinion addressing the state of the science on risk assessment of plant protection products for non-target arthropods. EFSA Journal 2015;13(2):3996, 212 pp. doi:10.2903/j.efsa.2015.3996

applied in conventional managed plots. Their direct (toxic) effect was not investigated. Drought may also have reduced herbicide effectiveness (by reducing absorption, translocation and metabolism of herbicides).

RMS considers that the appropriateness of applicant's proposal (concerning the additional risk mitigation measures to be required by risk managers at the Member States level to mitigate indirect effects resulting from in-crop weed control) should be discussed during the EU peer-review.
Based on direct effects assessment of the EU representative formulation to non-target arthropods, the applicant concluded that a combination of standard risk mitigation measures (e.g., in-field buffers and drift reduction technology) that may be required on the label to protect off-field NTTP communities could be protective of NTA too. The applicant indicated that non-standard mitigations are more likely to be required in simplified landscapes where the remaining refuge areas for insects, birds and mammals are limited (see later information on landsparing vs. landspering in NTTPs). Mitigation measures (e.g., semi-natural habitats, such as field margins) to support Integrated Pest Management (IPM), in simplified landscapes areas, where intensified field crop production occurs, is an important consideration in terms of biodiversity outcome.
Risk managers may consider the need of further mitigation measures to protect biodiversity in agricultural landscape from the effects of plant protection products. Please refer to "Additional mitigation measures to protect biodiversity" thereafter.

Soil meso-organisms - Biodiversity Assessment via indirect effects via and trophic interactions

Summary by the applicant:

The following assessment illustrates that ecological diversity and function of soil meso-organisms within spray zones will be sufficiently maintained to achieve the SPG for this taxa group according to the protection goals as defined in the Terrestrial guidance document (SANCO/10329/2000) sustains a food resource for other animals, primarily birds and mammals within in -field areas, sustains soil structure and function that has a knock on effect of enabling soil function of soil microbial communities. This in turn helps to maintain the community structure within the soil.

Table […]: The relationship between Specific Protection Goals, assessment and measurement endpoints for soil macro-organisms from foliar applications

Specific Protection Goals[1]	Assessment Endpoints	Measurement Endpoints	Glyphosate Study Types
Protection of structure (biodiversity) and function of soil macro-organism communities and function of soil micro-organism communities.	Structure and function of soil macro-organism communities Long-term effects on the *soil macro- organism communities**	Survival and reproduction	Earthworm chronic Collembola chronic Predatory mite chronic
Protection of soil services (e.g., decomposition and cycling of organic matter and nutrients)	Long-term effects on the function of soil macro-organism communities (decomposers).	Survival and reproduction	
Soil Organism Biodiversity Assessment Based on the direct effects' assessment, there is low to negligible risk to the structure and function of soil organism populations and communities (EFSA, 2015a) and the likelihood of indirect effects soil organism biodiversity is also considered to be negligible.			

*in italic: part missing from applicant's document added by RMS
[1] EFSA still needs to receive input from risk managers on the definition of specific protection goals being led by DG SANTE. In the draft Scientific Opinion addressing the state of the science on risk assessment of plant protection products for in-soil organisms, negligible effects are considered to be ≤ 10% and small effects are considered to be ≤ 35%.

Conclusion to the biodiversity assessment for soil meso-organisms
Glyphosate is a critical tool to enable conservation tillage systems, which can greatly improve the abundance and biodiversity of soil organisms. Based on the direct effects assessment, where no direct effects were

observed, it is therefore considered, that the risk of indirect effects via trophic interactions to soil community biodiversity and supporting/regulating services related to soil processes is considered to be low.

The indirect effects via trophic interaction, a biodiversity assessment and consideration of relevant and reliable literature, concerning soil meso-organisms are discussed and presented.

Comment and conclusion of RMS

The approach used by the applicant consisted of defining specific protection goal considering some of the recommendations of EFSA scientific opinion on soil organisms (2017). Focus was made on the protection of structure (biodiversity) and function of soil macro-organism communities and function of soil micro-organism communities and the protection of soil services (e.g., decomposition and cycling of organic matter and nutrients). Considering the direct effects on survival and reproduction of standard species, the applicant considered that SPG are met. RMS considers that the current standard risk assessment and protection goals address the direct effect only and have not been defined to address specifically indirect effects. RMS noted that the EFSA scientific opinion on soil organisms (2017) also considered additional organism groups such as enchytraeids, microarthropods, gastropods, nematodes that should be considered to properly address the effects on soil organisms, protection of soil biodiversity and provision of ecosystem services. In order to support the long term performance of the functional role of in-soil organisms in several ecosystem services in agricultural soils, it is recommended in this scientific opinion to define the service providing units as the abundance/biomass of the populations of species belonging to the different functional groups. For the off-field non-target areas, it is proposed that only negligible effects on the abundance/biomass of in-soil organisms' populations can be tolerated.

In view of the literature review of the previous RAR used in support to biodiversity assessment by the applicant, RMS considers that sublethal effects following short-term exposure should not be disregarded. The previous RAR further stated that it can not be excluded that with repeated applications of glyphosate containing plant protection products during the season or year by year will have negative effects on the biotic soil community. It was considered that herbicide application did not directly affect the mortality or reproduction but instead the biological activity of the animals (RAR 2015). The literature data submitted for the current renewal did not provide conclusive information on effects on soil organism communities and assemblage of communities.

Regarding monitoring data (Silva et al, 2018); 300 samples have been collected as part of the LUCAS topsoil project between April and October of 2015 and 17 samples are from three independent vineyards in north-central Portugal taken in September 2015. Results from these data indicate GLY is quantified in ~21% of 317 soil samples, AMPA is quantified in ~42% of 317 soil samples, with the maximum concentration being 2.05 mg/kg for GLY and 1.92 for AMPA, measured in the Portugese vineyard. RMS underlines that these maximum measured concentrations should be regarded with caution since the exact sampling depth is unknown (15/20cm), and in any case higher than the one that would be considered for risk assessment in permanent crops (5cm).
Also, this study concluded that maximum level of glyphosate detected is more than 2-times less than the predicted environmental soil concentration used for the standard glyphosate soil organism assessment, which considered a worst-case exposure scenario. However the direct comparison with expected PECsoil in vines is uncertain since this latter is calculated in the study on 5 cm depth while the sampling depth for the measured concentration is 15/20cm and cannot be related to a precise use pattern of the active substance (application rate, time passed since last application….).

From the regulatory risk assessment, no effect on survival and reproduction of standard test species of glyphosate is expected after application of MON52276 as intended. This conclusion was reached by considering tier 1 laboratory tests that are based on No Observed Effect Concentrations that considered survival and reproduction. For earthworms, effects on body weight as indicator for biomass is also part of the tier 1 laboratory test. However, no information on the other functional groups/organisms recommended in the scientific opinion has been proposed. Thus, risk managers may consider the need of further mitigation measures to protect biodiversity in agricultural landscape from the effects of plant protection products. Please refer to "Additional mitigation measures to protect biodiversity" thereafter.

Soil micro-organisms - Biodiversity Assessment

Summary by the applicant:

In the following table, the specific protection goals (SPGs) relevant to soil microflora are presented with the relationship between the SPGs, the direct effects study types, assessment and measurement endpoints. The assessment endpoint is an explicit expression of an environmental entity and the specific property of that entity to be protected. Measurement endpoints relate directly to the effects study endpoints.

Table […]: The relationship between Specific Protection Goals, assessment and measurement endpoints for soil micro-organisms from foliar applications

Specific Protection Goals[1]	Assessment Endpoints	Measurement Endpoints	Glyphosate Study Types
Protection of function of soil micro-organism communities.	Long-term effects on the function of soil micro-organism communities	N-transformation rate ≤25% difference from control at ≥28 days.	N-transformation rate
Protection of soil services (e.g., cycling of organic matter and nutrients)	Long-term effects on the function of soil micro-organism communities (i.e., Nitrogen cycling).	N-transformation rate ≤ 25% difference from control at ≥28 days	

Soil micro-organism Biodiversity Assessment
Based on the direct effects assessment, there is low risk to functioning of soil microbial populations and communities (EFSA, 2015a) and the likelihood of indirect effects on soil function due to effects on microbial or bacterial biodiversity is considered low to negligible.

[1] EFSA still needs to receive input from risk managers on the definition of specific protection goals being led by DG SANTE. In the draft Scientific Opinion addressing the state of the science on risk assessment of plant protection products for in-soil organisms, low to negligible effects are considered to be ≤ 10% and small effects are considered to be ≤ 35%.

Conclusion to the biodiversity assessment for soil micro-organisms;
Glyphosate is a critical tool to enable conservation tillage systems, which can greatly improve the abundance and biodiversity of soil organisms. There is low risk of direct effects to soil community biodiversity and supporting/regulating services related to soil processes. This conclusion is not changed after reviewing reported levels of glyphosate from soil monitoring studies. In addition, based on a review of the literature and considering the results of the direct effects assessment, the likelihood of indirect effects soil organism biodiversity is considered to be low.

The indirect effects via trophic interaction, a biodiversity assessment and consideration of relevant and reliable literature, concerning soil microflora are discussed and presented.

Comment and conclusion of RMS
The approach used by the applicant consisted of defining specific protection goal considering some of the recommendations of EFSA scientific opinion on soil organisms (2017). Focus was made on the protection of function of soil micro-organism communities and the protection of soil services (e.g., cycling of organic matter and nutrients). Considering the effects on soil nitrogen transformation, the applicant considered that SPG are met. RMS noted that the EFSA scientific opinion on soil organisms (2017) also considered the need of information on effect on community/structure for mycorrhiza, other fungi and protozoa and on community of soil bacteria and archea to properly address the effects on soil organisms, protection of soil biodiversity and provision of ecosystem services. In order to support the long term performance of the functional role of in-soil organisms in several ecosystem services in agricultural soils, it is recommended in this scientific opinion to define the service providing units as the community/structure of the different functional groups. For the off-field non-target areas, it is proposed that only negligible effects on the abundance/biomass of in-soil organisms' populations can be tolerated.

The literature data submitted for the current renewal did not provide conclusive information on effects on soil organism communities and assemblage of communities. Mijangos et al. (cited in Duke et al. 2012) examined glyphosate effects on glyphosate-sensitive plants (triticale and peas) and their rhizosphere microbial communities. Community diversity and richness were found to be reduced at the highest rate of glyphosate application in rhizospheres of killed glyphosate-sensitive pea and glyphosate-sensitive triticale, but not in soil from triticale grown alone. (data gap : applicant to provide study of Mijangos et al cited in Duke et al. 2012).
Newman M. et al., 2016 also investigated the rhizosphere bacterial community composition response to the application of glyphosate in formulation Roundup PowerMax. The study is then of limited relevance for the risk assessment of glyphosate and its representative formulation. Barcoded sequencing permitted detailed phylogenetic diversity analysis and was used to identify specific bacterial taxa shifts in the rhizosphere bacterial community in response to repeated glyphosate exposure on corn and soybeans. The authors hypothetised that long-term glyphosate application could affect rhizosphere nutrient status. RMS considers the parameters investigated (rhizosphere bacterial community) as relevant as the effects of glyphosate may be masked by "functional redundancy" where overall soil functions are unaffected while microbial community composition is altered and key functions mediated by specific microbial populations are affected. Alterations to soil microbial community composition and subsequent

changes in microbial diversity could potentially have pronounced long-term effects on soil quality and plant health. This study does not provide any information on whether this shift affects functional capability of the soil.
Thus, in view of the literature data submitted for the current reapproval dossier, and in view of the literature review of the previous RAR used in support to biodiversity assessment by the applicant, and the additional literature data available, RMS considers that a shift in their community structures of soil micro-organisms could not be excluded as glyphosate could be used as a source of P, C or N by soil micro-organisms.

From the regulatory risk assessment, no effect of glyphosate on nitrogen transformation is expected after application of MON52276 as intended. This conclusion was reached by considering tier 1 laboratory tests that are based on acceptable effects on nitrogen transformation rate. RMS considers that the current standard risk assessment and protection goals address the direct effect only and have not been defined to address specifically indirect effects. No information on community/structure for mycorrhiza, other fungi and protozoa and on community of soil bacteria and archea recommended in the scientific opinion of soil organisms (2017) has been proposed. Thus, risk managers may consider the need of further mitigation measures to protect biodiversity in agricultural landscape from the effects of plant protection products. Please refer to "Additional mitigation measures to protect biodiversity" thereafter.

Non-target Terrestrial Plants (NTTPs) – Biodiversity Assessment via indirect effects via and trophic interactions

Summary by the applicant:

In the following table, the specific protection goals relevant to non-target terrestrial plants are presented with the relationship between the SPGs, the direct effects study types, assessment and measurement endpoints. The assessment endpoint is an explicit expression of an environmental entity and the specific property of that entity to be protected. Measurement endpoints relate directly to the effects study endpoints.

Table […]: The relationship between specific protection goals and associated assessment and measurement endpoints for non-target terrestrial (NTTP) plants from off-crop spray drift

Specific Protection Goals[1]	Assessment Endpoints	Measurement Endpoints	Glyphosate Study Types
Negligible risk to off-field NTTP communities to support nutrient cycling, water regulation, food web, aesthetic values and genetic resources (biodiversity)	Protect 95% of the populations in 90% of the cases.	EC_{50} values for plant survival, height and weight.	Vegetative vigor Seedling emergence

NTTP Biodiversity Assessment
Based on the current direct effect assessment for the representative formulation, standard risk mitigation measures (e.g., in-field buffers, drift reduction technology nozzles, hooded sprayers) will be required on the label to protect NTTP communities outside the cropped area.

However, if additional risk mitigation measures are considered to be required by risk managers at the Member States level, to mitigate indirect effects resulting from in-crop weed control, risk mitigation options that maybe considered are presented in detail in the Biodiversity Assessment Report ([…]) A summary of main points and options is provided in […] "FURTHER INFORMATION TO BE SUBMITTED" in the sub-chapter "The benefit and contribution of Glyphosate to biodiversity (nature conservation and habitats) and to European environmental policy goals".

[1] It is assumed that the biodiversity is maintained when most of the plant populations will not be affected using plant protection products. It is assumed that this goal can be reached when the plant populations are protected off-crop.

Conclusion to the biodiversity Assessment for Non-target Terrestrial Plants (NTTPs)

The existing terrestrial ecotoxicology guidance for NTTP assessments provides risk assessment methods for evaluating potential direct effects to NTTP communities outside the cropped area. Historically, protection of

in-crop non-target plants / weeds has not been considered in ecological assessments for PPPs. However, in the revision of the PPP data requirements, the former phrase "*Non-target plants are non-crop plants located outside the treatment area*" was deleted. As an outcome of this revision, an EFSA Scientific Opinion (2014) was developed that defined SPGs for off-crop and in-crop NTTPs and linking them to biodiversity. In the Scientific Opinion (2014), NTTPs were newly defined as "*all plants growing outside fields, and those growing within fields that are not the intended pesticide target*"; though the Scientific Opinion (2014) does not have the status of an official guidance document. The derivation of SPGs for NTTPs requires further discussion and decision making between risk assessors and risk managers as well as risk mitigation options to address indirect effects. Holistically addressing potential indirect effects to birds and mammals by limiting in-crop weed control may be better handled through policies and programs outside the PPP framework.

Based on the current direct effect assessment for the representative formulation, standard risk mitigation measures (e.g., in-field buffers, drift reduction technology nozzles, hooded sprayers) will be required on the label to protect NTTP communities outside the target area.

The indirect effects via trophic interaction, a biodiversity assessment and consideration of relevant and reliable literature, concerning terrestrial non-target plants are discussed and presented.

Comment and conclusion of RMS on NTTPs biodiversity

The approach used by the applicant consisted of defining specific protection goals considering some of the recommendations of EFSA scientific opinion on Non Target Terrestrial Plants (2014)[36]. Focus was made on the protection of off-field NTTP communities to support nutrient cycling, water regulation, food web, aesthetic values and genetic resources (biodiversity). The ecosystem services to protect was indeed the one reflecting in the scientific opinion. However the Service Provided Units proposed by the applicant did not consider in-field NTTP for food web support, in-field NTTP for aesthetic values and genetic resources and endangered species.

The applicant indicated that indirect effects through trophic interactions to farmland birds by reducing in-crop food resource has still not been defined. However, following the approach to define SPG and considering the recommendations of the EFSA scientific Opinion on NTTPs (2014), in-field NTTP as food web could be used as pragmatic approach in context of regulatory risk assessment for taking into account those kind of indirect effects. From the Scientific Opinion on NTTPs, the protection of in-field NTTP for food web indicated that since the function of non-target plants as a food source is more relevant in this context than structural endpoints (plant diversity), the SPG should be aimed at the conservation or restoration of those functions as food or habitat sources rather than at the protection of the populations of single species. The functional group for food web support provides food (biomass of green material and seeds) and habitat (cover, host plant) provisioning for higher trophic levels. In this opinion, the magnitude of effects for food web support is proposed to be negligible at landscape scale to medium effects at field scale. Duration of effects above the magnitude of effects should not exceed weeks in general and should be limited to in time (no to few days) during breeding/chick phase. However this option should be agreed by risk managers. Moreover the current guidance on birds and mammals risk assessment is under revision.
RMS considers that given the efficacy spectrum of glyphosate (total herbicide), the in-crop protection goals for non-target plants should be considered depending on the crop groups and accounting for good agricultural practices. For example, in orchards and vineyards, application is generally made in the rows, below trees. The space between the rows should ideally be vegetated to allow the use of the part of the field by herbivorous vertebrates and arthropods and offer flowering resources for bees.

From the literature review of the previous RAR that could be used as evidence for assessing the risk to NTTPs biodiversity and potential effects on trophic interactions, the following main information could be retained:

- intrinsic variability in plant sensitivity to herbicides should be inquired
- reproductive endpoints in many cases were more sensitive that vegetative ones
- crops and wild plant species responded quite variably when they were tested in different seasons as well as when tested under different environmental conditions
- sublethal effects of repeated drift events or exposure to mixtures of herbicides are unaddressed
- herbicides can influence plant communities in terms of species composition and diversity
- foliar applied glyphosate to target plants is released into the rhizosphere and might negatively affect non-target plants, disease problems and nutritional status

[36] EFSA Scientific Opinion addressing the state of the science on risk assessment of plant protection products for non-target terrestrial plants. EFSA Journal 2014;12(7):3800, 163 pp. doi:10.2903/j.efsa.2014.3800

- disease development, including increasing soil pathogen populations immobilizing micronutrients involved in disease resistance
- even small effect of glyphosate itself on functionally important components of the agroecosystem can have an impact when bearing in mind the extensive usage of glyphosate in various indications and large area
- Ecological side effects might even be stronger in diverse and species rich forest ecosystems.
- glyphosate has several secondary or indirect effects on plant physiology which may also explain its herbicidal effects

In view of the updated literature review, two main articles are proposed as basis for further discussions by risk assessors and risk managers. In Colbach et al., (2018), the authors evaluated the strategies of land-sharing[37] and land-sparing[38] in silico, based on a case study with maize-based cropping systems including genetically modified varieties that allow the use of glyphosate. Three series of scenarios were simulated over 28 years and 10 weather repetitions in a small landscape consisting of four 3-ha fields in Aquitaine (South-Western France):

- "landsharing scenarios based on a single diverse rotation (soybean/maize/wheat/maize), with different crop patterns in the landscape",
- "landsparing scenarios with varying proportions (ranging from 0 to 100%) of contrasting cropping systems in the landscape, either cropping system aiming to maximise biodiversity or one aiming to maximise production",
- and "landsparing scenarios including permanent grass strips (10% of each field)."

The authors concluded that the "landsharing scenario combining fields aiming to maximise crop production with either fields aiming to maximise biodiversity (25% of landscape) or grass strips (10% of landscape) were best, resulting in high crop production and medium biodiversity at the landscape scale. Landsharing scenarios always produced less biodiversity and less production. When more crops and cropping systems were grown each year in the landscape, the weed impact on production and biodiversity was higher and more stable over the years." RMS considered the system-based approach used is interesting as tool for decision-making and discussions on how to better handle biodiversity in agricultural landscape in European legislation/regulation. However, RMS considers that this study is only a case-study not to be generalised to other crop systems. As stated by the authors, the "results are case-specific" and "new simulations and rules are needed for different types of cropping systems, landscapes and pedoclimates, and the performance of the best solutions should be tested in field studies."

Koning et al. 2019 investigated the effects of moldboard plowing, chisel plowing, and glyphosate herbicide application on weed species density and diversity in agricultural fields. This paper investigates the effects of glyphosate applications versus tillage on the weed vegetation in a field experiment. Two different glyphosate doses were included in the experiment, 100% and 50% of the recommended dose on the product label, in order to assess the effect of both a normal frequent application as well as the effect of a frequently applied reduced dose. Two different tillage methods were investigated, chisel plow and mould board plow, to evaluate the influence of a minimal versus a fully soil turning approach to plowing. Overall, any method employed influenced the weed composition in some way. Some species were favored over others depending on the weed management method, but the overall biodiversity of the weed community was not more negatively affected by one method compared to another.

The report of Arts et al. (2017) explores and presents SPG options and related Exposure Assessment Goals options from a Dutch perspective, which might be used to develop guidance on environmental risk assessment procedures for PPPs and arable weeds in in-field areas and non-target terrestrial plants in off-field areas. The described options serve to facilitate discussions at the EU level.

Three options for in-field specific protection goals (SPGs) for arable weeds are proposed:

1/ Maximal weed reduction option. This is the current option in the EU risk assessment.

Characteristics of this options are:

- Maximal provision of the ecosystem service 'crop production',
- Lowere priority for other ecosystem services provided by non-crop plants,
- No protection of non-crop plants in-field.

2/Moderate weed reduction option. Characteristics of this options are :

- Support of a certain moderate level of arable weeds in in-field areas.

[37] The concept of landsharing is an approach that aims to combine agricultural production and conservation of biodiversity in the same territories. It is generally associated with modification of agricultural practices and concepts of agroecology, conservation agriculture…

[38] Landsparing aimed to reconcile protection of biodiversity and agricultural production, by separating land between areas dedicated to the protection of biodiversity (without agricultural production) and land dedicated to agriculture.

- Support of several ecosystems services provided by non-crop plants, such as regulating services (e.g. prevention of erosion), supporting services (e.g. provision of habitat to invertebrates and food for farmland birds) and cultural services (e.g. protecting weeds of conservation concern).
- Improvement of local biodiversity relative to the current status.
- Effects on the ecosystem service 'crop production' is limited and controllable if implemented via a pre-defined in-field fraction of non-sprayed areas or conservation headlands.

3/ Beneficial weed protection option. Characteristics of this option are:

- Protection of 'beneficial' and low-competitive non-target plants that could potentially be managed to maintain diverse ecosystem services.
- Control of weeds that hamper growth of crop plants and thus need to be controlled to secure crop production.
- Improvement of local biodiversity relative to the current status.
- Effects on the ecosystem service 'crop production' are less quantifiable because they are dependent on the availability of selective herbicides that control pernicious weeds but spare 'beneficial' ones.

For the off-field area, three options for SPGs are also described.

1/ Population recovery option for non-target terrestrial plants. Characteristics of this option are:

- Effects on the vegetative growth/biomass of non-target terrestrial plants in the operational edge-of-field strip are accepted if:
- a) recovery takes place within an acceptable time frame
- b) effects in the operational nearby off-field strip are negligible.
- Effects on reproductive endpoints might occur in the operational edge-of-field strip.
- Least restrictive for the provision of the in-field ecosystem service 'crop production'.
- Sustainable plant populations at the landscape level are likely not at stake under the condition that in the agricultural landscape enough ecological focus areas are available (7% is proposed in the reform of the Common Agricultural Policy.

2/ Threshold option for vegetative growth of non-target terrestrial plants (this option is similar to the current procedure in the EU risk assessment). Characteristics of this option are:

- Effects on the vegetative growth/biomass of non-target terrestrial plants in the operational edge-of-field are negligible.
- Effects on reproductive endpoints might occur at the local level.
- Sustainable plant populations at the landscape level are likely not at stake.

3/ Threshold option for vegetative growth and generative reproduction of non-target terrestrial plants. Characteristics of this option are:

- Effects on the vegetative growth/biomass and on generative endpoints (flower and seed production; viability of seeds) of non-target terrestrial plants in the operational edge-of-field strip are negligible;
- Improvement of sustainability of plant populations and biodiversity at local and landscape level.

Within all three possibilities for off-field SPGs, two options are proposed for the spatial unit of the exposure assessment goal (EAG). The two options are either a 10-cm or a 2-m width of off-field strip in the edge-of-field area (and for SPG option 1 in the nearby off-field area as well) for which these three possible SPGs are assessed. This 10 cm is considered a minimum width from a scientific point of view because a plant cannot grow on e.g. a 1-mm strip. The background for offering these options is that spray drift is the most importanty exposure route and that spray drift deposition decreases sharply with distance from the treated field. Thus protecting a 10-cm-wide strip leads to higher exposure estimates (e.g. a factor of two) than protecting a 2–m- wide strip.
Agronomic consequences of the in-field and off-field options for specific protection goals have not been studied so far and need further elaboration and research.

Overall, research dealing with such subject could be interesting for risk managers and decision making in a context of comparative assessment of the different agricultural practices existing to manage in-field weeds development.

RMS wishes to remind the overall outcome of the extensive literature review conducted in the scope of the EFSA Opinion on NTTPs that are of interest when dealing with the question of impact on biodiversity and indirect effect through trophic interactions. The EFSA opinion stated that rare arable weeds are usually annual species that need regular soil disturbance and are preferably found in crop edges of conventional farming as well as in field centre and edges of organic fields. It was stated that many arable weeds have become rare owing to intensive management practices introduced in the last 50 years: extensive use of agrochemicals applied with ever increasing machinery size, increased field size and destruction of marginal habitats for the use of this machinery, better seed cleaning, use of high-density crop shading out weeds, and other modifications in crop types and management such as monoculture and timing of harvest. Studies pointed to the fact that uncropped cultivated (tilled) margins appear to be best for rare

arable weeds. Herbicides are very detrimental to rare arable weeds. However, uncropped cultivated margins are not a preferred option by the farming community and, consequently, are not practised much among farmers. Although rare arable weeds need protection from pesticide use, their management should be considered in the light of the overall agricultural practices of crop margins (buffer zones or in-field non-treated strips, etc.). Management practices that favour rare arable weeds have been identified, e.g. uncropped tilled field edges with no herbicide spray.

Considering the direct effects assessment of the EU representative formulation to non-target plants, the applicant concluded that a combination of standard risk mitigation measures (e.g., in-field buffers and drift reduction technology) will be required on the label to protect off-field NTTP communities. The applicant considered that "inclusion of no-spray buffer zones as a standard mitigation measure protects NTTP communities in off-target areas, which indirectly supports biodiversity by maintaining habitat as both a refuge and food source for other organisms in off-target areas." On this basis, the applicant considered that the standard risk assessment is protective of indirect effects occurring outside of the target area. RMS considers that the current standard risk assessment and protection goals address the direct effect only and have not been defined to address specifically indirect effects. RMS would like to highlight that a loss of plant biodiversity due to application of plant protection products may affect the entire food web, including birds and mammals. The presence of appropriate range of plants as food sources is vital to the survival of many bird and mammal species, as well as the presence of adequate habitat for a number of species, including leaf-dwelling arthropods that serve as food sources for birds and mammals, and small mammals.
The applicant proposed additional mitigation measures for consideration of Member States given their local requirement related to protection of biodiversity against indirect effects through trophic interaction. The applicant also refer to a communication on the benefits of spatially-targeted biodiversity conservation[39] in simplified landscapes (intensive management of agricultural field with limited habitats for wild species). He argued that "their support to biodiversity is local and can also contribute to improved landscape connectivity as part of a broader landscape planning approach." The authors of the communication, which is based on the study from Früh-Müller, A., et al. (2018)[40] indicated that this study suggests that agri-environmental measures "would be more effective if payments were targeted to areas under the greatest environmental pressures, such as intensive agricultural regions — to gain maximum environmental benefits."
Thus, risk managers may consider the need of further mitigation measures to protect biodiversity in agricultural landscape from the effects of plant protection products. Please refer to "Additional mitigation measures to protect biodiversity" thereafter.

Additional mitigation measures to protect biodiversity via indirect effects via and trophic interactions
The notifier also presented several options for additional mitigations measures to be considered by risk managers. A summary as proposed by the applicant is reported in the box below.

The benefit and contribution of Glyphosate to biodiversity (nature conservation and habitats) and to European environmental policy goals

- The Glyphosate Renewal Group (GRG) supports sustainable agriculture in the EU, specifically the contribution of the safe, effective and sustainable use of glyphosate-based products and recognises the importance of biodiversity protection as enshrined in European legislation (Sustainable Use Directive (2009/128/EC), Plant Protection Products Regulation (EC) No 1107/2009). Biodiversity is essential for sustainable farming. It provides farmers with essential ecosystem services like climate regulation, water retention, soil fertility, pollination and natural pest control that are significantly supporting food, feed and fibre production. In addition, biodiversity increases the resilience of cropping systems towards stresses like climate change.

- Biodiversity reflects a complex interaction of ecological, climatic and anthropogenic parameters. It is difficult to assess the impact of individual interventions. The scale of human activities increases rapidly with population, technology and economics, which inevitably impact biodiversity through pressures on the environment. Huge areas of land have been built upon for housing, industry and transport over the past 50 years. The area available for biodiversity is constantly being reduced and

[39] "Science for Environment Policy": European Commission DG Environment News Alert Service, edited by SCU, The University of the West of England, Bristol. https://ec.europa.eu/environment/integration/research/newsalert/pdf/maximum_benefit_aem_target_specific_environmental_pressures_germany_526na5_en.pdf
[40] Communication "Science for Environment Policy" based on Früh-Müller, A., Bach, M., Breuer, L., Hotes, S., Koellner, T., Krippes, C. and Wolters, V. (2018). The use of agri-environmental measures to address environmental pressures in Germany: Spatial mismatches and options for improvement. Land Use Policy, 84, pp. 347–362.

only recently have real efforts been made to mitigate these effects. Similarly, forestry and agricultural activity has converted primary and secondary landscapes to economic use on a large scale, particularly in low and middle income countries. Much of this activity has been driven by the food and feed needs of markets in high income countries, such as those of the EU, and by the growth of wealthy classes particularly in middle income countries. Amongst other problems of these complex issues has been 'land grabbing' in low and middle income countries by other countries which want to assure food supply for their growing populations.

- Agriculture in the EU is, itself, a complex mix of landscapes, cropping systems and land use intensities, with significant areas of agriculture protected under directives and regulations to support biodiversity. The complexity of the agricultural landscape is a key driver of biodiversity and, within that, crop choice and rotation and cultivation techniques have significant impacts. In temperate areas, the balance of spring and winter cropping has a major impact on farmland birds. Similarly, the increased area of maize, a relatively low biodiversity crop in most parts of the EU, may have affected biodiversity at a landscape level as well as at field level.

- Inputs to crops such as herbicides clearly impact the number of non-crop plants (weeds) in a field with potential effects on arthropods and soil organisms. However, weeds affect crop yield and quality, and interfere with harvest. Therefore, there is a conflict between biodiversity and human needs which must be carefully balanced. New technologies such as data and modelling to achieve thresholds for weed populations requiring intervention to protect yield and quality, and finely targeted herbicide application techniques may help achieve this balance.

- The potential impact of glyphosate has to be viewed bearing in mind these complexities. Changing its use will not be a panacea to resolve biodiversity issues.

- Biodiversity is a complex, multi-facetted topic, reaching beyond agriculture and any specific pesticide including glyphosate. Land use change and therefore loss of habitats is the main driver of biodiversity decline. Therefore, only part of the biodiversity topic can be addressed in the frame of an herbicide active ingredient renewal dossier and the GRG welcomes the drive to address biodiversity through an integrated European strategy.

- The use of glyphosate based products enables farmers to applying tangible good agricultural practices that support biodiversity, e.g.

    - practicing climate-friendly farming with reduced need for cultivations (ploughing), requiring less fuel and thereby decreasing the greenhouse gas emissions associated with weed control.
    - contributing to soil health and soil biodiversity by applying no-till practices
    - increasing the planting of cover crops, with corresponding habitat and trophic interaction advantages for biodiversity.

- By using glyphosate-based products farmers and professional users can actively contribute to European environmental goals as outlined in the European Green Deal. They help ensure that European food remains safe, nutritious and of high quality with minimum impact on nature.

- Glyphosate-based products can contribute to environmental policy goals in several ways:

    - Reducing the use and more importantly, the risks and impact of pesticides:
        - Glyphosate has undergone extensive environmental testing and assessments that support its intended uses. Glyphosate works by targeting a site that is essential for plant growth and is only found in plants, fungi and some bacteria. Glyphosate does not accumulate in animals, and readily binds to soil, further limiting exposure to wildlife. Because of these properties, glyphosate and glyphosate-based products pose negligible risk to non-target organisms such as birds, wild mammals, earthworms, beneficial insects including pollinators, and aquatic animals under good agricultural practices.
        - Glyphosate-based products work only on emerged green plants, which enables the use of precision application technology that actively contributes to the reduction of use and volume of pesticides. Weed recognition and precision applications are

developing at a rapid rate and are already been used to significantly reduce use in some sectors, such as railway.

- Integrated Weed Management (IWM)
  - A more comprehensive implementation of IWM practice is desired in EU and the revision of the Sustainable Use Directive (2009/128/EC) will provide opportunities to improve the implementation and improvement of IWM programs. GRG member companies support the sustainable use of pesticides and will contribute to the revision of the directive. Glyphosate-based products are an essential tool in IWM, due to the high efficacy and its unique mode of action and the fact that it is translocated throughout the plant, including down to roots, rhizomes and tubers below ground. A comprehensive access to and diversified use of the toolbox of chemical (including glyphosate) and non-chemical measures (e.g. crop rotation, cover crops, no-till practices) supports effective weed resistance management and the reduction of the weed seed bank in soil.

- Soil biodiversity (Soil Thematic Strategy COM/2006/0231)
  - Glyphosate-based products enable sustainable soil management practices, such as minimum and no-till practice and allow farmers to successfully manage cover crops. Without glyphosate the agronomic and environmental benefits of these practices will never be fully realised.
    - Glyphosate's unique properties make it ideally suited to promote conservation tillage, an agricultural practice based on the principles of minimal soil disturbance (no-till or reduced tillage), organic soil content (crop residues), and diversified crop rotation.
    - Conservation tillage is beneficial to nutrient cycling, reducing fertiliser demand, improving functional soil biodiversity, improving soil structure, supporting cover crop management, improving carbon sequestration, increasing water infiltration and conservation, and reducing soil erosion and thus improving water quality.

- Increased biodiversity in agricultural areas ('Bringing nature back into our lives' / 'EU Nature Restoration Plan' (EU draft biodiversity strategy) and EU Agri-environmental and greening measures under the EU Common Agricultural Policy (CAP))
  - Cover crops are "habitat" for soil microbes and thus lead to soil carbon sequestration depending on the class of cover crop (grasses, legumes, brassicas or non-legume broadleaves) and soil type with all its co-benefits such as erosion prevention, improved water quality, increased groundwater replenishment, soil moisture conservation, improved soil physical and biological properties, supply of nutrients to the following crop, cuts in fertiliser use and costs. In addition, cover crops help to suppress weeds, can provide cover and resources for wildlife, and can break pest cycles.
  - Cover crop mixtures which are planted between July and September in Europe can serve as habitats for bees, other insects, animals and birds as they provide food and shelter.
  - Glyphosate is the standard herbicide used for terminating all cover crops because its lack of residual activity in soil and favourable environmental safety profile make it ideally suited for this purpose. Failure to completely control cover crops through improper termination of the cover crop results in them acting as a weed and competing with the follow-on crop for nutrients in addition to being a potential host for pests and diseases.

- Measures against climate change, including carbon sequestration and reducing CO_2 during crop production:
  - According to the IPBES report (2019), climate change is a key driver for biodiversity loss.
  - Glyphosate-based products enable minimum and no-till farming practices and allow to manage cover crops, and by this significantly increase soil carbon sequestration. Soil carbon content is an important indicator for soil health. If soils are rich with organic matter and if they are not disturbed by tillage practices (ploughing) they

build up a favourable soil structure that facilitates water and nutrients storage and thus support agricultural production.
        - To the contrary, ploughing (inverting soil layers) stimulates microbial activity and consequently increases the breakdown of organic matter releasing carbon into the atmosphere, disturbing the soil structure and increasing susceptibility to erosion and droughts.

    - Safety of the public transportation infrastructure: Railway use
        - Glyphosate-based products are the product of choice of all European railway companies to keep the railway tracks free from weeds and by this guaranteeing safety and a reliable operation of the railway network. A well-functioning railway network is the backbone of public transportation and thus essential to maintain as an alternative to the individual use of cars. Holistically this indirectly supports biodiversity by limiting the loss of natural habitats to more roads.
        - In the representative use GAP table GRG included specific exemplary uses on railway tracks. Precision application technology is being introduced across Europe and can alone lead to use reduction rates of up to 70%. Reduced use rates result in reduced exposure of non-target plants and organisms.

    - Invasive Alien Species Regulation (Regulation (EU) No 1143/2014, fulfilling Action 16 of Target 5 of the EU 2020 Biodiversity Strategy)
        - Invasive alien species are one of the major causes of biodiversity loss. Glyphosate-based products actively support wildlife habitat maintenance and restoration by controlling difficult to eradicate invasive and noxious plant species that damage natural habitats in the most targeted and effective way. Due to glyphosate's systemic activity, lack of soil activity and favourable environmental safety profile, it has a long history of effectively controlling invasive species and successfully restoring a variety of habitats that host native species.
        - In the representative use GAP table GRG included specifically exemplary uses against Japanese knotweed and Giant hogweed.

    - Limiting the land used for agriculture and forestry
        - Glyphosate based products allow for an increase of land use efficiency in agriculture and forestry by enabling efficient farming and forestry practices to reduce their footprint. If these practices are embedded in corresponding legal frameworks that limit agricultural and forest land expansion, more land can be spared e.g. for wild nature conservation without compromising productivity. This is important as reducing productivity in Europe (a consequence of glyphosate not being available in EU anymore) will lead to a push to regain lost productivity, most likely through land expansion elsewhere with the respective negative impact on biodiversity.

- GRG proposes specific avenues to increase the contribution of glyphosate to biodiversity in Europe. These may be considered to be included in the product label or connected to Member State policy (*e.g.,* nature conservation plans), and thus contributing to reaching the EU environmental and biodiversity policy goals:
    - The representative use patterns included by the GRG in this 2020 renewal dossier depict the opportunity for significant rate reductions in Europe when compared to the 2012 Annex I renewal. In some cases the rates are reduced up to 50%, which has been made possible by advancements in application technology and differentiated application rates tailored to the growth stage of the weeds and the respective crop and weed situation, reflecting the specificities of European farming. This includes treated area reduction by precision applications such as targeted, spot or band application whenever possible in the crops concerned. In addition, the GRG is not considering preharvest uses and residential uses in our representative use pattern.
    - The GRG is committed to improving the use and adherence of glyphosate-based products as part of Integrated Pest and Integrated Weed Management (IPM/IWM) programs in Europe and emphasizing this by including clear and descriptive language on our product labels and instructions in a consistent way across the EU Member States. By this the contribution of glyphosate to supporting the goals of the Sustainable Use Directive (2009/128/EC, SUD) will be further increased. This includes the proper use of glyphosate and other herbicides,

integrating different herbicidal modes of action and combining them with other complementary agronomic, cultural and mechanical practices to managing weed growth, minimizing weed resistance and working towards reducing the weed seed bank in soil. In general, recommended IWM Best Management Practices are to regularly scout the field and become familiar with the weeds and their biological characteristics, set up a weed management plan for diversified crop rotations, reduce weed pressure and RMSressively manage weeds with the objective of reducing the weed seed bank for sustainable weed management.

- The GRG proposes to connect a specific biodiversity condition to the use of glyphosate-based products and to any other good agricultural practice dedicated to controlling weeds in the field with sufficient efficacy. This can be done in the frame of EU environmental policy, e.g. the SUD. To address the loss of biodiversity in Europe, the GRG sees opportunities to reintroduce more landscape features and non-productive areas (e.g. multi-functional field margins or compensation areas) to provide habitats for animal and plant species, including pollinators and pest antagonists that are associated with agriculture. This will be particularly impactful in simplified landscapes or intensified production areas, where otherwise refuge areas for insects, birds and mammals are limited. In a multi-stakeholder approach, this needs to be balanced against farmers' capacity to sustain the economic loss of taking areas out of production: here the available CAP tools might provide adequate financial support. To find the best balance between productivity and nature conservation, preferably areas of low productivity should be converted to habitats and other areas should be maintained at a high level of productivity. Through this the GRG is supporting the 'Bringing nature back into our lives' / 'EU Nature Restoration Plan' (EU draft biodiversity strategy) and EU Agri-environmental and greening measures under the EU Common Agricultural Policy (CAP). Examples could include compensation areas such as no-spray zones (either in-crop or off-crop) for biodiversity enhancement, to compensate for indirect effects through in-crop weed control.

Mitigation measures need to be proportionate, relevant and adapted to the landscape and agricultural context in respective Member States. If a risk to biodiversity via trophic interaction is locally established at the landscape level, and additional risk mitigation measures are considered necessary by risk managers at the Member States, the GRG proposes that risk mitigation options and biodiversity conservation measures as summarized in the following tables shall be considered for glyphosate-based product registrations.

These mitigation options will bring the greatest ecological benefits when implemented in simplified landscapes or in intensified production areas, where the refuge areas for insects, birds and mammals are limited. It is anticipated that this measure will not bring a high ecological benefit in complex landscapes where enough refuges are available off-field.

It needs to be noted that biodiversity related mitigation measures need to be adapted to the local Member State level, to the local environmental circumstances (e.g. landscape), to the local biodiversity conservation status and to the desired protection and conservation goals.

Table […]: Types of standard risk mitigation measures described in MAgPIE across the various Member States to mitigate effects on biodiversity and how they could be applied to glyphosate products. (Proceedings published in 2017: "Mitigating the Risks of Plant Protection Products in the Environment MAgPIE", Society of Environmental Toxicology and Chemistry (SETAC))

Type of Mitigation Measure	Risk Mitigation Measure	Benefits	Glyphosate renewal dossier (2020)
Restrictions or modifications of products' conditions of application	Application rate, Application frequency, application timing, and interval between applications	Lower transfers to groundwater and surface water; Reduces exposure of organisms in-crop and off-crop.	Significant reductions (50% in volume) in newly proposed application rates compared with the representative use presented in the 2012 renewal dossier. […] Treated area restriction

			1. for the representative use GAPs: applying to only 50% of the total area in orchard/vineyard area. 2. maximum of 50% of the total area for broad acre vegetable inter-row 3. Invasive species control e.g., couch grass – maximum of 20% of the cropland + extended application intervals. Limited frequency and timing of application: 28-day interval between applications and no pre-harvest applications
Application equipment with Spray Drift Reduction Technology (SDRT)	Spray drift reduction nozzles (SDRN), shields, Precision treatment, etc.	Reduces exposure of organisms in-crop (precision treatment) and off-crop	Reduction of spray drift to the off-field: 1. Use 75% drift reducing nozzles for pre-sowing/pre-planting in arable crops. 2. Use of ground directed, shielded spray for band application in orchards / vineyards and broad-acre vegetable inter-row application.
Buffer zones	Non-sprayed zone at the edge of a crop	Reduces exposure of organisms and off-crop	Establishment of buffer zones: Buffer zones of varying size (depending on the type of SDRT) are required as protection for off-crop NTTP communities from spray drift.

Table […]: Examples of non-standard mitigation described in MAgPIE across the various Member States and risk mitigation options, to address potential indirect effects and how they could be applied to glyphosate products. (Proceedings published in 2017: "Mitigating the Risks of Plant Protection Products in the Environment MAgPIE", Society of Environmental Toxicology and Chemistry (SETAC))

Type of Mitigation Measure	Risk Mitigation Measure	Benefits	Comments
Moderate weed reduction in-crop	A certain part of the in-crop area would not be sprayed.	Aims to support a moderate level of arable weeds in-crop to support provisioning of habitat to invertebrates and food for farmland birds / mammals to overcome indirect effects from in-crop weed control. The option for "moderate weed control" could be achieved by implementing non-spray crop areas along the edge and/or at the corners and/or in areas of lower productivity of an agricultural	Mitigation option for broad acre row crops to protect against indirect effects through trophic indirections. This measure will bring the greatest ecological benefit when implemented in simplified landscapes or in intensified production areas, where the refuge areas for insects, birds and mammals are limited. It is anticipated that this measure will not bring a high ecological benefit in complex landscapes where enough refuges are available off-field. The economic consequence of this option may be that the monetary value of the crop decreases due to competition of the crop with arable weeds. The agronomic consequence of this measure is the progressive increase in the weed seed bank in-field, which may increase the weed pressure and thereby the need for higher levels of weed control in subsequent years.

		field while for the remaining in-crop area a maximal weed reduction can apply.	
Multi-functional field margins	Supportive measures: Using seed mixtures: wild flower-sown mix, pollen and nectar flower mix, adapted wild bird cover mix, vegetated filter strips.	Reduces exposure of organisms in-crop and off-crop, provides habitat and food resources and mitigates indirect effects on biodiversity.	Field margins provide benefits for conservation in terms of biodiversity (species) and the provision of biotic and abiotic agro-ecosystem services. This measure will bring the greatest ecological benefit when their creation is spatially targeted in simplified landscapes and intensified crop production areas, where the refuge areas for insects, birds and mammals are limited. It has been shown that this measure will not bring a high ecological benefit in complex landscapes where enough refuges are available off-field Sown flower areas bear the risk of also generating disservices to crop management such as increased pest pressures and/or noxious or invasive alien plant species, if seed mix are not properly selected.
Compensation areas	Recovery areas (ecological focus areas, biodiversity refuge) => part of the agricultural area that is not cultivated anymore (land sparing) Supporting measures: hedges, trees, landscape features, biotopes, afforested areas.	Provides habitat and food resource, reduces exposure of organisms in-crop, and depending on location in the farmland, may reduce exposure of non-target organisms.	Availability of habitats is key to support food webs and biodiversity. The creation of semi-natural habitats and corridors across the landscape is especially important in intensively cropped areas, to ensure sufficient connectivity between available habitat patches. Since different species have different habitat requirements non-crop/semi-natural habitat creation measures should be adapted to the local situation. This measure will bring the greatest ecological benefit when implemented in simplified landscapes or in intensified production areas, where the refuge areas for insects, birds and mammals are limited. It is anticipated that this measure will not bring a high ecological benefit in complex landscapes where enough refuges are available off-field.

Table […]: Comparison of representative uses and rates between the 2012 and 2020 glyphosate EU renewal dossiers

Glyphosate uses	2012 renewal dossier	2020 renewal dossier
PRE-SOWING, PRE-PLANTING, Also applicable to renovation / change of land use applications.	Not separately considered	Maximum application rate of 1.44 kg as/ha glyphosate in any 12 month period.

Spring & autumn after harvest (incl stubble and/or seedbed prep)	Maximum application rate 4.32 kg/ha in any 12-month period	Maximum application rate of 2.16 kg as/ha glyphosate in any 12 month period.
Post-harvest, pre-sowing, Pre-planting – cereal volunteers	Not separately considered	Maximum application rate of 0.54 kg as/ha glyphosate in any 12 month period.
Post-harvest, pre-sowing, Pre-planting – Couch grass	Not separately considered	Maximum application rate of 1.08 kg as/ha glyphosate in any 12 month period.
Pre-harvest application in cereals and oilseeds	Maximum application rate 4.32 kg/ha in any 12-month period	Pre-harvest application is not defended as a representative use
Vegetable inter-row application	Not separately considered	Maximum application rate of 1.08 kg as/ha glyphosate in any 12 month period.
Orchard crops, vines, including citrus & tree nuts	Maximum cumulative application rate 4.32 kg/ha in any 12-month period	Maximum application rate of 2.88 kg as/ha treated area glyphosate in any 12 month period.
Railroad tracks	Not considered	Maximum application rate 3.6 kg as/ha glyphosate in any 12-month period.
Invasive species in agricultural and nonagricultural areas	Not considered	Maximum application rate 1.8 kg as/ha glyphosate in any 12-month period.

[…]

Comment and conclusion of RMS on risk mitigation proposals to protect biodiversity

The risk mitigation options presented are considered applicable and suitable to mitigate risks identified from standard risk assessments conducting with the EFSA guidance document for aquatic organisms (2013).

The applicant seems to state that drift reduction measures like buffer zones, drift reduction nozzles, (…) will be favourable for biodiversity. These buffer zones or other risk mitigation measures are necessary according to the risk assessment in order to reduce the risk to the off-crop community of plants to an acceptable level. They are considered to contribute to protection of biodiversity but may not be sufficient alone. In intensive agricultural areas, the extent of the off-field areas may be limited and it appears essential to create sufficient compensation areas to protect the biodiversity within the landscape.

Additional proposals for mitigation of risk from indirect effects should be discussed further during the EU peer review in order to establish the basis for harmonised set of measures to be implemented on MS level at product authorisation.

Overall conclusion of RMS on the assessment of risk to biodiversity via indirect effects and trophic interactions

The regulation (EU) 2017/2324 related to the approval of glyphosate stated that "Member States shall pay particular attention (…) to the risk to diversity and abundance of non-target terrestrial arthropods and vertebrates via trophic interactions". A loss of plant biodiversity following the application of plant protection products may affect the entire food web. It could affect the presence of adequate habitats for arthropods, as well as for birds and mammals. Moreover the presence of appropriate range of plants as food sources is vital to the survival of foliage eating arthropods, birds and mammals, as well as nectar and pollen sources for bees. However, there is currently no validated tools nor methodology for a European harmonized risk assessment of biodiversity and consideration of indirect effects via trophic interactions available. RMS considers that the current standard risk assessment and protection goals address the direct effect only and have not been defined to address specifically indirect effects.

Moreover, even if indirect effects and trophic interactions are linked to biodiversity, there is much more to consider to protect biodiversity and the providing ecosystem services in Europe in adequacy with the various EU and national legislations.

For aquatic organisms and bees, EFSA guidance documents proposed specific protection goals that followed methodology reported in the EFSA guidance on specific protection goals (2016). As the aim of this guidance is to make general protection goals operational for use in environment risk assessment and take into account biodiversity and ecosystem services, RMS considered that it is the most suitable approach available to assess biodiversity in the context of regulatory risk assessment.
For aquatic organisms, according to the guidance document in force for aquatic organisms (EFSA, 2013), risk assessment based on ecological threshold option could be considered protective of both direct effects as well as indirect effects including trophic interaction among the aquatic food chain when the magnitude of effects is considered as negligible for each ecological entity of each of the aquatic organisms. However, for glyphosate, it could not be considered that all indirect effects and food web interactions are addressed given that not all food sources are considered. For example, study to assess the effects on sediment-dwelling organisms is missing. Additionally, information on impact on decomposition processes in aquatic systems, or effects on the biofilm (algae, fungi and bacteria-matrix) would need to be considered. Further information on the effect to the aquatic community could also contribute to assess risk to biodiversity via indirect effects and trophic interactions. Thus some uncertainties remain.
For bees, risk to bee biodiversity from direct effects can be considered covered by the risk assessment for glyphosate that is based on standard laboratory tests. However indirect effects that may result to the reduction of weeds availability could not be addressed via current risk assessment. One option could be to implement compensatory area but for the time being the effectiveness of such method is only qualitative.

For soil organisms, non-target arthropods and non-target terrestrial plants, the applicant attempted to define what could be specific protection goals for these organisms by considering recent EFSA Scientific Opinions. However, RMS noted that functional/organism groups used are limited to the regulatory species. In view of the literature data, RMS considers that a shift in their community structures of soil micro-organisms could not be excluded as glyphosate could be used as a source of P, C or N by soil micro-organisms and should be further investigated. However the options proposed to set SPG for these organisms should be agreed by risk managers and guidance documents should have to be revised accordingly.
Regarding the indirect effects through trophic interactions to farmland birds by reducing in-crop food resource as consequence of glyphosate application, one option could be to consider additional mitigation measures that allow birds to find food resources from adjacent non-treated area. Considering this, a reflection should be made on the desired option manageable at the European landscape for approval of active substance as well as at more local level (MS, field…). System-based approach exist that may help risk managers to choose the more appropriate approach (landsharing *vs.* landsparing) considering the biodiversity goal of the European legislation.
Regarding the indirect effects linked to the loss of habitats for non-target arthropods and cascading effects to birds and mammals, one option could be to compensate this loss. Same concept as for indirect effects related to non-target plants as food source could be considered.

Overall, there is a need of practical harmonised risk assessment tools for the assessment of active substance and plant protection products before their placement on the market. For that purpose, guidance documents used for risk assessment should be revised to take into account specific protection goals as defined according to the principles of EFSA guidance (2016). Besides a protection goal for biodiversity, an agreed upon methodology for assessing biodiversity and the impact of pesticide use under the auspices of the regulatory assessment process would also have to be developed before a dedicated assessment could be performed.
In the meantime, given the importance of agroecosystems as habitats and food/ressources supply location, discussions among risk managers should be reinforce around the question of biodiversity in agricultural landscape. There is a balance to find between reducing indirect effects and impact on biodiversity and benefits to use plant protection products such as glyphosate to maintain agricultural food and livestock production sustainable.
Implementation of mitigation measures dedicated to biodiversity could be part of the environmental risk assessment in the context of plant protection products considering definition of SPG. Under Regulation (EC) No 1107/2009, the evaluation of effects on biodiversity via indirect effects and trophic interactions are limited to effects caused by the intrinsic properties of the active substance itself. The consideration of the extent of uses of a specific plant protection active substance should be considered by risk managers during the decision making process. The risk mitigation options presented are considered applicable and suitable to mitigate risks identified from standard risk assessments conducting with the EFSA guidance document for aquatic organisms (2013). Additional proposals for mitigation of risk from indirect effects should be discussed further during the EU peer review in order to establish the basis for harmonised set of measures to be implemented on MS level at product authorisation .

Monitoring programs and indicators such as farmland bird index, grassland butterfly index, (…) should be developed and harmonised. As reported Maes J. et al. (2020)[41] in a recent JRC report "*Monitoring biodiversity is essential to be able to assess if policy targets have been met (e.g. halting biodiversity loss).*" JRC also indicated that *"While availability of information on species and habitats is improving, indicators on genetic diversity are still missing from the overall picture, and in particular organised information, at the EU level, on the number, amount and geographical distribution of traditional breeds, cultivars, landraces, wild crop relatives, traditional and ancient varieties*." Harmonised approach to report results and assessment of monitoring programs and indicators will allow to have feedback on the effectiveness of mitigation measures taken. System-based approach could also be used for that purpose as they represent tools that could be used by both risk assessors and risk managers.

2.10 ENDOCRINE DISRUPTING PROPERTIES

2.10.1 Gather all relevant information

Standard toxicology and ecotoxicology studies conducted to meet to the data requirements under Regulation (EU) 283/2013 have been submitted. These data have been submitted in support of the glyphosate application for re-approval under Regulation EU No. 1107/2009.
A literature search has been conducted to identify the published data from the last 10 years in the open literature.

Data from relevant studies were added to the Excel template provided as Appendix E to the EFSA/ECHA guidance for the identification of endocrine disruptors (2018). Each study was given a unique identification number (study ID matrix) for its identification in the data matrix and the Lines of Evidence (LoE) spreadsheets of the Appendix E.

The applicant provided the Appendix E in two separate files (one for the mammalian toxicity data and another for the ecotoxicological data) while a single Appendix E compiling tox and ecotox data should have been provided. Furthermore the Tox Cast and *in vitro* data have not been included in Appendix E (for ecotoxicological data). Therefore a data gap is set to the applicant to provide a single updated Appendix E which must include all ecotoxicological data including Tox Cast and *in vitro* data. The ED assessment should be also updated to include the Tox Cast and *in vitro* data for the ecotoxicological assessments and lines of evidence.

2.10.2 ED assessment for humans

Please refer to separate document "Volume 1, 2.10.2 ED assessment for Humans".

2.10.3 ED assessment for non-target organisms

2.10.3.1 ED assessment for T-modality

2.10.3.1.1 Lines of evidence for adverse effects and endocrine activity related to T-modality

Amphibian data

An **amphibian metamorphosis assay (AMA)** according to OECD TG 231 is available ([redacted] 2012) and is evaluated in Vol. 3CA, section B.9.2.3.

Regarding developmental stages reached by the tadpoles till study end, the median tadpole stage at test end was for all treatments stage 57 according to Nieuwkoop and Faber (NF). When looking at the distribution of the treatment tadpole cohorts to the different stages, though, there was a slight shift towards later developmental stages reached by tadpoles exposed to higher glyphosate concentrations. In the control treatments, there were more tadpoles that at day 21 are in earlier developmental stages (e.g. NF 55) compared to the treatments with higher glyphosate concentrations. The tadpole group exposed to the highest glyphosate concentration tested was the only one with single animals reaching stage NF 61 and 62. The differences between stage distribution in the cohorts of the control treatment were however not statistically significantly different from the highest glyphosate treatment if tested with two sided Mann-Whitney-U test ($p = 0.052$).

[41] Maes, J., et al., 2020. Mapping and Assessment of Ecosystems and their Services: An EU ecosystem assessment, EUR 30161 EN, Publications Office of the European Union, Ispra, 2020, ISBN 978-92-76-17833-0, doi:10.2760/757183, JRC120383.

There were significant effects of glyphosate exposure on the endpoints tadpole growth and tadpole snout-to-vent length in the highest tested glyphosate treatment (nominal 90 mg a..e /L) when compared to the control treatment. In the two tested treatments below 90 mg a.e./L -which were 4.3 and 20 mg a.e./L – the snout-to-vent length showed an increasing trend compared to the control treatment (Jonckheere-Terpstra trend test; $p \leq 0.05$). However, while in the 4.3 mg a.e./L the tadpole snout-to-vent length was statistically significant different from the control (Dunnet test $p \leq 0.05$), the differences in tadpole length between the 20 mg a.e./L treatment and the control was not statistically significant. The reported differences could not be observed when snout-to-vent length was normalized to hind-limb length.

The incidence in diagnostic observation in the gross histopathology of thyroid gland of African Clawed Frog (*Xenopus laevis*) at test end showed no treatment related effects on the observed endpoints changes in thyroid gland size, follicle size and asymmetry. Thyroid follicular epithelium showed also no sign of hyperplasia or hypertrophy due to increased glyphosate concentrations.

The former RMS concluded that the results of this assay do not point at disturbed thyroidal activity due to glyphosate exposure of the tested species. It is not clear whether the tested concentrations were sufficiently high to cover the MTC level as recommended in the new ECHA/EFSA guidance document on endocrine disrupters (2018). However, in OECD 231 it is recommended that the highest tested dose should be "set by the solubility limit of the test substance; the maximum tolerated concentration (MTC) for acutely toxic chemicals; or 100 mg/L, whichever is lowest." It is noted that the range finding test was performed up to the limit dose of 100 mg/L and the final test was performed at 90 mg/L (measured), or 100 mg/L (nominal), since the range finder did not indicate significant acute toxicity below that level and the solubility of glyphosate is much higher (10.1 g/L). As a result, since the test was performed at the limit dose suggested by OECD 231, the study is considered to be valid.

Literature data on amphibians
The available dataset from open literature includes 27 studies on amphibians and one on reptiles. The majority of the studies investigated acute effects (survival) of juvenile stages of amphibians. However, the studies by Jones (2010), Williams et al. (2010), Navarro-Martín et al. (2014) and Lanctot et al. (2014), involved chronic effects related to growth (e.g. snout-to-vent length) and development (time to metamorphosis and metamorphosis success). An additional study, on the **reptile species** *Caiman latirotris* (Poletta et al. 2011), showed effects on total length and snout vent length (SVL).

In a study by Slaby et al. (2019), oocytes of *Xenopus laevis* were exposed to glyphosate and the formulation Roundup GT Max. The aim was to investigate the effects on the oocyte maturation, which is an essential preparation for the laying and the fertilization. Kinetics of the maturation process were assessed by determining GVBD (Germinal Vesicle Breakdown) ratios every 15 min for 13 h. The results showed that exposure to glyphosate as well as the formulation caused a delay of the hormone dependent process and were responsible for spontaneous maturation. Severe and particular morphogenesis abnormalities of the meiotic spindle were also observed, while the MAPK (mitogen-activated protein kinases) pathway and the MPF (mitosis-promoting factor) did not seem to be affected by exposures.

At a later stage of the evaluation, an additional study (Lanctot, 2013) was shown to be available from the literature search which might be relevant for the ED assessment. The study investigated the effects of the formulation Roundup WeatherMax on metamorphosis of wood frogs (*Lithobates sylvaticus*) in natural wetlands. From the results, the authors suggested that the resulting gene expression data (mRNA levels) indicate potential of glyphosate to alter hormonal pathways during tadpole development.
Overall, the RMS considers that the observed effects from the open literature studies summarized above can be regarded as 'sensitive to, but not diagnostic of' endocrine disruption according to the EFSA/Echa guidance document. As an AMA is already available, this should cover this point. Besides, the test material used in the literature studies were formulated products with glyphosate rather than the active substance itself. However, although the data from literature are considered less relevant but supplementary and not contributing to a pattern of ED activity nor counter the results of the available AMA study, they are included here for the sake of completeness.

The tables below presented the lines of evidence based on applicant's proposal updated by RMS to reflect RMS's conclusion for each study. As a consequence, studies judged invalid by RMS are not reported.

Table 2.10.3.1.1-1 Lines of evidence for adverse effects related to T-modality

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
6e	Sensitive to, but not diagnostic of, EATS	Body weight (fish)	*Oncorhynchus mykiss*	85 (60 post hatch)	days	Uptake from water	> 9.63	mg/L	No effect	No effect on fish growth and Gonado-somatic index up to highest test concentrations (9.63 - 100 mg/L) in three GLP studies and one publication. **RMS:** Agreed that no effect on fish growth and GSI observed. Study with *O.mykiss* (ID6): Study valid but results unreliable. "variability of wet and dry weight in control and at	In fish: Mostly no effects; very few systemic effects (mortality) or secondary effects (only in a range where systemic effects already occur).	No evidence for adversity observed.	N
7d	Sensitive to, but not diagnostic of, EATS	Body weight (fish)	*Pimephales promelas*	254	days	Uptake from water	> 25.7	mg/L	No effect				
7f	Sensitive to, but not diagnostic of, EATS	Body weight (fish)	*Pimephales promelas*	254	days	Uptake from water	> 25.7	mg/L	No effect				
7k	Sensitive to, but not diagnostic of, EATS	Body weight (fish)	*Pimephales promelas*	254	days	Uptake from water	> 25.7	mg/L	No effect				
8c	Sensitive to, but not diagnostic of, EATS	Body weight (fish)	*Pimephales promelas*	21	days	Uptake from water	> 33	mg/L	No effect				

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
15b	Sensitive to, but not diagnostic of, EATS	Body weight (fish)	*Gasterosteus aculeatus*	42	days	Uptake from water	> 100	mg/L	No effect	9.63 mg/L (2 replicates only). Study on *P.promelas* (ID7): "derivatisation efficiency of this analytical method can not be verified by RMS. 2 replicates per concentations. Study considered supportive" Study with *Gasterosteus aculeatus* (ID15): seawater used in the test (this fish species can be found in both freshwater and sea), interactions between the ions and the active			
7c	Sensitive to, but not diagnostic of, EATS	Length (fish)	*Pimephales promelas*	254	days	Uptake from water	> 25.7	mg/L	No effect				
7e	Sensitive to, but not diagnostic of, EATS	Length (fish)	*Pimephales promelas*	254	days	Uptake from water	> 25.7	mg/L	No effect				
7j	Sensitive to, but not diagnostic of, EATS	Length (fish)	*Pimephales promelas*	254	days	Uptake from water	> 25.7	mg/L	No effect				
8b	Sensitive to, but not diagnostic of, EATS	Length (fish)	*Pimephales promelas*	21	days	Uptake from water	> 33	mg/L	No effect				
15a	Sensitive to, but not diagnostic of, EATS	Length (fish)	*Gasterosteus aculeatus*	42	days	Uptake from water	> 100	mg/L	No effect				

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
8g	Sensitive to, but not diagnostic of, EATS	Gonado-somatic index	*Pimephales promelas*	21	days	Uptake from water	> 33	mg/L	No effect	substance cannot be discounted. Study considered relevant and reliable for seawater fish.			
6a	Sensitive to, but not diagnostic of, EATS	Hatching success	*Onorhynchus mykiss*	85 (60 post hatch)	days	Uptake from water	> 9.63	mg/L	No effect	No effect on fish hatching success up to highest test concentrations (9.63 and 25.7 mg/L) in 2 studies. **RMS:** Study with *O.mykiss* (ID6) is valid but results are unreliable. Variability of hatching success at 9.63 mg/L (2 replicates only)			
7a	Sensitive to, but not diagnostic of, EATS	Hatching success	*Pimephales promelas*	254	days	Uptake from water	> 25.7	mg/L	No effect				
7h	Sensitive to, but not diagnostic of, EATS	Hatching success	*Pimephales promelas*	254	days	Uptake from water	> 25.7	mg/L	No effect				

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
										Study on *P.promelas* (ID7): Study supportive. Number of eggs spawned per female and number of eggs per spawning with high variability (duplicate) particularly in control and highest tested dose 2 replicates per concentations. Derivatisation efficiency of this analytical method can not be verified by RMS.			
6c	Sensitive to, but not diagnostic of, EATS	Larval survival and length	*Oncorhynchus mykiss*	85 (60 post hatch)	days	Uptake from water	> 9.63	mg/L	No effect	No effect on fish survival and length up to highest test concentration (9.63 mg/L). <u>RMS:</u> Study valid but results unreliable.			
6d	Sensitive to, but not diagnostic of, EATS	Larval survival and length	*Oncorhynchus mykiss*	85 (60 post hatch)	days	Uptake from water	> 9.63	mg/L	No effect				

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
										Variability of egg viability at 9.63 mg/L (2 replicates only). Survival was found to be lower in the control than in the tested concentrations.			
6b	Sensitive to, but not diagnostic of, EATS	Morphological abnormalities	*Oncorhynchus mykiss*	85 (60 post hatch)	days	Uptake from water	> 9.63	mg/L	No effect	No morphological abnormalities up to highest test concentration (9.63 mg/L). RMS: Study valid but results unreliable (variability for other parameters)			
7g	Sensitive to, but not diagnostic of, EATS	Reproduction (fecundity, fertility)	*Pimephales promelas*	254	days	Uptake from water	> 25.7	mg/L	No effect	No effect on fish reproduction up to the highest test concentrations (25.7 and 33 mg/L) in 2 studies. RMS: Agreed. 254d study (ID7) is supportive.			
8d	Sensitive to, but not diagnostic of, EATS	Reproduction (fecundity, fertility)	*Pimephales promelas*	21	days	Uptake from water	> 33	mg/L	No effect				

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
										21d study (ID8) corresponds to FSTRA.			
5b	Sensitive to, but not diagnostic of, EATS	Behaviour (fish): Lethargy	*Brachydanio rerio*	168	hours	Uptake from water	3.2	mg/L	Increase	Systemic effect (secondary to systemic toxicity: Adverse effect on fish behaviour observed in only one study; effect occurs in the same concentration with increased mortality at the same concentration range (5.6 mg/L).			
5a	Systemic toxicity	Survival (fish)	*Brachydanio rerio*	168	hours	Uptake from water	5.6 (appl) 3.2 (RMS)	mg/L	Deacrease	Decrease in fish survival only observed in one study. No effects on survival up to the highest test concentrations			_
7b	Systemic toxicity	Survival (fish)	*Pimephales promelas*	254	days	Uptake from water	> 25.7	mg/L	No effect				
7i	Systemic toxicity	Survival (fish)	*Pimephales promelas*	254	days	Uptake from water	> 25.7	mg/L	No effect				

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
8a	Systemic toxicity	Survival (fish)	*Pimephales promelas*	21	days	Uptake from water	> 33	mg/L	No effect	(25.7 - 33 mg/L.) **RMS:** *D.rerio* (ID5) : 10% mortality at 3.2 mg/L (not statistically significant but considered biologically relevant by RMS) 254d study with *P. promelas* (ID7): derivatisation efficiency of analytical method can not be verified by RMS. 2 replicates per concentations. Study supportive			
9d	Sensitive to, but not diagnostic of, EATS	Body weight (amphibian)	*Xenopus laevis*	21	days	Uptake from water	90	mg/L	Increase	Slight increase in amphibian growth.	In amphibians: No adverse effects observed. The slight increase in amphibian		N

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
9e	Sensitive to, but not diagnostic of, EATS	Snout-vent length/growth	*Xenopus laevis*	21	days	Uptake from water	4.3	mg/L	Increase		growth is not cosidered to be T-mediated since no effects are observed on developmental stage, hind limb length or thyroid histopathology.		
9b	Sensitive to, but not diagnostic of, EATS	Malformations	*Xenopus laevis*	21	days	Uptake from water	> 90	mg/L	No effect	No effect on amphibian malformations and mortality up to the highest concentration (90 mg/L.)			
9a	Systemic toxicity	Mortality (amphibian)	*Xenopus laevis*	21	days	Uptake from water	> 90	mg/L	No effect				–
1c	Sensitive to, but not diagnostic of, EATS	Clinical observations, behaviour	*Colinus virginianus*	20	weeks	Oral	> 2250	ppm	No effect	No effect on avian behaviour and food consumption up to the highest test doses (1000 and 2250 ppm) in 3 studies.	In birds: No significant adverse effects observed in 3 studies.		N
2c	Sensitive to, but not diagnostic of, EATS	Clinical observations, behaviour	*Anas platyrhynchos*	21	weeks	Oral	> 2250	ppm	No effect				
1d	Sensitive to, but not diagnostic of, EATS	Food consumption	*Colinus virginianus*	20	weeks	Oral	> 2250	ppm	No effect				

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
2d	Sensitive to, but not diagnostic of, EATS	Food consumption	*Anas platyrhynchos*	21	weeks	Oral	> 2250	ppm	No effect				
4c	Sensitive to, but not diagnostic of, EATS	Food consumption	*Anas platyrhynchos*	17	weeks	Oral	> 1000	ppm	No effect				
1b	Sensitive to, but not diagnostic of, EATS	Adult body weight (bird)	*Colinus virginianus*	20	weeks	Oral	> 2250	ppm	No effect	No effect on avian adult body weight up the highest test doses (1000 and 2250 ppm) in 3 studies.			
2b	Sensitive to, but not diagnostic of, EATS	Adult body weight (bird)	*Anas platyrhynchos*	21	weeks	Oral	> 2250	ppm	No effect				
4b	Sensitive to, but not diagnostic of, EATS	Adult body weight (bird)	*Anas platyrhynchos*	17	weeks	Oral	> 1000	ppm	No effect				
1f	Sensitive to, but not diagnostic of, EATS	Egg production	*Colinus virginianus*	20	weeks	Oral	> 2250	ppm	No effect	No effect on avian reproduction up to the highest test doses (1000 and 2250 ppm) in 4 studies.			
2f	Sensitive to, but not diagnostic of, EATS	Egg production	*Anas platyrhynchos*	21	weeks	Oral	> 2250	ppm	No effect				

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
4d	Sensitive to, but not diagnostic of, EATS	Egg production	*Anas platyrhynchos*	17	weeks	Oral	> 1000	ppm	No effect				
1m	Sensitive to, but not diagnostic of, EATS	Offspring body weight (bird)	*Colinus virginianus*	20	weeks	Oral	> 2250	ppm	No effect	Decreased avian offspring body weight at the highest test dose of 2250 ppm in only 1 of 3 valid studies. No effects on offspring body weight up to the highest test doses (1000 and 2250 ppm) in 2 valid studies.			
2m	Sensitive to, but not diagnostic of, EATS	Offspring body weight (bird)	*Anas platyrhynchos*	21	weeks	Oral	2250	ppm	Decrease				
4l	Sensitive to, but not diagnostic of, EATS	Offspring body weight (bird)	*Anas platyrhynchos*	17	weeks	Oral	> 1000	ppm	No effect				
4h	Sensitive to, but not diagnostic of, EATS	Egg weight	*Anas platyrhynchos*	17	weeks	Oral	> 1000	ppm	No effect	No effect on avian egg weight at the highest test concentration of 1000 ppm in the valid study.			
1g	Sensitive to, but not diagnostic of, EATS	Cracked eggs	*Colinus virginianus*	20	weeks	Oral	> 2250	ppm	No effect	No effect on avian egg-shell breaking strength up to highest test			

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
2g	Sensitive to, but not diagnostic of, EATS	Cracked eggs	*Anas platyrhynchos*	21	weeks	Oral	> 2250	ppm	No effect	doses (1000 and 2250 ppm) in 3 valid studies.			
4e	Sensitive to, but not diagnostic of, EATS	Cracked eggs	*Anas platyrhynchos*	17	weeks	Oral	> 1000	ppm	No effect				
1i	Sensitive to, but not diagnostic of, EATS	Eggshell thickness	*Colinus virginianus*	20	weeks	Oral	> 2250	ppm	No effect	Avian eggshell thickness is not affected in a dose-dependent pattern in 3 valid studies. The decrease in study ID 3 only occurred at a dose of 50 ppm and not at higher doses.			
2i	Sensitive to, but not diagnostic of, EATS	Eggshell thickness	*Anas platyrhynchos*	21	weeks	Oral	> 2250	ppm	No effect				
4g	Sensitive to, but not diagnostic of, EATS	Eggshell thickness	*Anas platyrhynchos*	17	weeks	Oral	> 1000	ppm	No effect				
1e	Sensitive to, but not diagnostic of, EATS	Gross pathology (bird)	*Colinus virginianus*	20	weeks	Oral	> 2250	ppm	No effect	No effect on avian gross pathology up to the highest test dose (2250			

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
2e	Sensitive to, but not diagnostic of, EATS	Gross pathology (bird)	*Anas platyrhynchos*	21	weeks	Oral	> 2250	ppm	No effect	ppm) in 2 studies.			
1k	Sensitive to, but not diagnostic of, EATS	Hatchability	*Colinus virginianus*	20	weeks	Oral	> 2250	ppm	No effect	No effect on avian offspring hatchability up to the highest test doses (1000 and 2250 ppm) in 3 studies.			
2k	Sensitive to, but not diagnostic of, EATS	Hatchability	*Anas platyrhynchos*	21	weeks	Oral	> 2250	ppm	No effect				
4j	Sensitive to, but not diagnostic of, EATS	Hatchability	*Anas platyrhynchos*	17	weeks	Oral	> 1000	ppm	No effect				
1h	Sensitive to, but not diagnostic of, EATS	Egg viability (% viable embryo of egg set)	*Colinus virginianus*	20	weeks	Oral	> 2250	ppm	No effect	No effect on avian egg viability or offspring survival in 2 bird species up to the highest test doses (1000 and 2250 ppm) in 3 studies.			
2h	Sensitive to, but not diagnostic of, EATS	Egg viability (% viable embryo of egg set)	*Anas platyrhynchos*	21	weeks	Oral	> 2250	ppm	No effect				

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
4f	Sensitive to, but not diagnostic of, EATS	Egg viability (% viable embryo of egg set)	*Anas platyrhynchos*	17	weeks	Oral	> 1000	ppm	No effect				
1l	Sensitive to, but not diagnostic of, EATS	Number of 14 day-old survivors	*Colinus virginianus*	20	weeks	Oral	> 2250	ppm	No effect				
2l	Sensitive to, but not diagnostic of, EATS	Number of 14 day-old survivors	*Anas platyrhynchos*	21	weeks	Oral	> 2250	ppm	No effect				
4k	Sensitive to, but not diagnostic of, EATS	Number of 14 day-old survivors	*Anas platyrhynchos*	17	weeks	Oral	> 1000	ppm	No effect				
1j	Sensitive to, but not diagnostic of, EATS	Viable embryos	*Colinus virginianus*	20	weeks	Oral	> 2250	ppm	No effect				
2j	Sensitive to, but not diagnostic of, EATS	Viable embryos	*Anas platyrhynchos*	21	weeks	Oral	> 2250	ppm	No effect				

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
4i	Sensitive to, but not diagnostic of, EATS	Viable embryos	*Anas platyrhynchos*	17	weeks	Oral	> 1000	ppm	No effect				
1a	Systemic toxicity	Mortality	*Colinus virginianus*	20	weeks	Oral	> 2250	ppm	No effect	No mortality in birds observed up to the highest test concentrations (2250 and 1000 ppm) in 2 studies.			_
2a	Systemic toxicity	Mortality	*Anas platyrhynchos*	21	weeks	Oral	> 2250	ppm	No effect				
16a	Sensitive to, but not diagnostic of, EATS	Emergence ratio of chironomids	*Chironomus riparius*	28	days	Uptake from water	> 1000	other	No effect	No effect on chironomid development and sex ratio observed up to the highest test concentration (1000 mg/L). **RMS:** Agreed. study considered as supportive only (no analytics in sediment while Glyphosate partition to sediment)	In Chironomus: No adverse effects observed.		N
16b	Sensitive to, but not diagnostic of, EATS	Developmental rate of chironomids	*Chironomus riparius*	28	days	Uptake from water	> 1000	other	No effect				
16c	EATS-mediated	Sex ratio in chironomids	*Chironomus riparius*	28	days	Uptake from water	> 1000	other	No effect				E, A, S

Table 2.10.3.1.1-2 Lines of evidence for endocrine activity related to T-modality

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
9c	EATS-mediated	Developmental stage	*Xenopus laevis*	21	days	Uptake from water	> 90	mg/L	No effect	No effect on T-mediated parameters up to the highest test concentration (90 mg/L)	Sufficiently investigated according to ED Guidance: No evidence for EAS-mediated activity in amphibians (development, histopathology)	Sufficiently investigated according to ED Guidance: No evidence for T-mediated activity observed.	T
9f	EATS-mediated	Hind limb length	*Xenopus laevis*	21	days	Uptake from water	> 90	mg/L	No effect				
9g	EATS-mediated	Thyroid histopathology (amphibian)	*Xenopus laevis*	21	days	Uptake from water	> 90	mg/L	No effect				

2.10.3.1.2 Assessment of the integrated lines of evidence and weight

T-mediated adversity has not been observed in the Amphibian Metamorphosis Assay. A slight increase in amphibian growth was detected which is ranked as "sensitive to, but not diagnostic of EATS" parameters. This effect is not considered to be T-mediated since no effects are observed on developmental stage, normalized hind limb length or thyroid histopathology. Additionally, in ecotoxicological studies with birds and fish, no adverse effects on parameters rated as "sensitive to but not diagnostic of EATS" were found.

T-mediated activity was investigated within an Amphibian Metamorphosis Assay. No effect on relevant parameters rated as "T-mediated" was found.
T-related parameters "EATS-mediated": In amphibians: No effects on relevant parameters, thyroid histopathology, hind limb length or developmental stage were observed.
T-related parameters "sensitive to but not diagnostic of EATS": -In birds: No relevant effects were observed (e.g. body weight, egg production, eggshell thickness, hatchability, egg viability). Isolated effects on body weight can be considered as negligible (for details refer to LoE Table). -In fish: No effects on relevant parameters (e.g. growth, fecundity, behaviour) were observed. -In amphibians: No effects on relevant parameters. A slight increase in amphibian growth is not considered to be EATS-mediated since no effects are observed on developmental stage, normalized hind limb length or thyroid histopathology.
Other modes of action: - No conclusive information available from data on invertebrates from regulatory studies. Moreover the focus of the guidance is on vertebrates *(it should be noted that applicant has summarized the main information in his assessment of ED, except 4 of the 6 Daphnia studies)*
☐ **T-related adversity of glyphosate is not observed.**

2.10.3.1.3 Initial analysis of the evidence and identification of the relevant scenario

Selection of relevant scenario.

Adversity based on T-mediated parameters	**Positive mechanistic OECD CF level 2/3 Test**	**Scenario**	**Next step of the assessment**	**Scenario selected**
No (sufficiently investigated)	Yes/No	1a	Conclude: ED criteria not met because there is not "**T-mediated**" adversity	
Yes (sufficiently investigated)	Yes/No	1b	Perform MoA analysis	
No (not sufficiently investigated)	Yes	2a (i)	Perform MoA analysis (additional information may be needed for the analysis)	
No (not sufficiently investigated)	No (sufficiently investigated)	2a (ii)	Conclude: ED criteria not met because no **T-mediated endocrine activity** observed	X
No (not sufficiently investigated)	No (not sufficiently investigated)	2a (iii)	Generate missing level 2 and 3 information. Alternatively, generate missing "EATS-mediated" parameters. Depending on the outcome move to corresponding scenario	
Yes (not sufficiently investigated)	Yes/No	2b	Perform MoA analysis	

2.10.3.1.4 MoA analysis for T-modalities

Not relevant since not needed for the conclusion on T-modality.

2.10.3.1.5 Conclusion on the ED assessment for T-modality

The ED criteria for this modality is not met because no **T-mediated endocrine activity** was observed for non-target species other than mammals. Considering the conclusion of ED assessment for mammalian species (see ED assessment for humans), the ED criteria for T-modality is also considered not met for mammalian species.

2.10.3.2 ED assessment for EAS-modality

2.10.3.2.1 Lines of evidence for adverse effects and endocrine activity related to EAS-modalities

Avian data (EAS-modality)

Three valid studies are available in the dossier, investigating the effects of glyphosate on avian reproduction. The first two GLP studies were performed according to FIFRA 71-4 and OECD TG 206, and may provide information on parameters sensitive to, but not diagnostic of, endocrine disruption:

CA 8.1.1.3/001: [redacted]1999: Bobwhite quails: No treatment related mortalities, overt signs of toxicity or treatment related effects on body weight or feed consumption were detected at any of the tested doses. In the gross pathological examination at test termination, increased regressing and/or regressed ovaries were observed at 1000 and 2500 ppm (3 out of 14 birds at 1000 ppm, 1 out of 14 birds at 2500 ppm). In male animals, at 1000 ppm elevated numbers of small testes were observed (2 compared to 1 in the control). This effect was not observed in the highest does of 2500 ppm treatment. The findings were not dose related and considered by the authors to be incidental to treatment. Further, no apparent treatment related effects upon reproductive performance were observed up to the highest tested dose of 2250 ppm.

CA 8.1.1.3/004; [redacted].1999 Mallard: In this study, a slight but statistically significant ($p<0.05$) reduction in the mean body weight of 14-day old survivors when compared to the control was observed at the highest treatment rate 2500 ppm. No remarkable effects on ovaries or testes were observed compared to the control in the gross pathological examination at test termination, and no other apparent treatment related effects upon reproductive performance were observed up to the highest tested dose.

In the third study (CA 8.1.1.3/005: [redacted]1978), no apparent treatment related effects upon reproductive performance were observed up to the highest tested dose of 1000 ppm. However, no gross pathological examination was performed in this study and therefore the results are less useful for assessment of endocrine disruption.

Literature data on birds

From the literature search, there were no conclusive results regarding endocrine disruption in birds. However, in one study (Ruuskanen et al., 2020), reduced flight feather moult (females only) and delayed plumage development (regardless of sex and age) were observed among juvenile quail exposed to glyphosate. It is known that feather development is under the control of hormones (such as thyroxine and oestrogen, and indirectly by testosterone, Leeson and Walsh, 2003[42]). However, both endpoints can also be strongly influenced by other factors such as diet and stress. Hence, although the endpoints would be "sensitive to, but not diagnostic of" ED they do not provide any strong information to be used to determine ED in birds.

Overall, from the available standard avian data presented in the dossier for renewal, the potential for endocrine disruption appears to be low.

Fish

Four chronic regulatory studies on fish are available to assess the effects of glyphosate.

An early life stage toxicity test on fish has been performed according to OECD TG 210 and OPPTS 850.12400 ([redacted] 2010, CA 8.2.2.1/001). Eggs of rainbow trout (*Oncorhynchus mykiss*) have been exposed during 85 days to mean measured glyphosate acid concentrations ranging from 0.064 to 9.63 mg a.i./L No statistically

[42] S. Leeson &T. Walsh (2003): Feathering in commercial poultry II. Factors influencing feather growth and feather loss; https://www.tandfonline.com/doi/abs/10.1079/WPS20034

significant effects on the biological endpoints hatching, survival or growth were detected. However, only 2 replicates have been tested while 4 are required in OECD guideline. Thus, given the variability in the egg viability, hatching success and wet and dry weight at the highest concentration of 9.63 mg glyphosate acid/L, RMS considered the data as informative only. None of the relevant parameters possibly indicating endocrine activity (e.g. numbers of eggs, sex ratio or vitellogenin content) were determined in this test.

The second study investigated the toxicity of glyphosate on zebrafish larvae in a 168 hour study according to OECD TG 212 and IBAMA 1990 ([redacted] 2000, CA 8.2.2.1/002, CA 8.2.2.1/001). Fish larvae were exposed to concentrations ranging from 0.32 to 32 mg glyphosate/L (nominal concentrations). At 5.6 mg/L and higher concentrations, effects on mortality were observed. Sublethal or behavioural effects were found at concentrations of 3.2 mg/L and higher. As mortality is of 10% at this concentration, this effect on behaviour can be attributed to systemic effect (secondary effect). The study did not allow to assess endocrine mechanism of action in fish.

A third chronic study has been performed with fish (Anonymous, 1975, CA 8.2.2.2/001). Fathead minnow (*Pimephales promelas*) were exposed during the 255 days to concentrations of glyphosate ranging from 0.7 to 25.7 mg/L. The study have been performed under non-GLP conditions and followed the recommended bioassay procedure for fathead minnow chronic tests issued by the National Water Quality Laboratory, Duluth (US EPA 1971). Observations were made on survival, growth, egg production on the first generation and on hatchability survival and growth of second generation eggs and fry. No significant effects on any of the assessed parameters were reported during the 255 day exposure period. RMS has considered this study as informative as some parameters show high variability between duplicates (e.g. eggs spawned/♀) and results appears sometimes fluctuant even if no obvious trend for effect is observed. The reliability of the statistics is doubtful and potential effects could have been masked. Moreover the analytical method can not be validated. Nevertheless RMS agrees that this study does not show any evidence for effect even at the highest concentration (25.7 mg a.e./L).

Finally, a Fish Short Term Reproduction Assay (OECD TG 229) is available. In this study, groups of fathead minnows (*Pimephales promelas*) were exposed for a period of 21 days to the active substance glyphosate at mean measured concentrations ranging from 0.046 and 33 mg/L ([redacted] 2012, CA 8.2.3/001). The assessed endpoints determining a potential impact of glyphosate on the hypothalamus-pituitary-gonadal (HPG) endocrine axis of fish were fecundity (cumulative egg production and eggs per female reproductive day), fertilization success, secondary sex characteristics (including fatpad and tubercle scores), gonado-somatic index (GSI), vitellogenin (VTG) and gonad histopathology. Further endpoints were survival, body length and wet weight. The vitellogenin levels were reduced but the differences were not statistically significant. None of the reproductive parameters (fecundity, fertilization success, gonadosomatic index, gonad histology) were affected. In case of an endocrine mode of action, it would be expected to detect reproductive effects in this study.

Literature data on fish

For ED assessment RMS considers as relevant only the study performed with the active substance itself in accordance with the EFSA guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009.

The applicant considered only one study as relevant and reliable with restrictions for ED assessment of fish. Le Mer, C. et al. (2013[43]) studied the effects of glyphosate on early life stages of the fish species threespine stickleback (*Gasterosteus aculeatus*). During 42 days, larval sticklebacks (less than 24 h old) were exposed to 4 glyphosate concentrations (0.1, 1, 10 and 100 µg glyphosate/L) together with a seawater control, a carrier (acetone) control and positive controls for estrogenic (0.05 µg/L ethinylestradiol, EE2) and androgenic (3 µg/l dihydrotestosterone, DHT) effects. The survivors were measured (length, wet weight) and then conserved for biochemical (VTG, and the male nestprotein spiggin, SPG) and histological (phenotypic sex determination) analyses. No significant effects on larval survival or growth were detected. Exposure to 3 µg DHT/L resulted in a significant effect on growth (body lengths) but did not induce SPG, possibly because of DHT degradation after the 24 hour solution renewal. The low concentration of DHT is known to be at the threshold for effects and should not invalidate the SPG endpoint. VTG was induced after EE2 exposure; however, glyphosate did not induce production of VTG and SPG. The proportion of mixed sex individuals was higher in the positive controls compared to the negative controls. No mixed sex individuals were found in the glyphosate treatments.
Based on this study, it can be concluded that glyphosate does not show estrogenic or androgenic effects to early life stages of sticklebacks at environmentally realistic concentrations (0.1, 1, 10 and 100 µg/L). No induced production

[43] Le Mer C. et al. (2012). Effects of chronic exposures to the herbicides atrazine and glyphosate to larvae of the threespine stickleback (Gasterosteus aculeatus). Ecotoxicology and environmental safety (2013), Vol. 89, pp. 174

of vitellogenin or a change in sex ratio in early life stages of sticklebacks was observed. RMS however notes that seawater was used in the test (this fish species can be found in both freshwater and sea), interactions between the ions and the active substance cannot be discounted. RMS considers the study relevant and reliablefor seawater fish.

Two other studies were considered by RMS as relevant and reliable with restrictions:

In Zhang S. et al. (2017)[44], the authors studied the effects of glyphosate on early development of larval zebrafish were investigated via morphological, biomechanics, behavioral and physiological analyses. The main results are:

- NOEC for morphological alterations =10 mg/L (epiboly process and body length, eye and head area)
- NOEC Surface tension of chorion < 1 mg/L (not concentration dependant), the study author claims that it is not significant at concentrations below 1 mg/L but the data are not shown in this study
- NOEC hatching rate = 200 mg/L (increase with concentration)
- NOEC larvae abnormality = 10 mg/L

Gene expression (these genes were not related to endocrine activity) and locomotor activity was not considered relatable to the risk assessment and were not considered by RMS. It is hypothetized that the decreased surface tension of chorion and the increased locomotive activities may contribute to the hatching rates after glyphosate treatment. No standardized guideline was used, RMS cannot state on the reliability of the results and conclusion. This study was considered relevant and reliable with restrictions.

Rodrigues et al. (2019)[45] investigated the impact of the glyphosate on non-target aquatic organisms. The authors assessed the acute toxicity and genotoxicity of glyphosate on fish. The toxic effects were evaluated in the fish embryo acute toxicity test with zebrafish (*Danio rerio*), while genotoxic effects were investigated in the comet assays with cells from zebrafish larvae and rainbow trout gonad-2 (RTG-2). Glyphosate caused no acute toxic effect (LC_{50}-96 h > 100 mg/L) in zebrafish. Glyphosate induced some morphological abnormalities (from 10 mg/L to 100 mg/L), including pericardial and yolk sac edemas, spinal curvature, head and tail deformities in different exposure times; however, these malformations were not statistically significant when compared to their respective negative control. Potential effects on hatching were not investigated. The sensitivity of the fish strain cannot be verified as no data with reference toxic 3,4-dichloroaniline was reported. No analytical verification of test concentrations were reported, RMS considers this study as reliable with restrictions.

Other literature studies on fish performed with the active substance are available:

- Uren Webster T. M. et al. (2014). Effects of glyphosate and its formulation, Roundup, on reproduction in zebrafish (Danio rerio). Environmental science & technology (2014), Vol. 48, No. 2, pp. 1271 – 1279
- Xia S. et al. (2013). Induction of vitellogenin gene expression in medaka exposed to glyphosate and potential molecular mechanism. Zhongguo Huanjing Kexue (2013), Vol. 33, No. 9, pp. 1656-1663
- Smith C. M. et al. (2019). Developmental and epigenetic effects of Roundup and glyphosate exposure on Japanese medaka (Oryzias latipes). Aquatic toxicology (2019), Vol. 210, pp. 215-226

These studies are considered relevant for ED assessment. However these studies are not reliable. The details of assessment of this studies can be found in the addendum to Volume 3 (AS) B.9 related to literature review on ecotoxicology of glyphosate.

In the literature review on ED, the study of Xie L. et al. (2005)[46] was cited. This study was part of the previous ED assessment (addendum to RAR of 2017). In this study, estrogenic potencies of four herbicides (triclopyr, 2,4-dichlorophenoxyacetic acid (2,4-D), diquat dibromide, glyphosate), two alkylphenol ethoxylate-containing surfactants (R-11 and Target Prospreader Activator (TPA)), and the binary mixture of surfactants with the herbicides were evaluated using an in vivo rainbow trout vitellogenin assay. The conclusion was that glyphosate had no effect on vitellogenin-production in this in vivo assay in rainbow trouts.

The studies of Zhang S. et al. (2017), Rodrigues et al. (2019) and Xie L. et al. (2005) should be added in the Excel file (**datagap** for the applicant to provide an updated Excel File including all relevant literature data following assessment of RMS).

RMS did not find in the applicant's submission some of the articles listed by the applicant as relevant and reliable

[44] Zhang S. et al. (2017). Biological impacts of glyphosate on morphology, embryo biomechanics and larval behavior in zebrafish (Danio rerio). Chemosphere (2017), Vol. 181, pp. 270-280

[45] Rodrigues, L.B. et al. (2019) Impact of the glyphosate-based commercial herbicide, its components and its metabolite AMPA on non-target aquatic organisms (referenced CA 8.2.2.1/005, cited as de Brito Rodrigues L. et al., 2019 in literature review of ED, CA 5.8.3/016)

[46] Xie L. et al. (2005). Evaluation of Estrogenic Activities of Aquatic Herbicides and Surfactants Using an Rainbow Trout Vitellogenin Assay. Toxicol. Sci. (2005) 87:91-8.

(or supplementary) after full text analysis in the literature review of ED (CA 5.8.3/016). Thus a **datagap** is set for the applicant to provide the following studies:

- Maskey E. et al. (2019). Disruption of oocyte maturation by selected environmental chemicals in zebrafish. Toxicology in vitro: an international journal published in association with BIBRA, (2019) Vol. 54, pp. 123-129
- Quassinti L. et al. (2015). Toxicity of Cupside 480SL® Spray Mixture Formulation Of Glyphosate To Aquatic Organisms. Pesticide Biochemistry and Physiology, Vol. 93, pp. 91-95

Invertebrates

The applicant made reference to two studies for reproduction of *Daphnia magna* conducted under OECD 202: [redacted] S. (1993, CA 8.2.5.1/003) and [redacted] (1990, CA 8.2.5.1/004). Three tests on chronic effects of glyphosate to soil macroorganisms were also reported: one with *Eisenia fetida* ([redacted] 2009, CA 8.4.1/001), one with *Hypoaspis aculeifer* ([redacted] 2009, CA 8.4.2.1/002) and one with *Folsomia candida* ([redacted] 2010, CA 8.4.2.1/001).

It should be noted that four other reproductive studies with Daphnia are available in Volume 3 CA B.9: [redacted] (1999, CA 8.2.5.1/001), [redacted] (1995, CA 8.2.5.1/002), [redacted] (1989, CA 8.2.5.1/005) and [redacted] (1982, CA 8.2.5.1/006). [redacted] (1989, CA 8.2.5.1/005), was the only study where time to first brood is reported (and found not affected).

Given that the the focus of the EFSA/ECHA guidance is "on vertebrate organisms, for which the current understanding of the endocrine system and availability of test methods is most advanced, i.e. mammals, fish, and amphibians." The guidance indicated that "further research is recommended for a better understanding of the endocrinology of invertebrates in the light of developing test guidelines for the identification of ED, including also mechanistic parameters."
Thus, in view of the assessment of the current regulatory studies focusing on systemic toxicity and given the focus of the EFSA.ECHA guidance on vertebrates, RMS considered that there is no need to include invertebrates regulatory studies in ED assessment of glyphosate.

RMS therefore only reported the study on Chironomus in which sex ratio were noted in view of the estimation of emergence and development rate. In this sediment-water toxicity test using spiked water with *Chironomus riparius* was performed according to OECD 219 ([redacted] 2020, CA 8.2.5.3/001). In this test larvae were exposed during 28 days to concentrations of 100 and 1000 mg glyphosate /L. No effects on chironomid sex ratio, emergence ratio and development rate were observed after 28 days at concentrations up to 1000 mg/L. The study has been considered as supportive in the absence of measured concentration in the sediment to ensure that the mass balance is acceptable.

Some literature studies indicating information potentially relevant for ED assessment are available in the literature review. The relevance and the reliability of these studies for the ED assessment have been further considered in accordance with the guidance recommendation "if available, information on invertebrate non-target organisms (e.g. endocrine mechanistic and/or adverse effect data) should be considered in the assessment applying the general principles of this guidance."

Literature data on invertebrates

Literature studies on invertebrates are available:

- Omran N. E. et al. (2016). The endocrine disruptor effect of the herbicides atrazine and glyphosate on *Biomphalaria alexandrina* snails. Toxicology and industrial health (2016), Vol. 32, No. 4, pp. 656-665
- Reddy S. B. et al. (2018). Disturbances in reproduction and expression of steroidogenic enzymes in aquatic invertebrates exposed to components of the herbicide Roundup. Toxicology Research and Application (2018), Vol. 2, pp. 239784731880527 6/1
- Canosa I. S. et al. (2018). Ovarian growth impairment after chronic exposure to Roundup Ultramax® in the estuarine crab *Neohelice granulata*. Environmental science and pollution research international (2018), Vol. 25, No. 2, pp. 1568-1575
- Canosa I. S. et al. (2019). Imbalances in the male reproductive function of the estuarine crab *Neohelice granulata*, caused by glyphosate. Ecotoxicology and environmental safety (2019), Vol. 182, pp. 109405
- Druart C. et al. (2017). A full life-cycle bioassay with *Cantareus aspersus* shows reproductive effects of a glyphosate-based herbicide suggesting potential endocrine disruption. Environmental pollution (2017), Vol. 226, pp. 240-249
- Avigliano L. et al. (2014). Effects of glyphosate on egg incubation, larvae hatching, and ovarian rematuration in the estuarine crab *Neohelice granulata.* Environmental Toxicology and Chemistry (2014),

Vol. 33, no. 8, pp. 1879

- Avigliano L. et al. (2018). Effects of Glyphosate on Somatic and Ovarian Growth in the Estuarine Crab *Neohelice granulata*, During the Pre-Reproductive Period. Water, air, and soil pollution (2018), Vol. 229, No. 2, pp. 44

The studies of Omran (2016), Canosa (2018) and Druart (2017) were performed with formulations. RMS considers these studies as not relevant for identification of endocrine disruptors in the sense of the ECHA/EFSA guidance since results are not based on the active ingredient itself.

Regarding the other studies, they are relevant for ED assessment but considered by RMS as not reliable. Please refer to Table B.9.11.1-2 in Volume 3 CA B.9.

The tables below presented the lines of evidence based on applicant's proposal updated by RMS to reflect RMS's conclusion for each study. As a consequence, studies judged invalid by RMS are not reported.

Table 2.10.3.2.1-1 Lines of evidence for adverse effects

Please refer to Table 2.10.3.1.1-1

Table 2.10.3.2.1-2 Lines of evidence for endocrine activity related to EAS-modality

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
8h	In vivo mechanistic	Vitellogenin (VTG) in females	*Pimephales promelas*	21	days	Uptake from water	> 33	mg/L	No effect	No effect on VTG content in males and females in a concentration up to the highest test concentration (33 and 100 mg/L) one GLP study and one published study. **RMS:** Agreed. Study ID15 : seawater used in the test (this fish species can be found in both freshwater and sea), interactions between the ions and the active substance cannot be discounted	Sufficiently investigated according to ED Guidance: No evidence for effects on VTG content in fish.	Sufficiently investigated according to ED Guidance: No evidence for EAS-mediated activity observed.	E, A, S
8i	In vivo mechanistic	Vitellogenin (VTG) in males	*Pimephales promelas*	21	days	Uptake from water	> 33	mg/L	No effect				
15c	In vivo mechanistic	VTG induction in fish	*Gasterosteus aculeatus*	42	days	Uptake from water	> 100	mg/L	No effect				E, A, S

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
15e	In vivo mechanistic	spiggin	*Gasterosteus aculeatus*	42	days	Uptake from water	> 100	mg/L	No effect	RMS: No effect on SPG content up to the highest test concentration (100 mg/L)	No evidence for effects on spiggin content in fish		A
8e	EATS-mediated	Male 2nd sex characteristics in females	*Pimephales promelas*	21	days	Uptake from water	> 33	mg/L	No effect	No histopathological effects or effects on male 2nd sex characteristics in female and male fish in a concentration range of 0.046 - 33 mg/L.	Sufficiently investigated according to ED Guidance: No evidence for EAS-mediated activity in fish (histopathology, 2nd sex characteristics).		A
8f	EATS-mediated	Male 2nd sex characteristics in males	*Pimephales promelas*	21	days	Uptake from water	> 33	mg/L	No effect				E, A, S
8j	EATS-mediated	Specific gonad histopathology	*Pimephales promelas*	21	days	Uptake from water	> 33	mg/L	No effect				
15d	EATS-mediated	Sex ratio	*Gasterosteus aculeatus*	42	days	Uptake from water	> 100	mg/L	No effect	No effects on fish sex ratio			

2.10.3.2.2 Assessment of the integrated lines of evidence and weight

No EAS-mediated adversity has been observed in the ecotoxicological studies (regulatory and literature data) conducted with glyphosate in birds, fish and amphibians.

EAS-mediated adversity has not been observed in the Fish Short Term Reproduction Assay (FSTRA; OECD TG 229). The conclusion of RMS remains the same as for the previous assessment of ED (2017): *reduced vitellogenin levels were observed. These differences were not statistically significant. None of the reproductive parameters (fecundity, fertilization success, gonadosomatic index, gonad histology) were affected. In case of an endocrine mode of action, it would be expected to detect reproductive effects in this study*. In ecotoxicological studies, no adverse effects on parameters rated as "sensitive to but not diagnostic of EATS" were found.

EAS-mediated activity was investigated within an Fish Short Term Reproduction Assay. No effect on relevant parameters rated as "EAS-mediated" was found.
EAS-related parameters "EATS-mediated": In fish: No effects on relevant parameters (e.g. secondary sex characteristics, sex ratio) were observed.
EAS-related parameters "sensitive to but not diagnostic of EATS": -In birds: No relevant effects were observed (e.g. body weight, egg production, eggshell thickness, hatchability, egg viability). Isolated effects on body weight can be considered as negligible (for details refer to Table 2.10.3.1.1-1). -In fish: No effects on relevant parameters (e.g. growth, fecundity, behaviour) were observed at doses below MTC. -In amphibians: No effects on relevant parameters. A slight increase in amphibian growth is not considered to be EATS-mediated since no effects are observed on developmental stage, normalized hind limb length or thyroid histopathology.
Other modes of action: -No conclusive information available from data on invertebrates from regulatory studies. Moreover the focus of the guidance is on vertebrates *(it should be noted that applicant has summarized the main information in his assessment of ED, except 4 of the 6 Daphnia studies)*
☐ **EAS-related adversity of glyphosate is not observed.**

Regarding EAS-mediated adversity, only secondary effects linked to systemic toxicity of glyphosate are observed. The observed effects are classified as "sensitive to, but not diagnostic of EATS" modalities and "systemic toxicity".

A Fish Short-Term Reproduction Assay was provided to investigate theEAS-mediated activity. No indication for EAS-related endocrine activity was observed.

The following evidences related to investigation of EAS-mediated endocrine activity of glyphosate <u>in fish studies</u> are made by the applicant and agreed by RMS:

- E-modality:
  - Parameter "in vivo mechanistic": No VTG induction in males or females.
  - Parameter "EAS-mediated": No depression of male secondary sex characteristics, no histopathological effects, no fecundity depression.
- A-modality:
  - Parameter "in vivo mechanistic": No VTG depression in females.
  - Parameter "EAS-mediated": No induction of male secondary sex characteristics, no histopathological effects, no fecundity depression.
- S-modality:
  - Parameter "in vivo mechanistic": No VTG depression in females.
  - Parameter "EAS-mediated": No histopathological effects, no fecundity depression.

2.10.3.2.3 Initial analysis of the evidence and identification of the relevant scenario

Studies are available with birds, amphibians and fish (applicant cited also invertebrate species but information was not retained by RMS except Chironomus) to assess EAS related effects on non-target organisms. Based on these studies it can be concluded that no adversity and endocrine activities were observed in these studies. The fish life cycle study with fathead minnow *Pimephales promelas* (CA 8.2.2.2/001, Anonymous, 1975) investigated almost all parameters Medaka Extended One-Generation Test (MEOGRT, OECD TG 240). However some uncertainties are related to the observed effects and therefore the reliability is not satisfactory. Following the ECHA/EFSA ED Guidance, EAS-mediated adversity is therefore not sufficiently investigated.
Consequently, there is a need to investigate endocrine activity. A Fish Short-Term Reproduction Assay (OECD TG 229) has been performed for this purpose. The results of the test indicated that there is no indication of EAS activity.

To conclude, according to the ECHA/EFSA ED Guidance (section 3.4.2), the data set is considered sufficient (scenario 2a (ii)). Thus **the substance does not meet the ED criteria with regard to E, A and S modalities** (see table below).

As no EAS-mediated activity was observed, it is possible to conclude that glyphosate does not meet the ED criteria for EAS-modality for non-target organisms other than mammals.

Selection of relevant scenario

Adversity based on EAS-mediated parameters	**Positive mechanistic OECD CF level 2/3 Test**	**Scenario**	**Next step of the assessment**	**Scenario selected**
No (sufficiently investigated)	Yes/No	1a	Conclude: ED criteria not met because there is not "**EAS-mediated**" adversity	
Yes (sufficiently investigated)	Yes/No	1b	Perform MoA analysis	
No (not sufficiently investigated)	Yes	2a (i)	Perform MoA analysis (additional information may be needed for the analysis)	
No (not sufficiently investigated)	No (sufficiently investigated)	2a (ii)	Conclude: ED criteria not met because no **EAS-mediated endocrine activity** observed	**X**
No (not sufficiently investigated)	No (not sufficiently investigated)	2a (iii)	Generate missing level 2 and 3 information. Alternatively, generate missing "EATS-mediated" parameters. Depending on the outcome move to corresponding scenario	
Yes (not sufficiently investigated)	Yes/No	2b	Perform MoA analysis	

2.10.3.2.4 MoA analysis for EAS-modalities

Scenario 2(ii) being established for glyphosate, the mode of action analysis is not necessary for EAS-modalities.

2.10.3.2.5 Conclusion on the ED assessment for EAS-modality

The EAS-modalities criteria are not met. Therefore for non-target organisms other than mammals, glyphosate is not considered ED regarding EAS-modalities.
Considering the conclusion of ED assessment for mammalian species (see ED assessment for humans), the ED criteria for EAS-modality is also considered not met for mammalian species.

2.10.4 Overall conclusion on the ED assessment

Conclusion for humans

It is agreed with overall conclusion of the applicant regarding human health. Based on the available data on glyphosate, the ED criteria are not met.

Conclusion for mammalian species as non-target organisms

Considering the conclusion of ED assessment for mammalian species (see ED assessment for humans), the ED criteria for EATS-modality is considered not met for mammalian species as non-target organisms.

Conclusion for non-target organisms other than mammals

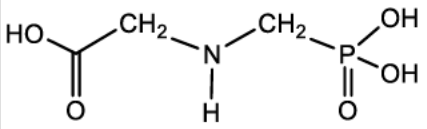
None of the ecotoxicological studies conducted with the active substance glyphosate was found to show EATS-mediated adversity to in birds, fish and amphibians. For EAS-mediated adversity, the effects are classified as "sensitive to, but not diagnostic of EATS" modalities and "systemic toxicity". Secondary effects were considered as a consequence of systemic toxicity.
Two studies are available that investigate EATS-related endocrine activity: an Amphibian Metamorphosis Assay for the T-modality and a Fish Short-Term Reproduction Assay for EAS-modality. Relevant parameters for T-modality and EAS-modality have been sufficiently investigated. There is no indication of EATS related endocrine activity.
Therefore, it is concluded that ED criteria with regards to non-target organisms are not met for glyphosate.

2.11 PROPOSED HARMONISED CLASSIFICATION AND LABELLING ACCORDING TO THE CLP CRITERIA *[SECTIONS 1-6 OF THE CLH REPORT]*

2.11.1 Identity of the substance [section 1 of the CLH report]

2.11.1.1 Name and other identifiers of the substance

Table 74: Substance identity and information related to molecular and structural formula of the substance

Name(s) in the IUPAC nomenclature or other international chemical name(s)	N-(phosphonomethyl)glycine
Other names (usual name, trade name, abbreviation)	Glyphosate
ISO common name (if available and appropriate)	Glyphosate
EC number (if available and appropriate)	213-997-4
EC name (if available and appropriate)	-
CAS number (if available)	1071-83-6
Other identity code (if available)	MON 77973
Molecular formula	$C_3H_8NO_5P$
Structural formula	
SMILES notation (if available)	-
Molecular weight or molecular weight range	169.1 g/mol
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	Not applicable
Description of the manufacturing process and identity of the source (for UVCB substances only)	Confidential information, please refer to Vol. 4
Degree of purity (%) (if relevant for the entry in Annex VI)	≥ 950 g/kg

2.11.1.2 Composition of the substance

Table 75: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	**Concentration range (% w/w minimum and maximum in multi-constituent substances)**	**Current CLH in Annex VI Table 3.1 (CLP)**	**Current self-classification and labelling (CLP)**
glyphosate	≥ 950 g/kg	H318 and H411	See ECHA C&L Inventory[1]

[1] https://echa.europa.eu/information-on-chemicals/cl-inventory-database

Table 76: Impurities (non-confidential information) if relevant for the classification of the substance

Impurity (Name and numerical identifier)	**Concentration range (% w/w minimum and maximum)**	**Current CLH in Annex VI Table 3.1 (CLP)**	**Current self-classification and labelling (CLP)**	**The impurity contributes to the classification and labelling**
IUPAC/CA/ISO common name: Formaldehyde CAS No: 50-00-0 EC No: 200-001-8	< 1.0 g/kg	Acute Tox. 3 *; H301 Acute Tox. 3 *; H311 Acute Tox. 3 *, H331 Skin Corr. 1B; H314 Skin Sens. 1; H317 Muta. 2; H341 Carc. 1B; H350 GHS08 GHS05 GHS06 Dgr	See ECHA C&L Inventory[1]	No Cut-off levels for classification are not exceeded with the proposed specification at < 1 g/kg
IUPAC name: N-nitroso-N-(phosphonomethyl)-glycine CA name: 2-[nitroso(phosphonomethyl)amino]-acetic acid ISO: not available CAS No:56516-72-4 EC No: not available	< 1 mg/kg	No current harmonized classification Known to be of concern because they can be activated to genotoxic carcinogens	No data available in ECHA's C&L Inventory	No According to SANCO/10597/2003 no additional concern when < 1 mg/kg.
IUPAC /CA/ISO common name: Triethylamine CAS No: 121-44-8 EC No: 204-469-4	Max. 2 g/kg	Acute tox. 4* ; H302 Acute Tox. 4*; H312 Acute Tox. 4*; H332 Skin Corr. 1A; H314 STOT SE 3; H335: C ≥ 1%	See ECHA C&L Inventory[1]	No Cut-off levels for classification or specific concentration limits set for triethylamine are not exceeded with the proposed specification at max. 2 g/kg
IUPAC /CA/ISO common name: Formic acid CAS No: 64-18-6 EC No: 200-579-1	Max. 4 g/kg	Skin Corr. 1A; H314	See ECHA C&L Inventory[1]	No Specific concentration limits for classification are indicated for formic acid, which are not exceeded at the proposed specification level of max. 4 g/kg.

[1] https://echa.europa.eu/information-on-chemicals/cl-inventory-database

Table 77: Additives (non-confidential information) if relevant for the classification of the substance

Additive (Name and numerical identifier)	**Function**	**Concentration range (% w/w minimum and maximum)**	**Current CLH in Annex VI Table 3.1 (CLP)**	**Current self-classification and labelling (CLP)**	**The additive contributes to the classification and labelling**
No additives	-	-	-	-	-

Table 78: Test substances (non-confidential information)

Identification of test substance	Purity	Impurities and additives (identity, %, classification if available)	Other information	The study(ies) in which the test substance is used

Glyphosate acid Technical Batch: P24	95.6% w/w a.i	Confidential		5.2.1/013 Glyphosate Acid: Acute Oral Toxicity Study In Rats [redacted]/P/4660 5.2.2/011 Glyphosate Acid: Acute dermal toxicity study in the rat [redacted]/P/4664 5.2.4/010 Glyphosate Acid: Skin Irritation to the Rabbit [redacted]/P/4695 5.2.5/011 Glyphosate Acid: Eye Irritation to the Rabbit [redacted]/P/5138 5.2.6/012 Glyphosate Acid: Skin Sensitisation To The Guinea Pig [redacted]/P/4699 5.3.2/033 Glyphosate Acid: 1 Year Dietary Toxicity Study in Dogs [redacted]/P/5079 5.3.2/034 Glyphosate Acid: 1 Year Dietary Toxicity Study in Dogs, Appendix [redacted]/P/5079 KCA 5.3.3/001 and KCA 5.3.3/002 Glyphosate Acid: 21 Day Dermal Toxicity Study in Rats [redacted]/P/4985 5.4.1/013 Glyphosate Acid: An Evaluation of Mutagenic Potential Using S. Typhimurium and E. Coli. CTL/P/4874 5.4.1/025 Glyphosate Acid: In Vitro Cytogenetic Assay In Human Lymphocytes CTL/P/6050 5.4.1/030 Glyphosate Acid: L5178Y TK+/- Mouse Lymphoma Gene Mutation Assay CTL/P/4991 5.4.2/009 Glyphosate Acid: Mouse Bone Marrow Micronucleus Test [redacted]/P/4954 5.5/006 Glyphosate Acid: One Year Dietary Toxicity Study in Rats [redacted]/P/5143 5.6.2/001 Glyphosate Acid: Developmental Toxicity Study in the Rat [redacted]/P/4819 5.6.2/009 Glyphosate acid: Developmental toxicity study in the rabbit [redacted]/P/5009 5.7.1/001 Glyphosate acid: Acute neurotoxicity study in rats [redacted]/P/4866 5.7.1/003 Glyphosate Acid: Subchronic Neurotoxicity Study In Rats [redacted]/P/4867

				5.7.2/001 Glyphosate acid: Acute delayed neurotoxicity study in domestic hen [redacted]/C/3122 5.8.2/003 Glyphosate Acid: Comparison of Salivary Gland Effects in Three Strains of Rat [redacted]/P/5160
HR-001 Batch: 940908-1	95.68 % w/w a.i	Confidential		5.2.1/015 HR-001: Acute Oral Toxicity Study In Rats [redacted] 94-0134 5.2.1/016 HR-001: Acute Oral Toxicity Study In Mice [redacted] 94-0133 5.2.2/012 HR-001: Acute dermal toxicity study in rats 94-0154 5.3.2/004 HR-001: 13-week Subchronic Oral Toxicity Study in Rats [redacted] 94-0138 5.4.1/015 HR-001: Reverse Mutation Test IET 94-0142 5.4.1/027 HR-001: In vitro cytogenicity test IET 94-0143 5.4.1/035 HR-001: DNA Repair Test (Rec-Assay) IET 94-0141 5.6.2/002 HR-001: Developmental toxicity study in rat [redacted] 94-0152

Glyphosate Technical Batch: 206-Jak-25-1	98.6%	Confidential	Supplementary data	5.1.1/011 [14C]-Glyphosate: Absorption and distribution in the rat – preliminary study 6365-676/1 5.1.1/012 [14C]-Glyphosate: Absorption, distribution, metabolism and excretion in the rat 7006-676/2 5.2.1/025 Assessment of acute oral toxicity of "Glyphosate technical" to mice 12321 5.2.1/029 Glyphosate Technical: Acute oral toxicity (limit) test in rats 5883 5.2.2/021 Glyphosate Technical: Acute dermal toxicity (limit) test in rats 5884 5.2.4/018 Glyphosate Technical: Primary Skin Irritation in Rabbits 5885 5.2.5/020 Glyphosate technical: Primary eye irritation test in rabbits 5886 5.2.6/020 Glyphosate Tech.: Magnusson & Kligman Maximisation Study in the Guinea Pig 5887 5.3.2/011 Glyphosate – 13 week dietary toxicity study in rats 7136 5.3.2/035 Glyphosate: 52 Week Oral Toxicity Study in Dogs 7502 5.4.1/020 Mutagenicity test: Ames Salmonella Assay with Glyphosate 12323 5.4.1/031 Mutagenicity test: In vitro Mammalian Cell Gene Mutation Test with Glyphosate 12325 5.4.2/012 Mutagenicity test: Micronucleus test with Glyphosate 12324 5.5/020 and 5.5/021 Glyphosate – 104 week dietary carcinogenicity study in mice 7793 5.6.1/009 Two-generation reproduction study in rat (dose-range-finding) [redacted] 42/90619 5.6.2/003 Developmental toxicity study in rat [redacted] 43 & 41/90716 5.6.2/014 Developmental toxicity study in rabbit [redacted] 45, 39 40 /901303

Glyphosate Technical Batch: 1071-83-6	96.8%	Confidential	Supportive data	5.4.3/001-3 Dominant lethal test in Wistar rats. TOXI: 888-DLT 5.6.2/004 Developmental toxicity study in rat [redacted].883.TER-R
Glyphosate Technical Batch: XHJ-64	98.7%	Confidential	Supportive data	5.3.2/019 A Three Month Feeding Study of Glyphosate (Roundup® Technical) in Mice 77-2111 5.4.1/032 CHO/HGPRT Gene Mutation Assay with Glyphosate ML-83-155 5.4.3/005 Dominant Lethal Study in Mice 401-064 5.6.1/014 A three generation reproduction study in rats with glyphosate 77-2063 5.6.2/008 Developmental toxicity study in rat 401-054 5.6.2/018/019 Developmental toxicity study in rabbit 401-055/401-056
Glyphosate Technical H95D161A	95.3%	Confidential		5.1.1/002 [14C]-Glyphosate: Absorption, distribution, metabolism and excretion following oral administration to the rat 1413/2-1011 5.4.1/014 Technical glyphosate: Reverse mutation assay "Ames test" using Salmonella typhimurium and Escherichia coli 434/014 5.4.1/026 Technical glyphosate: Chromosome aberration test in CHL cells in vitro 434/015 5.6.2/010 Developmental toxicity study in rabbit 434/020 5.8.2/004 Glyphosate Technical: Pharmacology Screening Study in the Rat 434/021

Glyphosate Technical Batch: T-941209	97.56%	Confidential		5.2.3/013 HR-001: Acute inhalation toxicity study in rats [redacted] 94-0155 5.2.4/011 HR-001: Primary Dermal Irritation Study in Rabbits [redacted] 95-0035 5.2.5/013 HR-001: Primary Eye irritation study in rabbits [redacted] 95-0034 5.2.6/013 HR-001: Dermal sensitisation study in Guinea pigs [redacted] 95-0036 5.3.2/017 HR-001: 13-week Subchronic Oral Toxicity Study in Mice [redacted] 94-0136 5.5/018 and 5.5/019 HR-001: 18-Month Oral Oncogenicity Study in Mice [redacted] 94-0151 5.5/004 HR-001: 24-Month Oral Chronic Toxicity and Oncogenicity Study in Rats [redacted] 94-0150 5.6.2/011 Developmental toxicity study in rabbit [redacted] 94-0153

Glyphosate Technical Batch: 60	96.8%	Confidential	 Supplementary data Supplementary data	5.2.1/026 Acute oral toxicity study with glyphosate technical (FSG 0309 H/05 March 90) in Wistar rats [redacted].874.AOR 5.2.1/027 Acute oral toxicity study with glyphosate technical in swiss albino mice [redacted].875.AOM 5.2.2/019 Acute dermal toxicity study with glyphosate technical (FSG 03090 H/05 March 90) in Wistar rats[redacted].876.ADR 5.2.4/015 Primary Skin Irritation Study with Glyphosate Technical (FSG 03090 H/05 March 90) in New Zealand White Rabbits [redacted].878.SKIN 5.3.1/001 28-Day Dietary Study in Wistar Rats. Test Compound Technical Glyphosate (FSG 03090 H/05 March 1990) [redacted].881.28 DDR 5.3.1/002 Amendment to Final Report. 28-Day Dietary Study in Wistar Rats. Test Compound Technical Glyphosate [redacted].881.28 DDR 5.3.1/003 28-Day Dietary Study in Wistar Rats. Test Compound Technical Glyphosate (FSG 03090 H/05 March 1990) [redacted].881.28 DDR 5.5/005 Combined Chronic Toxicity and Carcinogenicity Study with Glyphosate Technical in Wistar Rats 886.C.C-R 5.6.1/006 Two Generation Reproduction Study in Rats TOXI 885-RP-G2 5.6.2/004 and 5.6.2/005 Teratogenicity study in Wistar rats [redacted].883.TER-R 5.6.2/012 Developmental toxicity study in rabbit TOXI: 884-TER-RB

Glyphosate technical Lot/Batch#: H05H016A	95.7% (w/w)	Confidential		5.2.6/010 Glyphosate Technical: Skin Sensitisation in the Guinea Pig – Magnusson and Kligman Maximisation method SMK-PH-05/0218 5.3.2/020 Glyphosate Technical: 13-Week Toxicity Study by Oral Route (Capsule) in Beagle Dogs 29646 [redacted] 5.3.2/031 Glyphosate technical: 52-week Toxicity Study by Oral Route (Capsule) in Beagle Dogs 29647 [redacted] 5.4.2/007 Glyphosate Technical: Micronucleus Test In The Mouse 2060/014 5.5/001 Glyphosate Technical: Dietary Combined Chronic Toxicity/Carcinogenicity in the Rat 2060-0012 5.5/012-015 Glyphosate technical: Dietary Carcinogenicity Study in the Mouse 2060-0011 5.6.1/001-003 Two Generation Reproduction Study in the Rat 2060/0013 5.7.1/002 Ninety Day Repeated Dose Oral (Dietary) Neurotoxicity Study in the Rat 2060-0010
Glyphosate acid Lot/Batch#: Y04707/082	97.6% (w/w)	Confidential		5.6.1/004 Multigeneration reproduction toxicity study in rats [redacted]/P/6332
Glyphosate technical Lot No. T-950308	94.61 % (w/w)	Confidential		5.3.2/032 HR-001: 12-Month Oral Chronic Toxicity Study in Dogs [redacted] 94-0157 5.6.1/005 HR-001: A two-generation reproduction study in rats [redacted] 96-0031
Glyphosate technical Batch No.: 206-JaK-119-1	99.2 % (w/w)	Confidential		5.6.1/007-008 Two Generation Reproduction Study in Rats [redacted] 47/911129
Glyphosate Lot No.: XLI-203	97.67 % (w/w)	Confidential		5.3.1/005 Range-finding Study of Glyphosate Administered in Feed to Sprague-Dawley Rats [redacted]-8921 5.6.1/010 Two Generation Reproduction Study in the Rat [redacted]-10387

Glyphosate (powder) Batch: not stated	Not stated	Confidential		Mechanistic study CA 5.8.2 Toxicology, (2020) Vol. 439, Art. No. 152466
Glyphosate technical Batch 11493988	97.7 % (w/w)	Confidential		5.1.1/001 A GLP 14-Day Oral (Dietary) Toxicokinetic Study of Glyphosate in Sprague Dawley Rats 00050502
Glyphosate Lot/Batch#: Y04707/045	99.2 % (w/w)	Confidential		5.1.1/003 Glyphosate acid: Excretion and tissue retention of a single oral dose (10 mg/kg) in the rat [redacted]/P/4940 5.1.1/005 Glyphosate acid: Excretion and Tissue Retention of a Single Oral Dose (10 mg/kg) in the Rat Following Repeat Dosing [redacted]/P/4944 5.1.1/006 Glyphosate acid: Whole body autoradiography in the rat (10 mg/kg) [redacted]/P/4943
Glyphosate Lot/Batch#: Y04707/048	99.5 % (w/w)	Confidential		5.1.1/004 Glyphosate acid: Excretion and tissue retention of a single oral dose (1000 mg/kg) in the rat [redacted]/P/4942 5.1.1/007 Glyphosate acid: Biotransformation in the rat [redacted]/P/5058
Glyphosate Part 1: UN-NO: 1759 Part 2: 32140	98 % (w/w)	Confidential		5.1.1/008-009 Part 1: Metabolism Study of 14C-labelled glyphosate after single oral and intravenous administration to Sprague-Dawley Rats Part 2: Glyphosate - ADME-Study in Rats Part 1: 9202/95 Part 2: [redacted] 038/94
Glyphosate Lot/Batch#: 061221	98.9 % (w/w)	Confidential		5.1.1/010 HR-001: Metabolism in the rat [redacted]332/951256
Glyphosate Lot/Batch#: 04062014	85.79 % (w/w)	Confidential		5.4.1/001 Glyphosate: Reverse Mutation Assay ‘Ames Test’ using Salmonella typhimurium and Escherichia coli. 41401854

Glyphosate Lot/Batch#: 569753 (BX20070911)	96.3 % (w/w)	Confidential		5.2.1/002 Glyphosate technical: Acute oral toxicity study in the rat (up and down procedure) 10/218-001P 5.2.2/001 Glyphosate technical: Acute dermal toxicity study in rats; Final report amendment 1 10/218-002P 5.2.3/014 Glyphosate: Acute inhalation toxicity study four-hour (nose only) in the rat 710/16 5.2.4/001 Glyphosate Technical - Primary skin irritation study in rabbits 10/218-006N 5.2.5/001 Glyphosate technical: Acute eye irritation study in rabbits 10/218-005N 5.2.6/001 Glyphosate technical: Local lymph node assay in the mouse 10/218-037[E] 5.4.1/007 Salmonella Typhimurium and Escherichia Coli Reverse Mutation Assay 1264500 5.4.2/002 Glyphosate Technical – Micronucleus Assay in Bone Marrow Cells of the Mouse. 1479200
Glyphosate technical Lot/Batch#: 2009051501	96.4 % (w/w)	Confidential	All studies supplementary	5.2.1/003 Acute Oral Toxicity Study of Glyphosate TC in Rats 24874 5.2.2/002 Acute Dermal Toxicity Study of Glyphosate TC in CD Rats 24876 5.2.3/003 Acute Inhalation Toxicity Study of Glyphosate TC in Rats 24875 5.2.4/003 Acute Dermal Irritation/Corrosion Test (Patch Test) of Glyphosate TC in Rabbits 24877 5.2.5/003 Acute Eye Irritation/Corrosion Test of Glyphosate TC in Rabbits 24878 5.2.6/002 Examination Of Glyphosate TC In The Skin Sensitisation Test In Guinea Pigs According To Magnusson And Kligman (Maximisation Test) 24879 5.4.1/004 Salmonella typhimurium and Escherichia coli Reverse Mutation Assay with Solution of Glyphosate TC spiked with Glyphosine 1332300

Glyphosate Lot/Batch#: 20090506	97.3 % (w/w)	Confidential	All studies supplementary	5.2.1/004 Acute Oral Toxicity Study of Glyphosate TC in Rats 24602 5.2.2/003 Acute Dermal Toxicity Study of Glyphosate TC in CD Rats 24604 5.2.3/002 Acute Inhalation Toxicity Study of Glyphosate TC In Rats 24603 5.2.4/002 Acute Dermal Irritation/Corrosion Test (Patch Test) of Glyphosate TC in Rabbits 24605 5.2.5/002 Acute Eye Irritation/Corrosion Test of Glyphosate TC in Rabbits 24606 5.2.6/003 Examination of Glyphosate TC in Skin Sensitisation Test in Guinea Pigs according to Magnusson and Kligman (Maximisation Test) 24607
Glyphosate Lot/Batch#: 20080801	97.3 % (w/w)	Confidential	All studies supplementary	5.2.1/005 Acute Oral Toxicity Study of Glyphosate TC in Rats 23910 5.2.2/006 Acute Dermal Toxicity Study of Glyphosate TC in CD Rats 23912 5.2.3/004 Acute Inhalation Toxicity Study of Glyphosate TC in Rats 23911 5.2.4/004 Acute Dermal Irritation/Corrosion Test (Patch Test) of Glyphosate TC in Rabbits 23913 5.2.5/004 Acute Eye Irritation/Corrosion Test of Glyphosate TC in Rabbits 23914 5.2.6/005 Examination of Glyphosate TC in Skin Sensitisation Test in Guinea Pigs according to Magnusson and Kligman (Maximisation Test) 23915 5.4.2/014 Micronucleus test of Glyphosate TC in Bone Marrow Cells of the CD Rat by oral administration 23917

Glyphosate Lot/Batch#: GI-1045	96.66 % (w/w)	Confidential		5.2.1/006 Glyphosate Technical: Acute Oral Toxicity Study in Rats C22864 5.2.2/005 Glyphosate Technical: Acute dermal toxicity study in rats C22875 5.2.3/005 Glyphosate Tech: Acute Inhalation Toxicity (Nose only) Study in the Rat 2743/0001 5.2.6/004 Glyphosate Technical: Contact Hypersensitivity in albino guinea pigs – Maximization-Test C22908
Glyphosate Lot/Batch#: 080704-1 thru 5	96.40 % (w/w)	Confidential		5.2.1/007 Glyphosate: Acute Oral Toxicity Study (UDP) In Rats 12170-08 5.2.2/004 Glyphosate: Acute Dermal Toxicity Study in Rats 12171-08 5.2.3/006 Glyphosate – Acute Inhalation Toxicity Study in Rats 12107-08 5.2.4/005 Glyphosate – Acute Dermal Irritation Study in Rabbits 12173-08 5.2.5/006 Glyphosate – Acute Eye Irritation Study in Rabbits 12172-08 5.2.6/006 Glyphosate – Skin Sensitization Study in Guinea Pigs. Buehler Test 12174-08
Glyphosate Lot/Batch#: 20070606	98.05 % (w/w)	Confidential		5.2.1/008 Acute Oral Toxicity Study in Wistar Hannover Rats for Glyphosate Technical [redacted]-3996.305.475.07 5.2.2/007 Acute Dermal Toxicity Study in Wistar Hannover Rats for Glyphosate Technical [redacted]-3996.310.456.07 5.2.4/006 Acute Dermal Irritation/Corrosion Study in Rabbits with Glyphosate Technical [redacted]-3996.311.476.07 5.2.5/007 Acute Eye Irritation/Corrosion Study in Rabbits with Glyphosate Technical [redacted]-3996.312.599.07

Glyphosate Lot/Batch#: 0507	96.1 % (w/w)	Confidential		5.2.1/009 Glyphosate technical material: Acute oral toxicity study in the rat (up and down procedure) B02755 5.2.2/009 Glyphosate technical material: Acute dermal toxicity study in rats B02766 5.2.4/007 Glyphosate Technical Material: Primary Skin Irritation Study in Rabbits (4-Hour Semi-Occlusive Application) B02777 5.2.5/008 Glyphosate Technical Material: Primary Eye Irritation Study in Rabbits B02788 5.2.6/009 Glyphosate Technical Material: Skin Sensitisation (Local Lymph Node Assay In The Mouse) GM8048-REG
Glyphosate Lot/Batch#: 200609062	95.1 % (w/w)	Confidential		5.2.1/010 Glyphosate Technical (NUP05068): Acute Oral Toxicity Study in Rats B02272 5.2.2/008 Glyphosate Technical (NUP 05068): Acute dermal toxicity study in rats B02283 5.2.3/008 Glyphosate Technical (NUP05068) : 4-Hour acute inhalation toxicity study in rats B02327 5.2.4/008 Glyphosate Technical (NUP 05068): Primary Skin Irritation Study in Rabbits (4-Hour Semi-Occlusive Application) B02294 5.2.5/009 Glyphosate Technical (NUP 05068): Primary Eye Irritation Study in Rabbits B02305 Talvioja, K. 5.2.6/008 Glyphosate Technical (NUP 05068): Contact Hypersensitivity in Albino Guinea Pigs, Maximisation Test B02316 5.4.1/009 Salmonella typhimurium and Escherichia coli Reverse mutation assay with Glyphosate technical (NUP-05068) 1061401[redacted]

Glyphosate Lot/Batch#: 040205	97.23 % (w/w)	Confidential		5.2.1/011 Glyphosate Acid Technical – Acute Oral Toxicity Up and Down Procedure in Rats 15274 5.2.2/010 Glyphosate Acid Technical: Acute Dermal Toxicity Study in Rats – Limit Test15275 5.2.3/009 Glyphosate Acid Technical: Acute Inhalation Toxicity Study in Rats – Limit Test 15276 5.2.4/009 Glyphosate Acid Technical – Primary Skin Irritation Study in Rabbits 15278 5.2.5/010 Primary Eye Irritation Study in Rabbits 15277 5.2.6/011 Glyphosate acid technical – Dermal Sensitization in Guinea Pigs (Buehler Method) 15279
Glyphosate (NUP5a99 62% glyphosate MUP; IPA salt) Lot/Batch#: Drum Sample E	62 % (w/w)	Confidential		5.2.1/012 NUP5a99 62% glyphosate MUP: Acute oral toxicity study in rats – Limit test 7907 5.2.3/011 NUP5a99 62% glyphosate MUP: Acute inhalation toxicity study in rats – Limit test 7909
Glyphosate (MON-0139) Lot/Batch#: LBRV-11092	62.34 % (w/w)	Confidential		5.2.1/014 Acute Toxicity Study of MON 0139 by Oral Administration in Mice B-3101/XX-95-205
Glyphosate Lot/Batch#: 1073	97.6 % (w/w)	Confidential		5.2.1/017 Final report for “Oral and dermal LD50 tests with [redacted] Glyphosate acid technical in rats, limit test” 00917 5.2.2/013 Final report for “Oral and dermal LD50 tests with [redacted] Glyphosate acid technical in rats, limit test” 00917
Glyphosate (IPA salt) Lot/Batch#: 940950	62% (w/w)	Confidential		5.2.1/018 Final report for “Oral and dermal LD50 tests with [redacted] Glyphosate 62% IPA in rats, limit test” 00926 5.2.2/014 Final report for “Oral and dermal LD50 tests with [redacted] Glyphosate 62% IPA in rats, limit test” 00926

Glyphosate Premix / Glyphosate isopropyl-amine salt Lot/Batch#: 290-JaK-146-4	46.1 / 62.2% (w/w)	Confidential		5.2.1/020 Glyphosate Premix: Acute Oral Toxicity (Limit Test) in the Rat 545/37 5.2.3/016 Glyphosate premix: Acute inhalation toxicity study four-hour exposure (nose only) in the rat. 545/39 5.2.4/012 Glyphosate Premix: Acute Dermal Irritation Test in the Rabbit 545/40 5.2.5/014 Glyphosate premix: Acute eye irritation test in the rabbit 545/41 5.2.6/015 Glyphosate Premix: Magnusson & Kligman Maximisation Study in the Guinea Pig 545/42
Glyphosate Lot/Batch#: L3258	Not reported	Confidential		5.2.1/024 Glyphosate technical Acute oral toxicity (Limit test) in the rat 134/37 5.2.2/018 Glyphosate technical: Acute dermal toxicity (Limit test) in the rat 134/38
Glyphosate Lot/Batch#: 190 A	98.1 % (w/w)	Confidential		5.2.1/028 Acute oral toxicity study in the rat: Glyphosate technical [redacted]-900823B 5.2.2/020 Acute dermal toxicity study in the rat: Glyphosate Technical [redacted]-900823A 5.2.4/017 Acute Dermal Irritation/Corrosion of Glyphosate Technical in the Rabbit (Intact and Abraded Skin) [redacted]-900822A 5.2.5/019 Acute eye irritation/corrosion of glyphosate technical in the rabbit [redacted]-900822 5.3.2/012 Glyphosate Technical: 90 Day Oral Toxicity Study in the Rat [redacted]-900914
Glyphosate Lot/Batch#: XLI-55	97.76 % (w/w)	Confidential		5.2.1/031 Acute Oral Toxicity Study of Glyphosate Batch/Lot/NBR No. XLI-55 in Sprague-Dawley Rats 88.2053.007 5.2.2/023 Acute dermal toxicity study of glyphosate batch/lot/NBR No. XLI-55 in New Zealand White rabbits 88.2053.008 5.2.4/020 Primary Dermal Irritation Study of Glyphosate 88.2053.010 5.2.5/022 Primary Eye Irritation Study of Glyphosate Batch/Lot/NBR No. XLI-55 in New Zealand White Rabbits 88. 2053. 009

Glyphosate Lot/Batch#: XLG-255	90.8 % (w/w)	Confidential		5.2.1/032 Acute oral LD50 study of MON-8750 in Sprague-Dawley rats 9308A 5.2.4/021 Primary Dermal Irritation Study of MON 8750 in New Zealand White Rabbits [redacted]-86-431/9308A 5.2.2/025 Acute dermal toxicity study of MON-8750 in New Zealand White rabbits 9308A 5.2.5/024 Primary eye irritation study of MON-8750 in New Zealand White rabbits [redacted]-86-431/9308A
Glyphosate Lot/Batch#: 36300892	99.6 % (w/w)	Confidential		5.2.5/016 Primary eye irritation study in rabbits [redacted] 93-405/N
Glyphosate Lot/Batch#: XLG-256	70.7 % (w/w)	Confidential		5.2.2/024 Acute dermal toxicity study of MON 8722 in New Zealand White rabbits 9307A 5.2.5/023 Primary eye irritation study of MON 8722 in New Zealand White rabbits 9307A
Glyphosate Lot/Batch#: 614034 (20100609 \ Milled)	96.9 % (w/w)	Confidential		5.2.3/001 Glyphosate technical: Acute inhalation toxicity study (nose-only) in the rat 11/054-004P
Glyphosate Lot/Batch#: GLP-0306-14124-F	47.2 glyphosate (57.8 glyphosate K salt)% (w/w)	Confidential		5.2.3/010 An Acute Nose-Only Inhalation Toxicity Study in Rats with MON 78623 3044.969
Glyphosate Lot/Batch#: P25	95.6 % (w/w)	Confidential		5.2.3/012 Glyphosate Acid: 4-Hour Acute Inhalation Toxicity Study In Rats [redacted]/P/4882
Glyphosate Lot/Batch#: XLH-270	85.52 % (w/w)	Confidential		5.2.3/020 Acute inhalation study of MON-8750 Technical [redacted]-87-228

Glyphosate IPA salt Lot/Batch#: LHRO-12010 X	53.8 % (w/w)	Confidential		5.2.3/021 Acute Toxicity of Rodeo® Herbicide Administered by Inhalation to Male and Female Sprague-Dawley Rats [redacted]-6582
Glyphosate Lot/Batch#: 120594	98.2 % (w/w)	Confidential		5.2.5/012 Primary eye irritation study in rabbits 2981-96
Glyphosate Lot/Batch#: 161-JRJ-131-2	99.5 % (w/w)	Confidential		5.3.1/004 Glyphosate: 4 Week Dietary Toxicity Study in Rats 5626 5.3.1/007 Glyphosate: Oral Maximum Tolerated Dose Study in Dogs 5660 5.3.2/018 Glyphosate – 13 week dietary toxicity study in mice 7024
Glyphosate Lot/Batch#: 01.12.1997 & 01.06.1997	>95 % (w/w)	Confidential		5.3.2/021-024 Subchronic (90 Day) Oral Toxicity Study With Glyphosate Technical In Beagle Dogs AND Test compound stability in experimental diet (dog feed) 1816
Glyphosate Lot/Batch#: D4490/1, P18	99.1 % (w/w)	Confidential		5.3.2/025-0.26 First Revision to Glyphosate Acid: 90 Day Oral Toxicity Study in Dogs [redacted]/P/1802
Glyphosate Lot/Batch#: T-940308	94.61 % (w/w)	Confidential		5.3.2/027 HR-001: 13-week Subchronic Oral Toxicity Study in Dogs [redacted] 94-0158
Glyphosate (MON-0139) Lot/Batch#: LURT-12011	62.49 % (w/w)	Confidential		5.3.2/029 Six Month Study of Mon 0139 by Gelatin Capsule to Beagle Dogs 810166
Glyphosate Lot/Batch#: NBP 2472136	96.17 % (w/w)	Confidential		5.3.2/036 Twelve Month Study of Glyphosate Administered by Gelatin Capsule to Beagle Dogs[redacted]-4965
Glyphosate Lot/Batch#: 229-Jak-142-6	101.5 % (w/w)	Confidential		5.3.3/003 Glyphosate – 3 Week Toxicity Study in Rats with Dermal Administration 7839

Glyphosate Lot/Batch#: 229-JaK-5-1 / 229-Jak-142-6	98.9 / 98.7 % (w/w)	Confidential		5.5/007-009 Glyphosate – 104 week combined chronic feeding /oncogenicity study in rats with 52 week interim kill (results after 104 weeks) Vol 1,2,3 7867
Glyphosate Lot/Batch#: 39730494	99.6 % (w/w)	Confidential		KCA 5.3.3/004-006 Glyphosate technical ([redacted]): Repeated Dose Twenty-eight-Day Dermal Toxicity Study in Rabbits [redacted] 214/94 (Test Code: GLY-94-410/N)
Glyphosate Lot/Batch#: 20110107-2	97 % (w/w)	Confidential		5.4.1/002 Reverse Mutation Assay using Bacteria (Salmonella typhimurium) with Glyphosate Tech. 126159
Glyphosate Lot/Batch#: 200903051	98.2 % (w/w)	Confidential		5.4.1/005 Reverse Mutation Assay using bacteria (Salmonella typhimurium) with Glyphosate TC 101268
Glyphosate Lot/Batch#: 20060901	97.7 % (w/w)	Confidential		5.4.1/010 Salmonella typhimurium and Escherichia coli Reverse mutation assay with Glyphosate technical (NUP-05070) 1061402
Glyphosate Lot/Batch#: 0609-1	95.0 % (w/w)	Confidential		5.4.1/011 Salmonella typhimurium and Escherichia coli Reverse mutation assay with Glyphosate technical (NUP-05067) 1061403
Glyphosate Lot/Batch#: 046	96.0 % (w/w)	Confidential		5.4.1/018 Mutagenicity – Salmonella typhimurium reverse mutation assay (Ames test) 887-MUT.AMES 5.4.2/015 Genetic toxicology: In vivo mammalian bone marrow cytogenetic test – Chromosomal analysis. 890-MUT-CH.AB
Glyphosate Lot/Batch#: XHJ-46	98.4 % (w/w)	Confidential		5.4.1/024 The report of mutagenic study with bacteria for CP67573 ET-78-241

Glyphosate Lot/Batch#: 22021	96 % (w/w)	Confidential		5.4.1/028 Evaluation of the ability of glyfosaat to induce chromosome aberrations in cultured peripheral human lymphocytes (with independent repeat) 141918
Glyphosate Lot/Batch#: 20061109	98.9 % (w/w)	Confidential		5.4.2/001 Glyphosate TGAI: Micronucleus test of glyphosate TGAI in mice 485-1-06-4696
Glyphosate Lot/Batch#: 20070545	99.1 % (w/w)	Confidential		5.4.2/005 Glyphosate Technical – Micronucleus Assay in Bone Marrow Cells of the Mouse 1158500
Glyphosate Lot/Batch#: 3578/99	95 % (w/w)	Confidential		5.4.2/008 A micronucleus study in mice for glyphosate técnico Nufarm [redacted]-G12.79/99
Glyphosate Lot/Batch#: FSG 03090	96.8 % (w/w)	Confidential		5.4.2/010 Mutagenicity – Micronucleus test in Swiss Albino Mice. 889-MUT.MN
Glyphosate Lot/Batch#: T830044	98.7 % (w/w)	Confidential		5.4.2/016 In Vivo Bone Marrow Cytogenetics Study of Glyphosate in Sprague-Dawley Rats 830083
Glyphosate Lot/Batch#: FSG 03090 H/05 March 1990	96.8 % (w/w)	Confidential		5.3.2/008-010 90-Day Oral Toxicity Study with Glyphosate Technical in Wistar Rats (FSG 03090 H/05 March 1990) [redacted]-882 90 OR
Glyphosate Lot/Batch#: L1656	97.1 % (w/w)	Confidential		5.3.2/013 Glyphosate Technical: 90 Day Oral Toxicity study in the Rat [redacted]-891002
Glyphosate Lot/Batch#: XLG 161	95.21 % (w/w)	Confidential		5.3.2/014 90 Day Study of Glyphosate Administered in Feed to Sprague/Dawley Rats [redacted]-7375

Glyphosate Lot/Batch#: P30	97.6 % (w/w)	Confidential		5.5/002 Glyphosate Acid: Two Year Dietary Toxicity and Oncogenicity Study in Rats [redacted]/PR1111
Glyphosate Lot/Batch#: XLH-264	96.5 % (w/w)	Confidential		5.5/010 Chronic study of glyphosate administered in feed to Albino rats [redacted]-10495
Glyphosate Lot/Batch#: 01/06/97	>95.14 % (w/w)	Confidential		5.5/016 Carcinogenicity Study with Glyphosate Technical in Swiss Albino Mice TOXI: 1559.CARCI-M
Glyphosate Lot/Batch#: NB 1782608/3 and NB 1782610/7	99.7 % (w/w)	Confidential		5.5/023 A chronic feeding study of glyphosate (Roundup® technical) in mice 77-2061
Glyphosate Lot/Batch#: GLP-0807-19475-T	95.11 % (w/w)	Confidential		5.8.2/001 Glyphosate – A 28-Day Oral (Dietary) Immunotoxicity Study in Female B6C3F1 Mice [redacted]-50393
Glyphosate NH_4 salt Lot/Batch#: RUD-9201-3544F	94.78 % (w/w)	Confidential		5.8.2/005 Ammonium Salt of Glyphosate (Mon-8750): General Pharmacological Study [redacted] 90-0149/ET-92-15
Glyphosate Lot/Batch#: 72390788	96 % (w/w)	Confidential		5.8.2/006 Toxicodinamic study of glyphosate in rat N.A.

Glyphosate Lot/Batch#: GLP-1103-21149-T	95.93 glyphosate acid 85.14 calculated glyphosate content % (w/w)	Confidential		5.8.2/014 Glyphosate acid - In Vitro Absorption through Abraded Rabbit Skin using [14C]-glyphosate JV2182-REG 5.8.3/001 Glyphosate: Androgen Receptor Binding (Rat Prostate Cytosol) Screening Assay 6500V-100334ARB 5.8.3/002 Estrogen Receptor Transcriptional Activation (Human Cell Line (HeLa-9903)) Screening Assay with Glyphosate 6500V-100334ERTA 5.8.3/003 Glyphosate: Estrogen Receptor Binding (Rat Uterine Cytosol) Screening Assay 6500V-100334ERB 5.8.3/004 Glyphosate: Human Recombinant Aromatase Assay 6500V-100334AROM 5.8.3/005 A Uterotrophic Assay of Glyphosate Administered Orally in Ovariectomized Rats [redacted]-843002 5.8.3/006 A Hershberger Assay of Glyphosate Administered Orally in Peripubertal Orchidoepididymectomized Rats [redacted]-843003 5.8.3/007 A Pubertal Development and Thyroid Function Assay of Glyphosate Administered Orally in Intact Juvenile/Peripubertal Male Rats [redacted]-843005 5.8.3/008 A Pubertal Development and Thyroid Function Assay of Glyphosate Administered Orally in Intact Juvenile/Peripubertal Female Rats [redacted]-843007
Glyphosate Lot/Batch#: P15	97.4 % (w/w)	Confidential		5.3.2/001-002 First Revision to Glyphosate Acid: 90 Day Feeding Study in Rats [redacted]/P/1599
Glyphosate Lot/Batch#: H95D 161 A	95.3 % (w/w)	Confidential		5.3.2/003 Technical Glyphosate: Ninety Day Sub-Chronic Oral (Dietary) Toxicity Study in the Rat 434/016
Glyphosate Lot/Batch#: 46540992	97.5 % (w/w)	Confidential		5.3.2/005-007 90 Day Range Feeding Study of Glyphosate in Rats 011-0001

Glyphosate Lot/Batch#: AZM30320T0	91.8 % (w/w)	Confidential		5.4.1/040 Glyphosate: V79 HPRT Gene Mutation Assay 8441968 5.4.1/041 Glyphosate: Micronucleus Test in Human Lymphocytes in vitro 8441969
Glyphosate	-	-	Explosive properties- Statement	[redacted] (1984) Report no. 122377
Glyphosate	97.7 %	-	Flammability- UN test N.1	[redacted] (2019) Report no. PS20190309-1
Glyphosate	97.7 %	-	Self-heating- UN test N.4	[redacted] (2019) Report no. PS20190309-2
Glyphosate	96.6 %	-	Oxidizing- EEC A.17	[redacted] (1997) Report no. RJ2401B
Glyphosate acid	95.49 %		Chronic toxicity test on *Brachydanio rerio*	[redacted] C.M. (2000c), Report No.: [redacted]-D62.16/99 (CA 8.2.2.1/002)

2.11.2 Proposed harmonized classification and labelling

2.11.2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 79: Proposed harmonised classification and labelling according to the CLP criteria

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	607-315-00-8	glyphosate (ISO) N-(phosphonomethyl)glycine	213-997-4	1071-83-6	Eye Dam. 1 Aquatic Chronic 2	H318 H411	GHS09 GHS05 Dgr	H318 H411			
Dossier submitters proposal	607-315-00-8	glyphosate (ISO) N-(phosphonomethyl)glycine	213-997-4	1071-83-6	Eye Dam. 1 Aquatic Chronic 2 No changes to existing entry proposed	H318 H411	GHS09 GHS05 Dgr	H318 H411			
Resulting Annex VI entry if agreed by RAC and COM	607-315-00-8	glyphosate (ISO) N-(phosphonomethyl)glycine	213-997-4	1071-83-6	Eye Dam. 1 Aquatic Chronic 2	H318 H411	GHS09 GHS05 Dgr	H318 H411			

2.11.2.2 Additional hazard statements / labelling

Table 80: Reason for not proposing harmonised classification and status under CLH public consultation

Hazard class	Reason for no classification	Within the scope of CLH consultation
Explosives	Data conclusive but not sufficient for classification	Yes
Flammable gases (including chemically unstable gases)	Hazard class not applicable	No
Oxidising gases	Hazard class not applicable	No
Gases under pressure	Hazard class not applicable	No
Flammable liquids	Hazard class not applicable	No
Flammable solids	Data conclusive but not sufficient for classification	Yes
Self-reactive substances	Data conclusive but not sufficient for classification	Yes
Pyrophoric liquids	Hazard class not applicable	No
Pyrophoric solids	Data conclusive but not sufficient for classification	Yes
Self-heating substances	Data conclusive but not sufficient for classification	Yes
Substances which in contact with water emit flammable gases	Data conclusive but not sufficient for classification	Yes
Oxidising liquids	Hazard class not applicable	No
Oxidising solids	Data conclusive but not sufficient for classification	Yes
Organic peroxides	Data conclusive but not sufficient for classification	Yes
Corrosive to metals	Data conclusive but not sufficient for classification	Yes
Acute toxicity via oral route	Data conclusive but not sufficient for classification	Yes
Acute toxicity via dermal route	Data conclusive but not sufficient for classification	Yes
Acute toxicity via inhalation route	Data conclusive but not sufficient for classification	Yes
Skin corrosion/irritation	Data conclusive but not sufficient for classification	Yes
Serious eye damage/eye irritation	**Eye Dam. 1; H318**	Yes
Respiratory sensitisation	Data lacking	No
Skin sensitisation	Data conclusive but not sufficient for classification	Yes
Germ cell mutagenicity	Data conclusive but not sufficient for classification	Yes
Carcinogenicity	Data conclusive but not sufficient for classification	Yes
Reproductive toxicity	Data conclusive but not sufficient for classification	Yes
Specific target organ toxicity-single exposure	Data conclusive but not sufficient for classification	Yes
Specific target organ toxicity-repeated exposure	Data conclusive but not sufficient for classification	Yes

Hazard class	Reason for no classification	Within the scope of CLH consultation
Aspiration hazard	Data lacking	No
Hazardous to the aquatic environment	**Aquatic Chronic 2; H411**	Yes
Hazardous to the ozone layer	Data lacking	No

2.11.3 History of the previous classification and labelling

A CLH proposal based on the assessment made for the review of active substances in plant protection products under Regulation (EC) No 1107/2009 was submitted to ECHA in 2016. Following public consultation and discussions in the Committee for Risk Assessment (RAC), the existing harmonised classification (Eye. Dam. 1; H318 and Aquatic Chronic 2; H411) was established and included in Annex VI of Regulation (EC) No 1272/2008. The conclusion was preceded by discussions primarily in the areas of carcinogenicity and mutagenicity.

The International Agency for Research on Cancer (IARC) of the World Health Organization (WHO) published in 2015 a monograph on glyphosate stating that the substance is "probably carcinogenic to humans (Group 2A)" (IARC, 2015, ASB2015-8421). The IARC evaluation of the potential carcinogenicity and genotoxicity of glyphosate or glyphosate-containing plant protection products was taken into consideration during the peer-review for the renewal of approval of glyphosate under Regulation (EC) No 1107/2009, but the same conclusion was not reached (EFSA conclusion, 2015, ASB2015-11412).

Likewise, the Joint Meeting on Pesticide Residues (JMPR) administered jointly by the Food and Agriculture Organization of the United Nations (FAO) and WHO who re-evaluated glyphosate in May 2016 concluded that glyphosate is unlikely to pose a carcinogenic risk to humans from exposure through the diet." (JMPR, 2016, ASB2016-4292).

Based on a weight of evidence approach considering epidemiological data as well as data from long-term studies in rats and mice, RAC reached the conclusion that criteria for classification are not fulfilled. One of the differences between the assessments made by IARC and RAC, respectively, is the use of data related to glyphosate-based herbicidal products. The assessments made by RAC are restricted to evaluating hazards arising from the intrinsic properties of a specific chemical and therefore data for glyphosate-based herbicidal products are not taken into account except for epidemiological information.

2.11.4 Identified uses

Glyphosate is a systemic herbicide used for weed control in agriculture, forestry, residential, industrial, and aquatic situations. The substance is widely used all over Europe and populations potentially exposed following the intended uses of the substances include operators, workers, bystanders, residents and potentially also consumers via intake of residues in food.

2.11.5 Data sources

This CLH proposal is based on data submitted in the context of renewal of the existing active substance glyphosate under Article 1 of Regulation (EU) No 844/2012.

2.12 RELEVANCE OF METABOLITES IN GROUNDWATER

An assessment of the relevance of metabolites in groundwater according to the stepwise procedure of EC guidance document SANCO/221/2000 – rev.10 is presented below.

Based on available laboratory soil degradation studies and field dissipation studies, AMPA is formed in amounts triggering a groundwater risk assessment according to the criteria defined in Regulation 284/2013 and in guidance document SANCO/221/2000. No other metabolites reach amounts triggering a groundwater risk assessment according to these criteria.

2.12.1 STEP 1: Exclusion of degradation products of no concern

AMPA does not fulfil the criteria defined in SANCO/221/2000 for being excluded as a degradation product of no concern. Hence, it has to be considered further in Step 2 of the tiered relevance assessment.

2.12.2 STEP 2: Quantification of potential groundwater contamination

PECgw for AMPA from FOCUS modelling and from HardSPEC are below 0.1 µg/L.

An extended groundwater monitoring data set was also collected throughout 14 EU countries, representing >230 000 samples collected from > 34 400 sampling sites for AMPA (please refer to Vol. 3 CA B.8.5 for detailed assessment). Compliance with the regulatory threshold of 0.1 µg/L was very high (99.3% of samples). Among the few samples (0.7%) exceeding 0.1 µg/L, the maximum measured concentration for AMPA was 19 µg/L.

Based on the quantification of potential groundwater contamination by regulatory modelling and through observations from large scale monitoring programs, it can be concluded that AMPA has a low propensity to leach at levels exceeding 0.1 µg/L. Therefore, a relevance assessment is strictly not triggered. Nevertheless, for completeness, the stepwise procedure of guidance document SANCO/221/2000 is presented below as additional information, based on the maximum concentration reported from the monitoring data.

2.12.3 STEP 3: Hazard assessment – identification of relevant metabolites

2.12.3.1 STEP 3, Stage 1: screening for biological activity

AMPA does not have a comparable target activity as the parent active compound as it does not contain the functional moiety to cause the herbicidal action that glyphosate does (SANCO/221/2000 – rev 10) and is not expected to impact the Shikimic acid pathway.

Studies available on alga and aquatic macrophytes suggested that AMPA is 14 times less toxic than glyphosate on alga and 7 times less toxic on aquatic macrophytes.

In addition to differences in the mode of activity described above, the relative herbicidal activity between AMPA and glyphosate was reported [redacted] 2012 CP 10.6.2/004). In this report, relative post-emergence phytotoxicity between glyphosate and AMPA were compared for the following species: 9 Dicotyledons (cocklebur, hemp sesbania, lambsquarters, morning glory, smartweed, soybean, sugar beet, velvetleaf, wild buckwheat) and 8 Monocotyledons (barnyard grass, corn, crabgrass, green foxtail, proso millet, rice, sorghum, wheat).
EC50 molar ratios were calculated as EC50 AMPA/ EC50 glyphosate acid and ranged from 3.4 for hemp sesbania to 86.8 for common lambsquarters. In all cases, the ratios were greater than two, indicating that AMPA has less than 50% of the herbicidal activity of glyphosate. The endpoints presented above cannot be used for the risk assessment of non-target plants but are indicative of a lower toxicity of AMPA.

RMS considered that AMPA has lost the herbicidal activity of the parent glyphosate. The herbicidal activity of AMPA is considered to be below 50 % of the parent activity.

2.12.3.2 STEP 3, Stage 2: screening for genotoxicity

Four Ames tests are available for AMPA of which one was concluded to be unacceptable and the other three as acceptable (classified as reliable with restrictions). In two of these studies AMPA was considered to be non-mutagenic. The other study was concluded to be equivocal as some statistical significant increases were observed without a dose response which were difficult to assess due to the lack of historical control data. AMPA was negative in an *in vitro* mammalian gene mutation study. Overall, AMPA is concluded to be negative for gene mutations *in vitro*.
Two *in vitro* UDS studies were available which were both negative. However, these studies are no longer considered acceptable due to sensitivity issues of the study method.
No *in vitro* micronucleus study is available. AMPA was negative for clastogenic and aneugenic effects in two *in vivo* micronucleus studies. In both studies, the number of scored PCE was too low compared to the current OECD test guideline although the study was in line with the OECD test guideline valid at the time of conduct of the study (1983). In the first study bone marrow exposure was proven as a decrease in PCE/NCE ratios was observed. In the second study at slightly lower dose levels (up to 1000 mg/kg bw), no increase in the frequency of micronucleated PCEs was observed. However, no direct evidence of bone marrow exposure was available as no effect on PCE/NCE

ratio was observed. Systemic toxicity was however observed in the study including clinical signs and bodyweight losses. Higher dose levels could not be tested due to mortality observed in the dose range-finding study. Considering the systemic toxicity observed and the bone marrow toxicity at higher dose levels in the first study the RMS considers that the bone marrow was sufficiently exposed in the second study.
Based on the available information AMPA is concluded to be non-genotoxic.

2.12.3.3 STEP 3, Stage 3: screening for toxicity

The parent compound glyphosate is currently classified with H318 (Eye Damage 1). The eye damage classification is not considered relevant for the groundwater metabolite assessment.

Pending the outcome of the final proposal for classification of the active substance, in this non-relevance assessment it is assumed the current classification for glyphosate is maintained. **It is noted that in case of any changes in the proposed classification of the parent glyphosate, this section should be updated.**

The metabolite AMPA was extensively investigated for acute and sub-chronic effects, for skin sensitization and developmental toxicity. In acute oral rodent studies the median lethal dose had been identified with signs of no toxicity as greater than 2000 mg/kg bw/day in rats. Non-sensitizing potential had been demonstrated with guinea pigs in a Magnusson and Kligman Maximization test. Sub-acute studies had been evaluated with rats and dogs. The lowest sub-acute NOAEL value of 100 mg/kg bw/day based on kidney weight increase in male rats and decreased bw gain in female animals. In addition, two 90-day studies are available in rats and one study in dogs. In the first rat study and in the dog study, no adverse effects were noted up to the highest dose tested and the NOAEL was concluded to be 1000 and 300 mg/kg bw/day, respectively. In the second rat study, based on increased urothelial hyperplasia of the urinary bladder of both sexes at 1200 mg/kg bw/day, the NOAEL was concluded to be 400 mg/kg bw/day.
Two developmental toxicity studies are available. In the first developmental study, no adverse effects were reported in maternal animals or foetuses up to highest dose tested. In the second study, a maternal NOAEL value of 150 mg/kg bw/day was based on clinical signs of decreased food consumption and decreased body weight gain. The developmental NOAEL of 400 mg/kg bw/day was derived on mean foetal weight decrease.
Overall, it can be concluded that AMPA is of similar toxicity as glyphosate and its reference values can be applied. This conclusion is in line with the previous EU evaluation. The RMS notes that in contrast to the previous evaluation, the reference values of glyphosate have been lowered based on salivary gland findings after repeated oral dosing in rats. In order to determine whether or not AMPA shares this effect with parent glyphosate and whether or not (higher) substance-specific reference values might be set for AMPA, special attention was paid to salivary gland findings in the studies with AMPA. However, based on the available sub-chronic data package, it cannot be excluded that AMPA would case the same effect in the salivary gland. In rats, a 28-day and a 90-day study are available in which the salivary glands including the parotid gland were investigated (study report no. 148-GLY and No. 7866). In these studies no treatment-related histopathological findings in the salivary glands were reported. However, the RMS notes that the route of administration in these studies was by gavage, thus bypassing the mouth. Therefore, it is questioned whether or not these studies covers the salivary gland findings reported after oral administration of glyphosate in rats. One 90-day study in rats is available (study report no 401-050) in which AMPA was administered through diet and also a 90-day study in dogs using oral (capsule) administration, however, these studies did only investigate the submandibular (submaxillary) salivary gland and did not include the parotid salivary gland. Therefore, no conclusion can be drawn based on these studies. Overall, as based on the available toxicity studies it cannot be excluded that AMPA causes similar histopathological changes in the salivary gland as parent glyphosate, the same reference values should be applied for both parent glyphosate and metabolite AMPA.

2.12.4 STEP 4: Exposure assessment – threshold of concern approach

At this moment metabolite AMPA is not considered relevant. The highest maximum concentration of AMPA observed in monitoring data exceeds the threshold of toxicological concern of 0.75 µg/L, therefore a refined risk assessment is applied in step 5.

2.12.5 STEP 5: Refined risk assessment

The highest maximum concentration of AMPA observed in monitoring data is 19 µg/L. Since the ADI of the parent is used the concentration equivalent to the parent should be used. The molecular weight of parent is 169.1 g/mol and the MW of metabolite is 111 g/mol. The concentration equivalent then yields (19 µg/L / 111) * 169.1 = 28.95 µg/L.

Table 2.12.5-1: Assessment of AMPA contribution to the diet via drinking water

	Weight (kg)	Water Consumption (L/day)	Uptake (µg/ kg bw/day)	% of ADI (ADI = 100 µg/kg bw/day)
Adult	60	2	0.965	0.96
Children	10	1	2.895	2.89
Infants	5	0.75	4.342	4.34

The contribution of metabolite through the diet has been compared with the proposed ADI of the parent compound glyphosate (0.1 mg/kg bw/day).
As shown in Table 2.12.5-1, it is evident that the highest estimated exposure via the drinking water is 4.34% of the ADI, which is below the allocation factor of 20% set in the WHO Guidance for drinking-water quality.

2.12.6 Overall conclusion

Based on the quantification of potential groundwater contamination by regulatory modelling and through observations from large scale monitoring programs, it can be concluded that AMPA has a low propensity to leach at levels exceeding 0.1 µg/L. Therefore, a relevance assessment is strictly not triggered. Nevertheless, for completeness, the stepwise procedure of guidance document SANCO/221/2000 is presented as additional information.
The maximum concentration of 10 µg/L is usually considered as threshold for non-relevant metabolites for EU approval. The maximum concentration reported in the groundwater monitoring data was used as worst-case for this assessment.

Based on this non-relevance assessment, the metabolite AMPA is not considered relevant at the maximum observed concentration of 19 µg/L.

2.13 CONSIDERATION OF ISOMERIC COMPOSITION IN THE RISK ASSESSMENT

2.13.1 Identity and physical chemical properties

Not relevant.

2.13.2 Methods of analysis

Not relevant.

2.13.3 Mammalian toxicity

Not relevant.

2.13.4 Operator, Worker, Bystander and Resident exposure

Not relevant.

2.13.5 Residues and Consumer risk assessment

Not relevant.

2.13.6 Environmental fate

Not relevant.

2.13.7 Ecotoxicology

Not relevant.

2.14 RESIDUE DEFINITIONS

2.14.1 Definition of residues for exposure/risk assessment

Food of plant origin:
Conventional crops: sum of glyphosate and AMPA, expressed as glyphosate
However, an overall residue definition for all crops (both conventional and GMO crops) can be proposed as sum of glyphosate, AMPA, *N*-acetyl-glyphosate and *N*-acetyl-AMPA, expressed as glyphosate
The residue definition is pending data gaps on genotoxicity for *N*-acetyl glyphosate, *N*-glyceryl AMPA, *N*-acetyl AMPA, *N*-methyl AMPA and *N*-malonyl AMPA.
Honey and bee products: sum of glyphosate and AMPA, expressed as glyphosate

Food of animal origin:
Sum of glyphosate, AMPA, *N*-acetyl glyphosate and N-acetyl AMPA, expressed as glyphosate.
The residue definition is pending data gaps on genotoxicity for *N*-acetyl glyphosate and *N*-acetyl AMPA.

Soil: Glyphosate and AMPA

Groundwater: Glyphosate and AMPA

Surface water: Glyphosate, AMPA and HMPA

Sediment: Glyphosate, AMPA and 1-oxo-AMPA

Air: Glyphosate

2.14.2 Definition of residues for monitoring

Food of plant origin:
Conventional crops: glyphosate
GMO crops: sum of glyphosate, AMPA and *N*-acetyl-glyphosate, expressed as glyphosate
The residue definition is pending data gaps on genotoxicity for *N*-acetyl-glyphosate.
Honey and bee products: glyphosate

Food of animal origin:
Sum of glyphosate, AMPA and *N*-acetyl glyphosate, expressed as glyphosate.
The residue definition is pending data gaps on genotoxicity for *N*-acetyl-glyphosate.

Soil: Glyphosate and AMPA

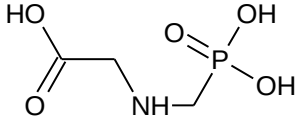
Groundwater: Glyphosate and AMPA

Surface water: Glyphosate and AMPA

Sediment: Glyphosate and AMPA

Air: Glyphosate

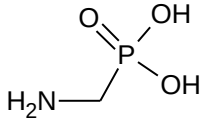
2.15 SUBSTANCES AND METABOLITES; STRUCTURES, CODES, SYNONYMS

Code Number (Synonyms) That indicated in bold was used in the summary dossier	**(IUPAC name /SMILES notation /InChiKey)**	**Structural formula**	**Compound found in:**
Glyphosate -Parent	IUPAC/CA name: N-(phosphonomethyl)glycine PMG CP 67573 SMILES notation: OC(=O)CNCP(=O)(O)O		**animal:** rat: Rate and extent of oral absorption: Rapid ($T_{max:}$ 2 - 8 h) but limited, about 20 %, based on urinary excretion and comparison of kinetic behaviour after oral and iv administration. Distribution: Wide, highest concentration after 7 d in bone, liver and kidney (< 1 % of applied radioactivity). C_{max} in plasma separated by dose and application route: Single gavage application of: 1mg/kg bw: 0.03 µg/mL 10 mg/kg bw: 0.25 µg/mL 30 mg/kg bw: 1.2 µg/mL 100 mg/kg bw: 8.3 µg/mL 600 mg/kg bw: 27.5 µg/mL Repeated dietary application (14 days) of: 72 mg/kg bw/d: 0.74 µg/mL 385 mg/kg bw/d: 5.0 µg/mL. AUC (to infinity or to last detectable concentration) in plasma separated by dose and application route: Single gavage application of: 1mg/kg bw: 0.33 µg × h/mL 10 mg/kg bw: 3.5 µg × h/mL 30 mg/kg bw: 20.8 µg × h/mL (only two animals) 100 mg/kg bw: 54.4 µg × h/mL (0-24 h -value)

			600 mg/kg bw: 377.6 µg × h/mL (0-24 h -value) Repeated dietary application (14 days) of: 72 mg/kg bw/d: 9.3 µg × h/mL 385 mg/kg bw/d: 50.7 µg × h/mL $t_{1/2}$: 6-12 h Metabolism: Poorly metabolised with the only biotransformation product aminomethylphosphonic acid (AMPA) accounting for up to 1% of the total excreted amount detected in faeces and urine in various studies. Rate and extent of excretion: Virtually complete within 7 d with major portion excreted within 48 h; absorbed amount eliminated via urine, unabsorbed via faeces; biliary excretion and exhalation negligible. Potential for accumulation: No evidence for accumulation (after 7 d total residues ≤1 % of the administered dose). laying hen (maximum values depicted): administration of mixture of glyphosate/AMPA: 67.1 % TRR (egg yolk); 76.1 % TRR (fat); 93.2 % TRR (kidney); 74.8 % TRR (thigh muscle); 71.0 % TRR (breast muscle); 68.6 % TRR (liver); 59.6 % TRR (gizzard) administration of glyphosate only: 59.54 % TRR (egg yolk); 21.48 % TRR (egg white); 60.97 % TRR (liver); 61.00 % TRR (thigh muscle); 39.05 % TRR (breast muscle); 40.66 % TRR (fat) lactating goat: administration of mixture of glyphosate/AMPA: 47.8 % TRR (milk), 48.0 % TRR (milk, depuration exp.); 83.7 % TRR (fat); 89.6 % TRR (kidney); 77.8 % TRR (liver); 74.5 % TRR (muscle)

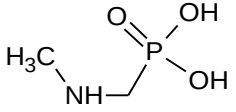
			administration of glyphosate only: 22.3 % TRR (milk); 91.3 % TRR (fat); 86.6 % TRR (kidney); 59.4 % TRR (liver); 87.1 % TRR (muscle) **plant: foliar treatment:** walnut: 63.22 % TRR (treated leaves); 81.60 % TRR (other tops); 85.36 % (roots) almond: 41.58 % TRR (treated leaves); 13.70 % TRR (other tops); 62.65 % (roots) pecan: 62.06 % TRR; (treated leaves); 61.74 % TRR (other tops); 59.85 % TRR (roots) apples: 96.1 % TRR (treated leaves); 101.3 % TRR (new growth above treatment); 95.1 % TRR (other new growth (leaves and stem)); 64.4 % TRR (trunk and branches); 66.4 % TRR (roots) grapes: 97.1 % TRR (treated leaves); 103.1 % TRR (new growth); 90.2 % TRR (roots and old stock); 79.44 % TRR (fruit) wheat (desiccation treatment): 85.0 %TRR (chaff); 82.6 % TRR (straw); 90.8 % TRR (grain) coffee: >91.1 % (treated leaves); >95.0 % (roots); >90.0 % (stems); >71.7 % TRR (untreated leaves); 98.0 % (coffee beans); 91.2 % (ripe coffee beans); 94.0 % (ripe pods) **plant: soil treatment:** soybean: 3.3 % TRR (forage); 0.6 % TRR (straw); 4.1 % TRR (hull), 2.6 % TRR (seed); 2.1 % TRR (hay) **plant: hydroponic treatment:** soybean: 85.5 % TRR (forage), 73.0 % TRR (roots) barley: 73.25 % TRR (aerial part/tops), 52.60 % TRR (roots) oats: 76.63 % TRR (aerial parts), 35.70 % TRR (roots) rice: 73.75 % TRR (aerial parts), 19.10 % TRR (roots) sorghum: 76.23 % TRR (aerial parts), 44.80 % TRR (roots) maize: 70.6 % TRR (forage), 61.1 % TRR (roots) wheat: 70.7 % TRR (forage), 38.5 % TRR (roots) cotton: 80.0 % TRR (forage), 38.8 % TRR (roots)

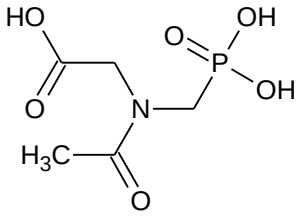
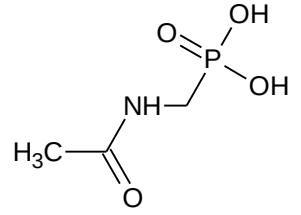
			coffee: 74.0 % TRR (aerial parts), 81.9 % TRR (roots) **rotational crops:** lettuce: 3.8 % TRR wheat: 0.5 % TRR (forage); 3.7 % TRR (grain); 0.4 % TRR (straw), 9.9 % (whole plant) radish: 1.1 % TRR (tops); 1.8 % TRR (roots) barley: 9.8 % TRR (grain); 1.0 % TRR (straw) carrot: 19.6 % TRR (roots); 21.1 % TRR (leaves); 6.9 % TRR (unknown part); 30.2 % TRR (leaves, primary crop) cabbage: 10.0 % TRR (whole plant), 46.4 % TRR (unknown part) beet: 2.0 % TRR (foliage); 7.1 % TRR (roots) peas: 11.1 % TRR (leaves); 15.6 % TRR (pods); 62.1 % (tops, primary crop) string bean: 23.8 % TRR (leaves); 40.1 % TRR (pods), 1.0 % TRR (leaves, primary crop); 7.9 % TRR (pods, primary crop), 54.2 % TRR (unknown part, primary crop) sweet corn: 3.2 % TRR (cob); 12.0 % TRR (forage/first harvest); 5.6 % TRR (second harvest) lettuce: 4.9 % TRR identified as glyphosate/AMPA mixture wheat: 3.7 % TRR identified as glyphosate/AMPA mixture (grain) **tolerant crops:** CP4-EPSPS or CP4-EPSPS and GOX modified crops: **Foliar application:** sugar beet: 79.65 % TRR (tops), 95.31 % TRR (roots) wheat: 89.44 % TRR (forage), 83.86 %TRR (hay), 69.19 % TRR (straw), 72.40 % TRR (grain) maize: 80.9 % TRR (forage); 77.9 % TRR (silage); 83.3 % TRR (fodder); 7.4 % TRR (grain) soybean: 89.1 % TRR (forage); 64.7 % TRR (hay); 25.2 % TRR (seeds) cotton: 95.7 % TRR (forage), 23.7 % TRR (seed) **GAT modified crops:**

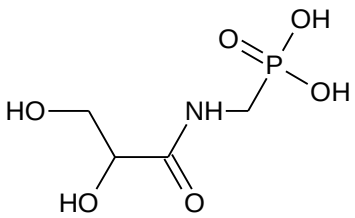
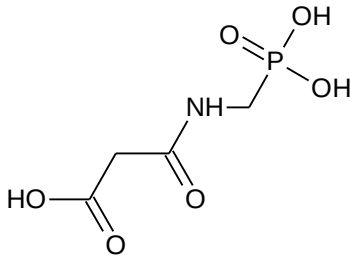
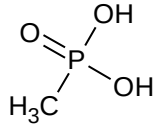
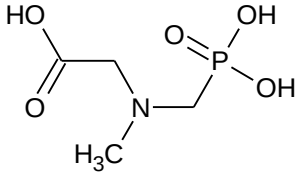
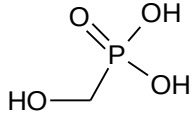
			Soil application followed by foliar applications maize: 58.0 % TRR (forage); 74.9 % TRR (stover); 0.1 % TRR (grain) canola: 3.0 % TRR (immature foliage); 20.8 % TRR (seeds) soybean: 9.1 % TRR (forage); 72.5 % TRR (hay); 22.7 % TRR (seeds); 56.9 % TRR (pods); 53.4 % TRR (foliage)
AMPA - QSAR number M02	IUPAC/CA name: Aminomethylphosphonic acid CP 50435 SMILES notation: NCP(=O)(O)O		**animal:** rat: As biotransformation product aminomethylphosphonic acid (AMPA) accounting for up to 1% of the total excreted amount 0.6 % TRR (plasma) in 14-day repeated dose study laying hen (maximum values depicted): administration of mixture of glyphosate/AMPA: 14.3 % TRR (egg yolk); 11.7 % TRR (fat); 14.1 % TRR (fat, depuration exp.); 5.3 % TRR (kidney); 9.8 % TRR (kidney, depuration exp.); 14.8 % TRR (thigh muscle); 17.3 % TRR (breast muscle); 32.6 % TRR (thigh muscle, depuration exp.); 42.2 % TRR (breast muscle, depuration exp.); 31.8 % TRR (liver); 53.1 % TRR (liver, depuration exp.); 40.1 % TRR (gizzard) administration of glyphosate only: 2.28 % TRR (egg yolk), 0.82 % TRR (egg white); 22.53 % TRR (liver); 4.06 % TRR (thigh muscle); 5.00 % TRR (breast muscle); 3.31 % TRR (fat) lactating goat: administration of mixture of glyphosate/AMPA: 4.9 % TRR (milk); 7.4 % TRR (whole milk, depuration exp.); 9.6 % TRR (fat); 6.2 % (kidney); 13.4 % TRR (kidney, depuration exp.); 12.7 % (liver); 30.7 % TRR (liver, depuration exp.); 7.5 % TRR (muscle), 10.4 % (muscle, depuration experiment) administration of glyphosate only: 7.5 % TRR (kidney); 21.4 % TRR (liver); 2.4 % (milk); 6.3 % (muscle); 4.7 % (fat))

			plant: foliar treatment: walnut: 6.56 % TRR (treated leaves); 1.70 % TRR (other tops); 1.92 % (roots) almond: 4.32 % TRR (treated leaves); 2.47 % TRR (other tops); 4.66 % (roots) pecan: 4.32 % TRR; (treated leaves); 1.4 % TRR (other tops) grapes: 9.2 % TRR (treated leaves), 2.5 % TRR (fruit) potato: 35.3 % TRR (tubers) wheat (desiccation treatment): 3.9 %TRR (chaff); 3.3 % TRR (straw); 2.8 % TRR (grain) apples: 6.5 % TRR identified as AMPA/N-methyl-AMPA mixture (treated leaves) coffee (identified as AMPA/N-methyl AMPA mixture): <0.9 % (treated leaves); <1 % (roots); <0.9 % (stems); 4.8 % (ripe coffee beans); 5.0 % (ripe pods) **plant: soil treatment:** potato: 6.6 % TRR (tuber); 31.0 % TRR after foliar application to the weeds followed by incorporation in soil soybean: 5.7 % TRR (forage); 2.7 % TRR (straw); 1.5 % TRR (hull), 1.6 % TRR (seed); 2.0 % TRR (hay) **plant: hydroponic treatment:** soybean: 9.2 % TRR (forage), 5.6 % TRR (roots) barley: 13.97 % TRR (aerial part/tops), 3.77 % TRR (roots) oats: 6.51 % TRR (aerial parts), 2.54 % TRR (roots) rice: 8.62 % TRR (aerial parts), 7.42 % TRR (roots) sorghum: 12.67 % TRR (aerial parts), 2.18 % TRR (roots) maize: 27.9 % TRR (forage), 10.1 % TRR (roots) wheat: 8.0 % TRR (forage), 8.8 % TRR (roots) cotton: 8.0 % TRR (forage), 8.9 % TRR (roots) coffee: 14.1 % TRR (aerial parts), 8.1 % TRR (roots) soybean: 16.6 % (forage, AMPA/N-methyl-AMPA) **rotational crops:** lettuce: 20.4 % TRR

			wheat: 20.5 % TRR (forage); 34.0 % TRR (grain); 12.7 % TRR (straw), 9.8 % (whole plant) radish: 12.3 % TRR (tops); 11.0 % TRR (roots) barley: 17.9 % TRR (grain); 16.6 % TRR (forage); 9.6 % TRR (straw) carrot: 1.4 % TRR (tops); 11.1 % TRR (roots); 2.9 % TRR (leaves); 9.0 % TRR (unknown part); 3.3 % TRR (leaves, primary crop) cabbage: 6.7 % TRR (whole plant), 8.4 % TRR (unknown part) beet: 4.6 % TRR (foliage); 12.5 % TRR (roots) peas: 11.1 % TRR (leaves); 15.6 % TRR (pods) string beans: 3.5 % TRR (leaves); 7.9 % TRR (pods), 1.9 % TRR (leaves, primary crop); 22.5 % TRR (pods, primary crop), 6.0 % TRR (unknown part, primary crop) sweet corn: 9.0 % TRR (cob); 11.1 % TRR (forage/first harvest) lettuce: 4.9 % TRR identified as glyphosate/AMPA mixture wheat: 3.7 % TRR identified as glyphosate/AMPA mixture (grain) **tolerant crops:** CP4-EPSPS or CP4-EPSPS and GOX modified crops: Foliar application sugar beet: 1.84 % TRR (tops), 3.79 % TRR (roots) wheat: 0.76 % TRR (forage), 3.45 %TRR (hay), 5.08 % TRR (straw), 10.77 % TRR (grain) maize: 15.9 % TRR (forage); 13.1 % TRR (silage); 11.2 % TRR (fodder); 60.3 % TRR (grain) canola: 7.7 % TRR soybean: 6.8 % TRR (forage); 12.8 % TRR (hay); 49.1 % TRR (seeds) cotton: 1.6 % TRR (forage), 1.4 % TRR (seed) **GAT modified crops:** Soil application followed by foliar applications

			maize: 4.0 % TRR (forage); 3.4 % TRR (stover); 6.1 % TRR (grain) canola: 1.4 % TRR (immature foliage); 1.9 % TRR (seeds) soybean: 39.3 % TRR (forage); 5.3 % TRR (hay); 11.2 % TRR (seeds); 10.2 % TRR (pods); 10.3 % TRR (foliage) soil: 50.1 % AR (aerobic lab) 30.2 % AR (anaerobic lab) 8.2 % AR (soil photolysis) 63.0 % of applied glyphosate (field) water: 16.0 % AR (aqueous photolysis, pH 5) 11.6 % AR (aqueous photolysis, pH 7) 42.7 % AR (aerobic mineralisation study) 15.7 % AR (water/sediment study) sediment: 18.7 % AR (water/sediment study)
N-methyl AMPA - QSAR number M03	IUPAC/CA name: [(Methylamino)methyl]phosphonic acid CP 70948 SMILES notation: CNCP(=O)(O)O		**plant: hydroponic treatment:** soybean: 1.1 % TRR (forage); 2.3 % TRR (roots) barley: 3.50 % TRR (aerial part/tops), 0.43 % TRR (roots) oats: 1.69 % TRR (aerial parts), 1.09 % TRR (roots) rice: 1.41 % TRR (aerial parts), 1.56 % TRR (roots) sorghum: 5.43 % TRR (aerial parts), 0.50 % TRR (roots) maize: 4.2 % TRR (forage), 0.6 % TRR (roots) wheat: 2.0 % TRR (roots) cotton: 2.0 % TRR (forage); 0.8 % TRR (roots) **rotational crops:** lettuce: 4.9 % TRR identified as glyphosate/AMPA mixture wheat: 3.7 % TRR identified as glyphosate/AMPA mixture (grain) **tolerant crops:** CP4-EPSPS modified crop, foliar application soybean: 0.6 % TRR (forage); 1.3 % TRR (hay); 0.8 % TRR (seeds)

N-acetyl glyphosate - QSAR number M04	IUPAC/CA name: N-acetyl-N-(phosphonomethyl)glycine SMILES notation: OC(=O)CN(CP(=O)(O)O)C(C)=O		**animal:** laying hen (administration of N-acetylglyphosate): 41.48 % TRR (egg white); 68.40 % TRR (egg yolk); 23.45 % TRR (abdominal fat); 25.22 % TRR (muscle); 63.81 % TRR (liver) lactating goat (administration of N-acetylglyphosate): 39.98 % TRR (milk); 21.43 % TRR (omental fat); 73.19 % TRR (renal fat); 64.73 % TRR (subcutaneous fat); 77.12 % TRR (kidney); 55.51 % TRR (liver); 16.70 % TRR (muscle) **tolerant crops:** GAT modified crops, soil application followed by foliar applications: maize: 27.0 % TRR (forage); 17.8 % TRR (stover); 63.8 % TRR (cobs); 51.2 % TRR (grain) canola: 89.5 % TRR (immature foliage); 79.6 % TRR (pods with seeds); 93.0 % TRR (foliage); 51.1 % TRR (seeds) soybean: 1.9 % TRR (forage); 19.2 % TRR (hay); 60.6 % TRR (grain); 27.7 % TRR (pod); 42.0 % TRR (foliage)
N-acetyl AMPA - QSAR number M05	IUPAC/CA name: [(Acetylamino)methyl]phosphonic acid SMILES notation: CC(=O)NCP(=O)(O)O		**animal:** laying hen (administration of N-acetylglyphosate): 4.34 % TRR (egg white); 1.10 % TRR (egg yolk); 10.18 % TRR (abdominal fat); 1.89 % TRR (muscle); 4.04 % TRR (liver) lactating goat (administration of N-acetylglyphosate): 4.31 % TRR (omental fat); 0.59 % TRR (renal fat); 14.86 % TRR (subcutaneous fat) **tolerant crops:** CP4-EPSPS or CP4-EPSPS and GOX modified crops, foliar application: canola: 0.9 % TRR (seed) soybean: 1.4 % TRR (seeds) GAT modified crops, soil application followed by foliar applications: maize: 1.7 % TRR (forage); 1.3 % TRR (stover); 5.0 % TRR (cobs); 9.4 % TRR (grain)

			canola: 3.4 % TRR (foliage); 14.7 % TRR (seeds) soybean 0.7 % TRR (hay); 2.2 % TRR (foliage); 23.5 % TRR (grain); 3.3 % TRR (pods)
N-glyceryl AMPA - QSAR number M06	IUPAC/CA name: (2,3-dihydroxypropanoyl-amino)methylphosphonic acid SMILES notation: O=C(NCP(=O)(O)O)C(O)CO		**tolerant crops:** CP4-EPSPS or CP4-EPSPS and GOX modified crops, foliar application: wheat: 0.34 % TRR (grain) maize: 0.5 % TRR (forage); 1.5 % TRR (silage); 1.6 % TRR (fodder); 6.9 % TRR (grain) canola: 3.9 % TRR soybean: 0.8 % TRR (hay); 1.6 % TRR (seeds)
N-malonyl AMPA - QSAR number M07	IUPAC/CA name: 3-oxo-3-(phosphonomethyl-amino)propanoic acid SMILES notation: O=C(CC(=O)O)NCP(=O)(O)O		**tolerant crops:** CP4-EPSPS modified crops, foliar application: soybean: 1.8 % TRR (seeds)
Methyl-phosphonic acid - QSAR number M08	IUPAC/CA name: Methylphosphonic acid SMILES notation: CP(=O)(O)O		**plant: hydroponic treatment:** soybean: 0.3 % TRR (roots) cotton: 2.0 % TRR (roots)
N-methyl glyphosate - QSAR number M09	IUPAC/CA name: 2-[methyl(phosphonomethyl)amino]acetic acid SMILES notation: CN(CC(=O)O)CP(=O)(O)O		**plant: hydroponic treatment:** cotton: 0.3 % TRR (roots)
HMPA - QSAR number M10	IUPAC/CA name: Hydroxymethylphosphonic acid SMILES notation: OCP(=O)(O)O		animal: - plant: - soil: - water: 10 % AR (water/sediment study) sediment: -

Level 3

Glyphosate

3 PROPOSED DECISION WITH RESPECT TO THE APPLICATION

3.1 BACKGROUND TO THE PROPOSED DECISION

3.1.1 Proposal on acceptability against the decision making criteria – Article 4 and annex II of regulation (EC) No 1107/2009

3.1.1.1 Article 4				
		Yes	No	
i)	It is considered that Article 4 of Regulation (EC) No 1107/2009 is complied with. Specifically the RMS considers that authorisation in at least one Member State is expected to be possible for at least one plant protection product containing the active substance for at least one of the representative uses.	X		
3.1.1.2 Submission of further information				
		Yes	No	
i)	It is considered that a complete dossier has been submitted	X		Please refer to data gaps under 3.1.4.
ii)	It is considered that in the absence of a full dossier the active substance may be approved even though certain information is still to be submitted because: (a) the data requirements have been amended or refined after the submission of the dossier; or (b) the information is considered to be confirmatory in nature, as required to increase confidence in the decision.			Not applicable
3.1.1.3 Restrictions on approval				
		Yes	No	
	It is considered that in line with Article 6 of Regulation (EC) No 1107/2009 approval should be subject to conditions and restrictions.		X	
3.1.1.4 Criteria for the approval of an active substance				
Dossier				
		Yes	No	
	It is considered the dossier contains the information needed to establish, where relevant, Acceptable Daily Intake (ADI), Acceptable Operator Exposure Level (AOEL) and Acute Reference Dose (ARfD).	X		
	It is considered that the dossier contains the information necessary to carry out a risk assessment and for enforcement purposes (relevant for substances for which one or more representative uses includes use on			Residues: It is considered that sufficient plant metabolism studies are available to cover glyphosate metabolism in conventional crops after both soil as well as foliar

	feed or food crops or leads indirectly to residues in food or feed). In particular it is considered that the dossier: (a) permits any residue of concern to be defined; (b) reliably predicts the residues in food and feed, including succeeding crops (c) reliably predicts, where relevant, the corresponding residue level reflecting the effects of processing and/or mixing; (d) permits a maximum residue level to be defined and to be determined by appropriate methods in general use for the commodity and, where appropriate, for products of animal origin where the commodity or parts of it is fed to animals; (e) permits, where relevant, concentration or dilution factors due to processing and/or mixing to be defined.			application. In addition, metabolism studies with crops with CP4 EPSPS or CP4 EPSPS and GOX modification, and GAT modified plants have been submitted. On the basis of the plant metabolism studies, the residue definition for enforcement of conventional crops is proposed as 'glyphosate', while the residue definition for enforcement of genetically modified crops is proposed as 'sum of glyphosate, AMPA and N-acetyl glyphosate, expressed as glyphosate'. For risk assessment, an overall residue definition for all crops has been proposed: sum of glyphosate, AMPA, N-acetyl glyphosate and N-acetyl AMPA, expressed as glyphosate. The metabolites N-acetyl glyphosate and N-acetyl AMPA are not relevant for conventional crops and crops with the CP4 EPSPS modification or CP4 EPSPS modification/GOX modification. Sufficient livestock metabolism studies are available. Based on these studies, the residue definition for enforcement of animal commodities is proposed as 'sum of glyphosate, AMPA and N-acetyl glyphosate, expressed as glyphosate'. The residue definition for risk assessment of animal commodities is proposed as 'sum of glyphosate, AMPA, N-acetyl-glyphosate and N-acetyl AMPA expressed as glyphosate'. The residue definitions are pending data gaps on genotoxicity for N-acetyl glyphosate and N-acetyl AMPA. Sufficient supervised residue trials are available in support of all intended uses, and existing MRLs are sufficiently high to cover the intended uses. However, additional supervised residue trials are required for the intended NEU use on olives. In addition, full acceptability of the residue data needs to be confirmed by additional information on extraction efficiency in all supervised residue trials. The calculated dietary burdens for all groups of livestock were found to exceed the trigger value of 0.004 mg/kg bw. Feeding studies are available, demonstrating that no residues are expected at the calculated dietary burden within the framework of the current renewal of glyphosate. Based on the available data, glyphosate, AMPA and N-Acetyl AMPA were shown stable during processing conditions simulating pasteurisation, baking/brewing/boiling, and sterilisation. Since residues were always <LOQ in the supervised residue trials, no further processing studies are required. It can be concluded that the metabolism in rotational crops is similar to the metabolism in primary crops. Field rotational crop studies are still required. The PECaccumulation value of AMPA should be taken into account to decide on an appropriate dose rate in these field studies. Subsequently, input values for the dietary burden calculation and the consumer risk assessment need to be derived from these field studies.

				The existing MRL for honey needs to be raised, however, one additional trial is still required. Furthermore, additional information regarding the extraction efficiency of the analytical method is needed for confirmation of the results from the available tunnel residue trials. No chronic or acute consumer risk has to be expected resulting from treatment of crops with glyphosate according to the GAP of the representative use for the current renewal of glyphosate. However, the consumer risk assessment is considered indicative and probably an underestimation, pending rotational crop field trials (not required for the defended use on orchards), and confirmation of the extraction efficiency in all supervised residue trials.
	It is considered that the dossier submitted is sufficient to permit, where relevant, an estimate of the fate and distribution of the active substance in the environment, and its impact on non-target species.	X		The information submitted is suitable to permit an estimate of the fate and distribution of the active substance in the environment and its impact on non-target species.
Efficacy				
		Yes	No	
	It is considered that it has been established for one or more representative uses that the plant protection product, consequent on application consistent with good plant protection practice and having regard to realistic conditions of use is sufficiently effective.	X		Glyphosate, as the active substance of MON 52276 is specific in its mode of action as it offers systemic control of a broad spectrum of weed species and is effective at a range of growth stages and timings. For further details see point 2.3.
Relevance of metabolites				
		Yes	No	
	It is considered that the documentation submitted is sufficient to permit the establishment of the toxicological, ecotoxicological or environmental relevance of metabolites.	X		It is considered that the documentation submitted is sufficient to permit the establishment of the toxicological, ecotoxicological or environmental relevance of metabolites.
Composition				
		Yes	No	
	It is considered that the specification defines the minimum degree of purity, the identity and maximum content of impurities and, where relevant, of isomers/diastereo-isomers and additives, and the content of impurities of toxicological, ecotoxicological or environmental concern within acceptable limits.	X		EU agreed minimum purity of glyphosate is 950 g/kg (95.0 % w/w) according to Commission Implementing Regulation (EU) 2017/2324. There are no additives intentionally added to the glyphosate technical. Impurities N-nitroso-glyphosate (NNG) and formaldehyde have been identified as being of (eco)toxicological relevance according to Commission Implementing Regulation (EU) 2017/2324. The level of NNG and formaldehyde in glyphosate technical are less than 1 mg/kg and 1 g/kg, respectively. Two new relevant impurities have been identified, trimethylamine and formic acid with levels are less than 2 g/kg and 4 g/kg respectively.

	It is considered that the specification is in compliance with the relevant Food and Agriculture Organisation specification, where such specification exists.	X		FAO Glyphosate: ≥ 950 g/kg
	It is considered for reasons of protection of human or animal health or the environment, stricter specifications than that provided for by the FAO specification should be adopted			Not applicable
Methods of analysis				
		Yes	No	
	It is considered that the methods of analysis of the active substance, safener or synergist as manufactured and of determination of impurities of toxicological, ecotoxicological or environmental concern or which are present in quantities greater than 1 g/kg in the active substance, safener or synergist as manufactured, have been validated and shown to be sufficiently specific, correctly calibrated, accurate and precise.	X		
	It is considered that the methods of residue analysis for the active substance and relevant metabolites in plant, animal and environmental matrices and drinking water, as appropriate, shall have been validated and shown to be sufficiently sensitive with respect to the levels of concern.	X		The monitoring methods are validated.
	It is confirmed that the evaluation has been carried out in accordance with the uniform principles for evaluation and authorisation of plant protection products referred to in Article 29(6) of Regulation 1107/2009.	X		
Impact on human health				
Impact on human health - ADI, AOEL, ARfD				
		Yes	No	
	It is confirmed that (where relevant) an ADI, AOEL and ARfD can be established with an appropriate safety margin of at least 100 taking into account the type and severity of effects and the vulnerability of specific groups of the population.	X		See section 2.6.10 The proposed ADI is 0.1 mg/kg bw/day and is based on a NOAEL of 10 mg/kg bw/day from a 2-year rat study with a standard assessment factor of 100. The proposed ARfD is 1.5 mg/kg bw/day and is based on an overall NOAEL of 150 mg/kg bw/day for developmental toxicity in the rabbit with a standard assessment factor of 100. The proposed AOEL is 0.03 mg/kg bw/day. The point of departure is a LOAEL of 30 mg/kg bw/day obtained from a 90-day rat study. By using an additional assessment factor of 2, a standard assessment factor of 100 and a correction for oral absorption of 20%, the resulting AOEL is 30 / (2*100) * 0.2 = 0.03 mg/kg bw/day.

				The proposed AAOEL is 0.3 mg/kg bw/day and is based on an overall NOAEL of 150 mg/kg bw/day for developmental toxicity in the rabbit, a standard assessment factor of 100 and a correction for oral absorption of 20%.
Impact on human health – proposed genotoxicity classification				
		Yes	No	
	It is considered that, on the basis of assessment of higher tier genotoxicity testing carried out in accordance with the data requirements and other available data and information, including a review of the scientific literature, reviewed by the Authority, **the substance SHOULD BE classified or proposed for classification**, in accordance with the provisions of Regulation (EC) No 1272/2008, **as mutagen category 1A or 1B.**		X	See section 2.6.4. Based on the available *in vitro* and *in vivo* genotoxicity studies (GLP-compliant guideline studies and public literature), no classification for germ cell mutagenicity is warranted.
Impact on human health – proposed carcinogenicity classification				
		Yes	No	
i)	It is considered that, on the basis of assessment of the carcinogenicity testing carried out in accordance with the data requirements for the active substances, safener or synergist and other available data and information, including a review of the scientific literature, reviewed by the Authority, **the substance SHOULD BE classified or proposed for classification**, in accordance with the provisions of Regulation (EC) No 1272/2008, **as carcinogen category 1A or 1B.**		X	See section 2.6.5. Based on data from long-term studies as well as on the epidemiological data as well as on in rats and mice, taking a weight of evidence approach, no hazard classification for carcinogenicity is warranted for glyphosate according to the CLP criteria.
ii)	Linked to above classification proposal. It is considered that exposure of humans to the active substance, safener or synergist in a plant protection product, under realistic proposed conditions of use, is negligible, that is, the product is used in closed systems or in other conditions excluding contact with humans and where residues of the active substance, safener or synergist concerned on food and feed do not exceed the default value set in accordance with Article 18(1)(b) of Regulation (EC) No 396/2005.			Not applicable
Impact on human health – proposed reproductive toxicity classification				
		Yes	No	
i)	It is considered that, on the basis of assessment of the reproductive toxicity testing carried out in accordance with the data requirements for the active substances, safeners or synergists and other available data and information, including a review of the scientific literature, reviewed by the Authority, **the substance SHOULD BE classified or proposed for**		X	See sections 2.6.6.1.2, 2.6.6.2.2, 2.6.6.3.2 and 2.6.6.4 of Vol 1. Based on the available reproductive toxicity studies, no classification for reproductive toxicity is warranted.

	classification, in accordance with the provisions of Regulation (EC) No 1272/2008, **as toxic for reproduction category 1A or 1B.**			
ii)	Linked to above classification proposal. It is considered that exposure of humans to the active substance, safener or synergist in a plant protection product, under realistic proposed conditions of use, is negligible, that is, the product is used in closed systems or in other conditions excluding contact with humans and where residues of the active substance, safener or synergist concerned on food and feed do not exceed the default value set in accordance with Article 18(1)(b) of Regulation (EC) No 396/2005.			Not applicable
Impact on human health – proposed endocrine disrupting properties classification				
		Yes	No	
i)	It is considered that **the substance SHOULD BE identified as having endocrine disrupting properties** in accordance with the provisions of point 3.6.5 in Annex II of Regulation (EC) No 1107/2009		X	The EATS modalities were sufficiently investigated and glyphosate does not induce EATS-mediated adversity and no EATS-related endocrine activity was observed *in silico, in vitro,* and *in vivo.*
ii)	Linked to above identification proposal. It is considered that exposure of humans to the active substance, safener or synergist in a plant protection product, under realistic proposed conditions of use, is negligible, that is, the product is used in closed systems or in other conditions excluding contact with humans and where residues of the active substance, safener or synergist concerned on food and feed do not exceed the default value set in accordance with Article 18(1)(b) of Regulation (EC) No 396/2005.			Not applicable
Fate and behaviour in the environment				
Persistent organic pollutant (POP)				
		Yes	No	
	It is considered that the active substance **FULFILS** the criteria of a persistent organic pollutant (POP) as laid out in Regulation 1107/2009 Annex II Section 3.7.1.		X	The criterion for persistence is fulfilled (please refer to level 2.8 for details). The criterion for potential for long-range environmental transport is not fulfilled. The criterion for bioaccumulation is not fulfilled.
Persistent, bioaccumulative and toxic substance (PBT)				
		Yes	No	

	It is considered that the active substance **FULFILS** the criteria of a persistent, bioaccumulative and toxic (PBT) substance as laid out in Regulation 1107/2009 Annex II Section 3.7.2.		X	The criterion for persistence is fulfilled (please refer to level 2.8 for details). The criterion for bioaccumulation and toxicity (environmental hazard) are not fulfilled.
Very persistent and very bioaccumulative substance (vPvB).				
		Yes	No	
	It is considered that the active substance **FULFILS** the criteria of a a very persistent and very bioaccumulative substance (vPvB) as laid out in Regulation 1107/2009 Annex II Section 3.7.3.		X	The criterion for persistence is fulfilled (please refer to level 2.8 for details). The criterion for bioaccumulation is not fulfilled.
Ecotoxicology				
		Yes	No	
i	It is considered that the risk assessment demonstrates risks to be acceptable in accordance with the criteria laid down in the uniform principles for evaluation and authorisation of plant protection products referred to in Article 29(6) under realistic proposed conditions of use of a plant protection product containing the active substance, safener or synergist. The RMS is content that the assessment takes into account the severity of effects, the uncertainty of the data, and the number of organism groups which the active substance, safener or synergist is expected to affect adversely by the intended use.		X	For non-target organisms other than aquatic organisms and wild mammals, the risk can be considered acceptable for all representative uses. For aquatic organisms, several data gaps have been identified. The finalisation of the risk assessment is pending the submission of these new data, particularly the ones related to algae and aquatic plants. For wild mammals, further information is needed to support the kinetic analysis of the residue decline data used for the higher tier risk assessment.
ii	It is considered that, the substance **SHOULD BE identified as having endocrine disrupting properties** that may cause adverse effects on non-target organisms in accordance with the provisions of point 3.8.2 in Annex II of Regulation (EC) No 1107/2009.		X	Glyphosate is not expected to have endocrine disrupting properties according to the EFSA/ECHA guidance (2019) for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009.
iii	Linked to the consideration of the endocrine properties immediately above. It is considered that the exposure of non-target organisms to the active substance in a plant protection product under realistic proposed conditions of use is negligible.			Not applicable
iv	It is considered that it is established following an appropriate risk assessment on the basis of Community or internationally agreed test guidelines, that the use under the proposed conditions of use of plant protection products containing this active substance, safener or synergist: — will result in a negligible exposure of honeybees, or	X		The proposed conditions of use of plant protection products containing glyphosate is expected to have no unacceptable acute or chronic effects on colony survival and development, taking into account effects on honeybee larvae and honeybee behaviour.

	— has no unacceptable acute or chronic effects on colony survival and development, taking into account effects on honeybee larvae and honeybee behaviour.			
Residue definition				
		Yes	No	
	It is considered that, where relevant, a residue definition can be established for the purposes of risk assessment and for enforcement purposes.	X		A residue definition can be established for environmental compartments (see level 2.8.5). Residues: reference is made to 2.2.14
Fate and behaviour concerning groundwater				
		Yes	No	
	It is considered that it has been established for one or more representative uses, that consequently after application of the plant protection product consistent with realistic conditions on use, the predicted concentration of the active substance or of metabolites, degradation or reaction products in groundwater complies with the respective criteria of the uniform principles for evaluation and authorisation of plant protection products referred to in Article 29(6) of Regulation 1107/2009.	X		PECgw for glyphosate and AMPA are below 0.1 µg/L for the available simulated uses (late application on orchards and potatoes; application on railway). A data gap is set for the applicant to provide PECgw calculations for all intended uses.

3.1.2 Proposal – Candidate for substitution

Candidate for substitution				
		Yes	No	
	It is considered that the active substance shall be approved as a candidate for substitution		X	The ADI, ARfD or AOEL of glyphosate are not significantly lower than those of the majority of the approved active substances. There is no indication that glyphosate is developmental neurotoxic or immunotoxic. Currently glyphosate is not classified for mutagenicity, carcinogenicity or reproductive toxicity and RMS remains of the opinion that classification is not required. For environment and ecotoxicology, the active substance does not fulfill the e-fate/ecotox criteria to be identified as candidate for substitution.

				Glyphosate does not meet the criteria for an endocrine disruptor.

3.1.3 Proposal – Low risk active substance

Low-risk active substances				
		Yes	No	
	It is considered that the active substance **shall be considered of low risk.** If the active substance is not a micro-organism, in particular it is considered that: (a) the substance **should NOT be classified or proposed for classification** in accordance to Regulation (EC) No 1272/2008 as any of the following: — carcinogenic category 1A, 1B or 2, — mutagenic category 1A, 1B or 2, — toxic to reproduction category 1A, 1B or 2, — skin sensitiser category 1, — serious damage to eye category 1, — respiratory sensitiser category 1, — acute toxicity category 1, 2 or 3, — specific Target Organ Toxicant, category 1 or 2, — toxic to aquatic life of acute and chronic category 1 on the basis of appropriate standard tests, — explosive, — skin corrosive, category 1A, 1B or 1C; (b) it has **not been identified as priority substance under Directive 2000/60/EC**; (c) it is **not deemed to be an endocrine disruptor** in accordance to Annex II of Regulation (EC) No 1107/2009; (d) it **has no neurotoxic or immunotoxic effects**;		X	Glyphosate is currently classified for Eye Damage, Category 1 (H318) and the RMS is of the opinion that classification should be retained, therefore the substance cannot be considered of low risk. Currently glyphosate is not classified for carcinogenicity, mutagenicity, reproductive toxicity, skin or respiratory sensitisation, acute toxicity, STOT-SE or STOT-RE and the RMS remains of the opinion that classification is not required. There is no indication that glyphosate is developmental neurotoxic or immunotoxic. Glyphosate is proposed for classification as: Aquatic chronic category 2 for environmental hazard. Glyphosate is not persistent and its bio-concentration factor is lower than 100. Glyphosate does not meet the criteria for an endocrine disruptor.

	(e) it **is not persistent** (half-life in soil is more than 60 days) or its **bio-concentration factor is lower than 100**. (f) it is a **semiochemical** and verifies points (a) to (d). Paragraph (e) doesn't apply to naturally occurring active substances. If the active substance is a micro-organism, in particular it is considered that at strain level the micro-organism has not demonstrated multiple resistance to anti-microbials used in human or veterinary medicine. If the active substance is a baculovirus, in particular it has not demonstrated adverse effects on non-target insects.			

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

Data gap	Relevance in relation to representative use(s)	Study status		
		No confirmation that study available or on-going.	Study on-going and anticipated date of completion	Study available but not peer-reviewed
3.1.4.1 Identity of the active substance or formulation				
See volume 4 of each source	Relevant for all uses			
3.1.4.2 Physical and chemical properties of the active substance and physical, chemical and technical properties of the formulation				
None				
3.1.4.3 Data on uses and efficacy				
None				
3.1.4.4 Data on handling, storage, transport, packaging and labelling				
None				
3.1.4.5 Methods of analysis				
See volume 4 of each source concerning data gaps on significant impurities.	Relevant for all uses			
Barclay: J-CA 4.1.1/001; Study No.OS-012Determination of active content and impurity profile of glyphosate; [redacted]2009f Analytical method on formaldehyde and NNG. A validated LOQ for NNG and formaldehyde in agreement with specifications should be provided	Relevant for all uses	x		
Barclay:	Relevant for all uses	x		

J-CA 4.1.1/002; Qualitative and quantitative profile of the test substance glyphosate technical (five batch analysis); [redacted].; 2008 Analytical method on NNG and formaldehyde. A validated LOQ for NNG in agreement with specification should be provided. The precision sample should be demonstrated for formaldehyde.				
Barclay: J-CA 4.1.1/005 G. L., report 15846.030.002.16; analytical method for the determination of formaldehyde; [redacted] The precision sample should be demonstrated for formaldehyde	Relevant for all uses	x		
[redacted] J-CA 4.1.1/023 ; Five Batches Analysis of Technical Grade Active Ingredient (TGAI) Glyphosate; [redacted] ; 2020 Analytical method on formaldehyde and NNG	Relevant for all uses	x		
Industrias Afrasa: J-CA 4.1.1/001; Study No.OS-012Determination of active content and impurity profile of glyphosate; [redacted] 2009f Analytical method on formaldehyde and NNG. A validated LOQ for NNG and formaldehyde in agreement with specifications should be provided	Relevant for all uses	x		
Industrias Afrasa: KCA section 1/021; study SSL04409; [redacted] (2010) The Horrat value reported for acccuracyis above 1 (but < 2) for the content 0.025 g/kg (formaldehyde) and 0.61 mg/kg (for NNG). An explanation should be provided	Relevant for all uses	x		
Multiresidue method for plant and food of animal origin	Relevant for all uses	x		
Plant matrices : Monitoring methods - A cross validation with incurred residue in plants to compare extraction with	Relevant for all uses	x		

dichloromethane and without dichloromethane taking into account the ratio between solvent sample				
Animal matrices : Monitoring methods - A strong argumentation to demonstrate that pH does not affect the extraction efficiency	Relevant for all uses	x		
Extraction efficiency of the analytical method for determination of residue in honey	Relevant for all uses	x		
The demonstration of derivatisation efficiency for methods CA 4.1.2/119, CA 4.1.2/128 and CA 4.1.2/129	Relevant for all uses	x		
3.1.4.6 Toxicology and metabolism				
1) Volume 1, section 2.6.1.1 short summary on toxicokinetic information. A public literature study is available in which 13 poisoning incidents with glyphosate-based herbicides in France (Zouaoui *et al.*, 2012) were analysed. This publication was evaluated during the previous assessment of glyphosate by RMS DE. However, it is not re-submitted by the applicant. The applicant is requested to submit this publication together with a summary and a relevance and reliability assessment of this publication.	Relevant for all uses	x		
2) Volume 1, section 2.6.2 acute toxicity The applicant is requested to justify why for the same batch different conclusions are drawn regarding the purity and the acceptability of acute toxicity studies. Study CA 5.2.1/020 acceptable Study CA 5.2.3/016 acceptable Study CA 5.2.4/012 supportive due to low purity Study CA 5.2.5/015 supportive due to low purity Study CA 5.2.6/016 acceptable	Relevant for all uses	x		
3) Volume 1, Section 2.6.2.10.1	Relevant for all uses	x		

During the previous assessment, it was noted that for formulations, Burger et al. (2009, refer to Volume 1 2.6.9) reported cases from Germany that might indicate respiratory irritation but these findings were considered to be likely due to POEA surfactants (tallowamines) present in the formulation. The RMS notes that this study was not re-submitted for the present evaluation. The applicant is requested to submit this publication together with a summary and a relevance and reliability assessment of this publication.				
4) Vol 3 CA B.6.2.3.13 (CA 5.2.3/013) In the study report two different lot numbers and purities are reported for the test substance. As no certificate of analysis was attached to the study report, the applicant is asked to further clarify which lot and purity has been used for the test	Relevant for all uses	x		
5) Volume 3 CA B.6.3.2.6 and B.6.3.2.13 and Volume 1 sections 2.6.3.1.1, 2.6.8.2 and 2.6.10 Cellular alterations in the parotid gland were also reported in a NTP study in rats and mice (Chan and Mahler, 1992). However, this study was not submitted. The applicant is requested to submit this study with an OECD summary and an evaluation of the results in rats and mice including the mechanistic study on the salivary gland and including effects on toxicity to reproduction.	Relevant for all uses	x		
6) Volume 3 CA B.6.3.2.24 (CA 5.3.2/033) Plasma phosphorus levels were lower in the male treated groups at week 52 but this was due, in part, to slightly higher individual control values. The RMS notes that the same pattern was observed during week 4, 13, 26 and 52. The applicant is requested to provide HCD on phosphorus levels in blood in order to determine whether this was indeed due to higher (individual) control values.	Relevant for all uses	x		
7) Volume 3 CA B.6.3.2.26 (CA 5.3.2/036) Decreased phosphorus levels, although statistically significant in females at 3 and 12 months, did not appear to be related to compound administration since the values were within the normal	Relevant for all uses	x		

range. The applicant is requested to provide HCD on phosphorus levels in blood in females.				
8) Volume 3 CA B.6.4.2.1 (CA 5.4.2/001) This study was not included in the reference list. The applicant is requested to add this study to the reference list.	Relevant for all uses	x		
9) Volume 3 CA B.6.4.2.2 (CA 5.4.2/002) This study was not included in the reference list. The applicant is requested to add this study to the reference list.	Relevant for all uses	x		
10) Volume 3 CA B.6.4.2.10 (CA 5.4.2/010) AGG notes that a discrepancy was seen regarding the batch number and purity reported in the study report and in the certificate of analysis that is attached to the study report. The applicant is asked to clarify this.	Relevant for all uses	x		
11) Volume 1, section 2.6.4.1 and Volume 3, CA B.6.10 The applicant provided a justification for the 1 mM concentration threshold as a criterium for relevance of public literature publication. The RMS largely agrees with the justification, however, a reference should be provided for the study in which an oral dose of 1,430 mg/kg bw (given as a formulation of 71.7% w/w glyphosate) resulted in plasma levels of 38.1 µg/mL in the rat. If the study is not already included in the dossier, the study should be submitted and evaluated. In addition, a further justification should be given on whether locally higher levels of glyphosate at cellular level could be reached (e.g. in intestinal epithelial cells and/or in the local lymphatic vessels of the intestinals).	Relevant for all uses	x		
12) Volume 3 CA B.6.5.5 (CA 5.5/005) The applicant is asked to provide historical control data for the effect on mandibular lymph node lymphoma, if available.	Relevant for all uses	x		
13) Volume 3 CA B.6.5.18.13 (CA 5.5-038 Alavanja)	Relevant for all uses	x		

The provided reference (K-CA 5.5-038) only concerns a correspondence to the article by Alavanja et al. 2013. Although the full article is publicly available online and could be reviewed by the AGG, the applicant is requested to submit the full publication to complete the dossier.				
14) Volume 1, section 2.6.5.1 – skin keratoacanthomas The applicant is requested to provide a trend test for the incidences of skin keratoacanthomas for the [redacted] study.	Relevant for all uses	x		
15) Volume 1, section 2.6.5.1.2.2. summary of epidemiological studies The applicant is requested to submit a full assessment including a relevance and reliability assessment of the following studies: Chang and Dellzell (2016) Zhang et al. (2019) Leon et al. (2019)	Relevant for all uses	x		
16) Volume 1, section 2.6.7 neurotoxicity During the previous assessment, several additional public literature studies were evaluated. These were not included in the evaluation of the applicant for the AIR-5 renewal. The applicant is requested to submit these publications together with an evaluation (including a relevance and reliability assessment) and an overall assessment.	Relevant for all uses	x		
17) Volume 3, CA B.6.8.1.1.5 skin sensitisation AMPA study 2 (CA 5.8.1/012) For challenge, the test material was selected at a concentration of 25%. The applicant is kindly asked to provide an argumentation why a higher concentration was not tested, also taking into account that higher concentrations were achieved in other studies (CA 5.8.1/011).	Relevant for all uses	x		

18) Volume 3, CA B.6.8.1.1.8 genotoxicity *in vivo* – AMPA study 2 (CA 5.8.1/027) The applicant is kindly asked to provide more detailed information on the historical negative control data, for instance when data were generated.	Relevant for all uses	x		
19) Volume 3, CA B.6.8.1.1.11 QSAR and read-across (submitted as CA 6.7.1/001) a) The applicant mentions that experimental genotoxicity data is available for N-methyl glyphosate (M09). The applicant is requested to submit the data as these were not included in the dossier. b) The applicant proposed a grouping approach for read-across for the other metabolites, however, this approach was not accepted by the RMS	Relevant for all uses	x		
20) Volume 1, section 2.6.8.1.2 N-acetyl AMPA The applicant is requested to provide an *in vitro* micronucleus study to address aneugenicity for N-acetyl AMPA.	Relevant for all uses	x		
21) Volume 1, section 2.6.8.1.3 N-acetyl glyphosate The applicant is requested to provide an *in vitro* micronucleus study to address aneugenicity for N-acetyl glyphosate	Relevant for all uses	x		
22) Volume 3, CA B.6.8.3.2 *in vitro* estrogen receptor alpha transcriptional activation assay The applicant is asked to provide support for the statement that minor deviations from the performance criteria do not affect the validity of these studies. The RMS considers that the deviation from the acceptance criteria for 17α-methyltestosterone may indicate a decreased sensitivity of the study for weak agonists.	Relevant for all uses	x		

23) Volume 1, section 2.6.9 Reports on medical surveillance on manufacturing plant personnel The absence of occupational exposure data from the European plants in the current dossier needs a further clarification from the applicant.	Relevant for all uses	x		
24) Volume 1, section 2.6.9 glyphosate in human breast milk The RMS noted that in Volume 3 section B.6.10 one additional study was reported investigating glyphosate in human breast milk samples (Abdel-Halim, 2019). The applicant reported that the reason for not submitting this study was that this study was considered supplementary due to several limitations. AGG disagrees and requests the applicant to submit this publications and to provide an assessment of the findings in order to evaluate the findings.	Relevant for all uses	x		
25) Volume 3, CA B.6.10 literature search The RMS notes that the search terms used are focussed on the data requirements and some specific search term which are considered relevant for human health are missing. For example, a quick search by the RMS retrieved the following publications which were not found in the literature search by the applicant: 1) Rueda-Ruzafa, L., Cruz, F., Roman, P., Cardona, D. Gut microbiota and neurological effects of glyphosate. (2019) NeuroToxicology 2) Pu Y, Yang J, Chang L, Qu Y, Wang S, Zhang K, Xiong Z, Zhang J, Tan Y, Wang X, Fujita Y, Ishima T, Wang D, Hwang SH, Hammock BD, Hashimoto K. Maternal glyphosate exposure causes autism-like behaviors in offspring through increased expression of soluble epoxide hydrolase. Proc Natl Acad Sci U S A. 2020 May 26;117(21):11753-11759. The applicant is requested to provide an additional literature search using endpoint specific search terms related to human health which are outside the data requirements such as autism, asthma, ADHD, coeliac disease, inflammatory bowel disease and obesity. The applicant is requested to submit all relevant	Relevant for all uses	x		

<table>
<tr><td>publications obtained from this search including a summary and an evaluation of these publications (including a relevance and reliability assessment).</td><td></td><td></td><td></td><td></td></tr>
<tr><td>26) Volume 3, CA B.6.10 literature search general

The applicant is requested to submit a summary and an evaluation of the following Category B (supplementary) studies as AGG disagrees that the studies should be considered as supplementary only. An evaluation and conclusion of these studies should be provided by GRG.

- No. 37 Tang et al., 2020; Glyphosate exposure induces inflammatory responses in the small intestine and alters gut microbial composition in rats.

- No 13 Donato et al., 2020; Exposure to glyphosate and risk of non-Hodgkin lymphoma and multiple myeloma: an updated meta-analysis.</td><td>Relevant for all uses</td><td>x</td><td></td><td></td></tr>
<tr><td>27) Volume 3 CA B.6.10 literature search Endocrine Disruption

To allow for a transparent evaluation of the possible endocrine disruption properties of glyphosate, the applicant is requested to (re-) submit the studies in the table below including (more) detailed studies summaries and an evaluation.

<table><tr><th>Author</th><th>Year</th><th>Title</th><th>Source</th></tr><tr><td>Abarikwu S. O. et al.</td><td>2015</td><td>Combined effects of repeated administration of Bretmont Wipeout (glyphosate) and Ultrazin (atrazine) on testosterone, oxidative stress and sperm quality of Wistar rats.</td><td>Toxicology mechanisms and methods (2015), Vol. 25, No. 1, pp. 70-80</td></tr><tr><td>Avila-Vazquez M. et al.</td><td>2015</td><td>Cancer and detrimental reproductive effects in an Argentine agricultural community environmentally exposed to glyphosate</td><td>Journal of Biological Physics and Chemistry, (2015) Vol. 15, No. 3, pp. 97-110.</td></tr><tr><td>Bernieri T. et al.</td><td>2019</td><td>Occupational exposure to pesticides and thyroid function in Brazilian soybean farmers.</td><td>Chemosphere, (2019) pp. 425-429</td></tr></table></td><td>Relevant for all uses</td><td>x</td><td></td><td></td></tr>
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Parvez S. et al.	2018	Glyphosate exposure in pregnancy and shortened gestational length: a prospective Indiana birth cohort study	Environmental Health, (2018) Vol. 17, pp. 23				
Owagboriaye F. et al.	2019	Comparative studies on endogenic stress hormones, antioxidant, biochemical and hematological status of metabolic disturbance in albino rat exposed to roundup herbicide and its active ingredient glyphosate.	Environmental science and pollution research international, (2019) Vol. 26, No. 14, pp. 14502-14512				
Kass L. et al.	2020	Relationship between agrochemical compounds and mammary gland development and breast cancer.	Molecular and cellular endocrinology, (2020) Vol. 508, Art. No. 110789				
George A. et al.	2018	The effect of glyphosate on human sperm motility and sperm DNA fragmentation	International Journal of Environmental Research and Public Health (2018) Vol. 15, 1117				
Santos R. et al.	2019	Thyroid and reproductive hormones in relation to pesticide use in an agricultural population in Southern Brazil.	Environmental Research, (2019) pp. 221-231				
28) Volume 1, section 2.10 Endocrine disrupting properties; section on "Lines of evidence for adverse effects and endocrine activity related to EAS-modalities". It is noted that three additional studies from public literature are available, which were submitted at a later time point. These studies (B.6.8.3.17, B.6.8.3.18, B.6.8.3.19) were not included in appendix E by the applicant. The applicant is requested to add these studies to appendix E.					Relevant for all uses	x	
29) Study summary for Abarikwu et al. (2015). Toxicology Mechanisms and Methods, 25 :1, 70-80. A brief study summary was submitted October 2020. A more detailed summary is requested.					Relevant for all uses	x	

30) Volume 1, section 2.6.5.1 The applicant is requested to provide the 2-year study in rats ([redacted] 1987) and the 2-year study in mice ([redacted] 1987), if possible, and an assessment of the studies.	Relevant for all uses	x		
31) Provide a single Appendix E compiling toxicological and ecotoxicological data.	Relevant for all uses			
3.1.4.7 Residue data				
Field rotational crop studies	The 'post-harvest, pre-sowing, pre-planting, pre-emergence outdoor use' & the inter-row use.		x (only a limited field rotational crop study)	
Supervised residue trials with olives in NEU	The NEU use on olives.	x		
One additional tunnel residue trial	Relevant for all uses	x		
A public literature study is available (Krüger *et al.* (2014)), which was evaluated during the previous assessment of glyphosate in 2015. The applicant is requested to submit this publication together with a summary and an assessment of this publication.	Relevant for all uses	x		
3.1.4.8 Environmental fate and behaviour				
For field studies performed outside EU, provide a comparison of actual field sites properties instead of default root ecoregions of the trial soils	Relevant for all uses	x		
For studies [redacted], 1993 and [redacted], 1993 (field studies), the distance of the weather station from the sites (when not onsite) should be provided	Relevant for all uses	x		
For study [redacted] 2020, 1- provide kinetic fittings for AMPA for Egerkingen soil 2- explanations and examples of the data processing are required. 3- justification should be given on the use of MARS database instead of measured data at nearest station for Egerkingen site	Relevant for all uses	x		

4- clarify the differences observed between weather values presented in the studies [redacted], 1992c and [redacted], 2020 for station Schallstadt-Mengen (Bad Krozingen site). 5- justify the use of data from "Löningen" site for normalization of data in Menslage soil in [redacted], 2020 study, instead of "Menslage-Borg" station mentioned in [redacted], 1992d. 6- for data processing and normalization, justify the use of different approaches for bulk density estimation and rationale behind the choice of a default data or a calculated value 7- Justify the rationale behind the estimation of organic matter in 30-100 cm horizon 8- justify the choice of the lower boundary condition (free drainage) for each site.				
For study [redacted], 2020 1- provide further kinetic fittings for glyphosate for New York site 2-provide a decline fit for AMPA for Ohio site 3- explanations and examples of the data processing are required. 4- clarify the approach used for processing of the data at T0 and relevant bulk density used in California site ([redacted], 1993). 5- update kinetics for the four sites from [redacted], 1993, CA 7.1.2.2.1/006 and [redacted], 1993, CA 7.1.2.2.1/005 considering replicate values. 6- provide a normalisation of data from sites Ohio ([redacted] 1993b) and Ontario ([redacted] 1993), if reliable data can be obtained from available weather stations, and provide a kinetic assessment to derive modelling endpoints. 7- justify the choice of the lower boundary condition (free drainage) for each site.	Relevant for all uses	x		
Field dissipation studies to determine the degradation rates of AMPA (and covering a sufficient range of soil pH)	Relevant for all uses	x		
Provide the literature article Dollinger et al. (2016) mentioned in the Dollinger et al. (2018) article	Relevant for all uses	x		
For study [redacted], 2020, confirm that the LOQ is at least two orders of magnitude below the lowest nominal concentration tested.	Relevant for all uses	x		

For study [redacted], 1993, confirm that the LOQ is at least two orders of magnitude below the lowest nominal concentration tested.	Relevant for all uses	x		
For study [redacted] (2005), update the kinetic analysis according to FOCUS guidance for light exposed systems with natural water.	Relevant for all uses	x		
For study [redacted] (1992), update the kinetic analysis according to FOCUS guidance for light exposed systems and provide data on the equivalence between continuous artificial sunlight used in the study and natural sunlight conditions.	Relevant for all uses	x		
For study [redacted] (2020), provide the amended report with information of the characterization of the unknown radioactivity.	Relevant for all uses	x		
For study [redacted] 1993 (with amendment [redacted] 1995) the low mass balance should be further justified	Relevant for all uses	x		
For study [redacted] (2003), updated kinetic evaluation should be provided using the HPLC analysis results.	Relevant for all uses	x		
Further address quantitatively or qualitatively metabolite 1-oxo-AMPA, quantified in sediment in Feser-Zügner 2002. Unless it is shown that the trigger is not exceeded or the ecotoxicological risk can be addressed qualitatively, PECsed calculations should be provided for 1-oxo-AMPA, based on default conservative substance properties in the absence of data.	Relevant for all uses	x		
Update PECgw and PECsw/PECsed for glyphosate and metabolites, considering the application schemes initially proposed, the endpoints agreed during the peer review and using all relevant models.	Relevant for all uses	x		
Monitoring data for groundwater: provide additional information on the measured concentration above the trigger of 0.1 µg/L for glyphosate. Additional assessment is required to confirm the exceedances are not related to long-term contamination in some locations and/or could not be attributed to particular context.	Not applicable			
Monitoring data for groundwater: provide further information on the outlier exclusion procedure in [redacted], 2020, CA7.5/002, and provide details of the values excluded.	Not applicable			
Monitoring data for surface water: provide further information on the outlier exclusion procedure in [redacted]s, 2020, CA7.5/002,	Not applicable			

and provide details of the data excluded. This is to confirm which maximum concentration should be retained for both ghlyphosate and AMPA in surface water, within the data set of [redacted], 2020 CA7.5/002 and [redacted], 2016, CA7.5/010				
Monitoring data for drinking water: clarify the definition of drinking water considered in the monitoring data collection of drinking water, at least from the aggregated data that may be clearer on the origin of the water types taken as supply for drinking water.	Not applicable			
3.1.4.9 Ecotoxicology				
Provide an update of the ED assessment in order to include the Tox Cast and *in vitro* data for the ecotoxicological assessments and lines of evidence. Provide a single Appendix E compiling toxicological and ecotoxicological data.	Relevant for all uses			
Full-text article and Study summary for Bolis *et al.* (2020), Environmental Pollution, Vol. 263, No. Part_B, pp. 114395	Relevant for all uses	x		
Full-text article and Study summary for Freitas *et al.* (2020), Human and Experimental Toxicology, Vol. 39, No. 5, pp. 596-604	Relevant for all uses	x		
Full-text article and Study summary for Moutinho *et al.* (2020), Ecotoxicology, 29, pages 1043–1051	Relevant for all uses	x		
Further information to support non-relevance for Imre *et al.* (2020), Novenyvdelem, Vol. 56, No. 1, pp. 1-9. ISSN: 0133-0829.	Relevant for all uses	x		
Full-text article and Study summary for Riaño *et al.* (2020), Chemosphere, Vol. 250, pp. 126287	Relevant for all uses	x		
Study summary for Ruamthum *et al.* (2011), Commun Agric Appl Biol Sci 2011;76(4):923-30.	Relevant for all uses	x		

Full-text article and Study summary for Szabo *et al.* (2019), AGROFOR International Journal, Vol. 4, No. 3, pp. 76-82.	Relevant for all uses	x		
Full-text article and Study summary for Ujhegyi *et al.* (2020), Ecological Indicators, Vol. 113, 106175	Relevant for all uses	x		
Study summary for Mestre et al. (2020) Chemosphere, (2020) Vol. 252, Art. No. 126433	Relevant for all uses	x		
Study summary for Odetti et al. (2020) Ecotoxicology and environmental safety, (2020) Vol. 193, Art. No. 110312	Relevant for all uses	x		
Ruuskanen *et al.* (2020), Environmental science & technology (2020), Vol. 54, No. 2, pp. 1128-1135: Request biological raw data, at least for food consumption and body weight; Rewrite the study summary to clearly reflect the differences between the 2 experiments, i.e., the short term food preference and the long term dietary exposure. Further consideration on potential impact of the effects observed in Ruuskanen et al. (2020) on avian populations.	Relevant for all uses	x		
Study summary for Ruuskanen *et al.* (2020), Scientific reports, Vol. 10, No. 1, pp. 6349	Relevant for all uses	x		
Further information related to the kinetic evaluation of the available residue decline data used for the higher tier risk assessment for wild mammals..	Relevant for all uses	x		
Further consideration on possible risk for amphibians and reptiles. (EFSA Scientific Opinion on the state of the science on pesticide risk assessment for amphibians and reptiles (2018) may provide useful information).	Relevant for all uses	x		
Provide a statistical power analysis as presented in appendix 5 of the OECD 210 (2013) guideline to confirm the robustness of the NOEC for the ELS study on fish with AMPA ([redacted], 2011, CA 8.2.2.1/004)	Relevant for all uses	x		

Provide toxicity tests on *Lemna* and emergent macrophytes with an exposure via overspray with the active substance and the formulation.	Relevant for all uses	x		
Provide a toxicity test on sediment dwelling organisms for glyphosate, AMPA and 1-oxo-AMPA.	Relevant for all uses	x		
In relation with e-fate data gap, provide further information to assess the risk assessment for metabolite 1-oxo-AMPA for sediment dwelling organisms.	Relevant for all uses	x		
Provide calculation of 96h-ECx values, NOEC and LOEC for the toxicity study on *Anabaena flos-aquae* with glyphosate [redacted], 1987, CA 8.2.6.2/002)	Relevant for all uses	x		
Provide calculation of 72h-ECx values NOEC and LOEC based on yield and growth rate for the toxicity study on *Navicula pelliculosa* ([redacted] 1987, CA 8.2.6.2/005)	Relevant for all uses	x		
Provide calculation of growth rate ECx values based on dry weight for the test on Lemna minor with glyphosate ([redacted] 2002, CA 8.2.7/001)	Relevant for all uses	x		
Provide a toxicity test on rooted macrophytes for glyphosate	Relevant for all uses	x		
Explain the differences in toxicity between the studies for the dossier and the public literature and to further investigate herbicide effects of glyphosate to phytoplankton, algae and macrophytes (data gap).	Relevant for all uses	x		
Provide an english certified translation of the article of Yanhui et al., 2015 (CA 8.2.7/013) related to toxicity of glyphosate to *Spirodela polyrhiza*	Relevant for all uses	x		
Provide a new toxicity test on alga with the representative formulation MON52276.	Relevant for all uses	x		

Provide 7d ECx (EC10, EC20 and EC50) based on growth rates for dry weight parameter for the study of [redacted] 2002 (CP 10.2.1/005)	Relevant for all uses	x		
Provide a statistical reanalysis (NOEC, LC10/20) and information on the extent of lethargy of the study of [redacted] 2000 (CA 8.2.2.1/002)	Relevant for all uses	x		
In relation with the request to update PECsw/PECsed for glyphosate and metabolites, considering the endpoints agreed during the peer review, provide an updated risk assessment for: -Aquatic organisms -Bees (surface water and puddle).	Relevant for all uses	x		
In relation to the data gap set for rotational crops in the residue section, provide further consideration of the relevance of metabolites for bees	Relevant for all uses	x		
Provide EC10/EC20 estimates for the chronic toxicity test on earthworms with AMPA ([redacted] 2003, CA 8.4.1/003)	Relevant for all uses	x		
Provide clarification for the study on effect on Soil Microbial Nitrogen Transformations with glyphosate of [redacted] 2014 (CA 8.5/001) regarding the lack of measurments at day 7.	Relevant for all uses	x		
Provide calculation of soil nitrogen transformation rate expressed in mg nitrate/kg dry weight soil/day between each measurement day for the study on effects on the Activity of Soil Microflora of AMPA ([redacted] 2010, CA 8.5/004)	Relevant for all uses	x		
Provide clarification for the study on effect on Soil Microbial Activity, Carbon and Nitrogen Transformations of MON 52276 ([redacted] 2012, CP 10.5/001) regarding the lack of measurments at day 7 in all treatments including control.	Relevant for all uses	x		
Provide ECx estimates based on phytotoxicity for the vegetative vigour study with glyphosate ([redacted] 1994, CA 8.6.2/001)	Relevant for all uses	x		

Provide further information to investigate the effects on soil microorganisms. Indeed, in view of the literature data, a shift in the community structures of soil micro-organisms could not be excluded as glyphosate could be used as a source of P, C or N by soil micro-organisms.	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of Abalaka M. E. et al.2015. Advance in Agriculture and Biology (2015), Vol. 4, No. 3, pp. 106-113	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of Abdulkareem S. I. et al.2014. Egyptian Academic Journal of Biological Sciences B Zoology (2014), Vol. 6, No. 2, pp.47-54	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of Abraham J. et al.2018. Entomologia Experimentalis et Applicata (2018), Vol. 166, No. 8, pp. 695-702	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of Achiorno C. L. et al..2018. Environmental pollution (2018), Vol. 242, No. Pt B, pp. 1427-1435	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of Aguilar-Dorantes K. et al..2015. American fern journal (2015), Vol. 105, No. 3, pp. 131	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of Ahemad M. et al.2012. Annals of microbiology (2012), Vol. 62, No. 4,pp. 1531-1540	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of Akcha F. et al.2012. Aquatic toxicology (2012), Vol. 106-107, pp.104-13	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of Albajes R. et al.2011. Biological Control (2011), Vol. 59, No. 1, pp.30-36	Relevant for all uses	x		

Study summary and a detailed assessment of reliability for the paper of Al-Daikh E. B. et al.2016. Advance in Agriculture and Biology (2016), Vol. 5, No. 1, pp. 14-19	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of Allegrini M. et al..2015. The Science of the total environment (2015), Vol. 533, pp. 60-8	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of Allegrini M. et al..2019. PloS one (2019), Vol. 14, No. 10, pp. e0223600	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of Allegrini M. et al. 2017. Soil biology & biochemistry (2017), Vol. 105, pp. 206-215	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of Allison J. E. et al.2013. Ecotoxicology ((2013), Vol. 22, No. 8, pp. 1289	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of Amid C. et al.2018. Environmental science and pollution research international (2018), Vol. 25, No. 14, pp. 13360-13372	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of Antunes S. C. et al..2010, Journal of hazardous materials (2010), Vol. 184, No. 1-3, pp. 215-25	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of Baglan H. et al.2018, The Journal of experimental biology (2018), Vol. 221, No. 20, pp 1	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of Banaee M. et al.2019, Comparative biochemistry and physiology. Toxicology & pharmacology (2019), Vol. 222,pp. 145-155	Relevant for all uses	x		

Study summary and a detailed assessment of reliability for the paper of Barbukho O. V. et al..2011. Gidrobiologicheskii Zhurnal (2011), Vol. 47, No. 3, pp. 74-79	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of Barriuso J. et al...2011. Microbes and environments (2011), Vol. 26, No. 4, pp. 332	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of Bortoli P. V. et al.2012. Ecologia Austral (2012), Vol. 22, No. 1, pp. 33	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of Bott S. et al. 2011. Plant and soil (2011), Vol. 342, No. 1-2, pp. 249	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of Boufleuer E. M. S. et al. 2016. Acta Iguazu (2016), Vol. 5, No. 5, pp. 25-33	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of Boutin C. et al. 2010. Environmental toxicology and chemistry (2010), Vol. 29, No. 2, pp. 327-37	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of Bridi D. et al. 2017. Toxicology (20171), Vol. 392, pp. 32-39	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of Bruckner A. et al. 2019. Ecotoxicology and environmental safety (2019), Vol. 174, pp. 506-513	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of Buch A. C. et al. 2013. Applied soil ecology (2013), Vol. 69, pp. 32-38	Relevant for all uses	x		

Study summary and a detailed assessment of reliability for the paper of Carmo E. L. et al. 2010. BioControl (2010), Vol. 55, No. 4, pp. 455-464	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of Carvalho L. B. et al. 2016. Planta Daninha (2016), Vol. 34, No. 4, pp. 815	Relevant for all uses	x		
Summary and assessment of relevance and reliability for Castilho A. F. et al.2016. Revista de Ciencias Agrarias / Amazonian Journal of Agricultural and Environmental Sciences (2016), Vol. 59, No. 3, pp. 302-309	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of Cavusoglu K. et al. 2011. Tarim Bilimleri Dergisi (2011), Vol. 17, No. 2, pp. 131	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of Claassens A. et al. 2019. Plant and Soil (2019), Vol. 438, No. 1/2, pp. 393	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of Zanuncio C. J. et al. 2018. Ecotoxicology and environmental safety (2018), Vol. 147, pp. 245-250	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of Condrosari P. et al. 2018. International Journal of ChemTech Research (2018), Vol. 11, No. 5, pp. 240-248	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of Cuhra M. et al. 2013. Ecotoxicology (2013), Vol. 22, No. 2, pp. 251-62	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of Currie Z. et al. 2015. Environmental toxicology and chemistry (2015), Vol. 34, No. 5, pp. 1178-84	Relevant for all uses	x		

Study summary and a detailed assessment of reliability for the paper of Dabney B. L. et al. 2018. Harmful algae (2018), Vol. 80, pp. 130	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of Damgaard C. et al. 2014. Journal of environmental science and health. Part. B, Pesticides, food contaminants, and agricultural wastes (2014), Vol. 49, No. 12, pp. 897	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of de Brito Rodrigues L. et al. 2017. Environmental toxicology and chemistry (2017), Vol. 36, No. 7, pp. 1755-1763	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of Demetrio P. M. et al. 2014. Bulletin of environmental contamination and toxicology (2014), Vol. 93, No. 3, pp. 268	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of Dennis P. G. et al. 2018. Scientific Reports (2018), Vol. 8, pp. 1	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of De Stefano L. G. et al. 2018. Ecological indicators (2018), Vol. 85, pp. 575-584	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of Druille M. et al. 2015. Agriculture, ecosystems & environment (2015), Vol. 202, pp. 48-55	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of Druille M. et al. 2013. Applied soil ecology (2013), Vol. 72, pp. 143-149	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of Dumitru G. et al. 2019. Revista de Chimie (2019), Vol. 70, No. 2, pp. 518-521	Relevant for all uses	x		

Study summary and a detailed assessment of reliability for the paper of El Sebai O. A. et al. 2012. Academic Journal of Entomology (2012), Vol. 5, No. 1, pp. 1-10	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of Emmanuel L. D.A. et al. 2015. International Journal of Tea Science (2015), Vol. 11, No.3/4, pp. 16	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of Enemaduku A. M. et al. 2015. Journal of Scientific and Engineering Research (2015), Vol. 2, No. 4, pp. 55-63	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of Fagundez G. A. et al. 2016. Spanish Journal of Agricultural Research (2016),Vol. 14, No. 1, p. e0301	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of Falis M. et al. 2014. Acta Veterinaria Brno (2014), Vol. 83, No. 2, pp. 95-99	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of Felline S. et al. 2019. Environmental pollution (2019), Vol. 254, No. Pt A, pp. 112977	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of Ferreira E. A. et al. 2015. Semina: Ciencias Agrarias (2015), Vol. 36, No. 2, pp. 645-655	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of Frontera J. L. et al. 2014. Open Environmental Sciences (2014), Vol. 8,pp. 49-53	Relevant for all uses	x		
Justification that the formulation Glyphos used in the paper of Galin R. R. et al. 2019 contains POEA. Bulletin of experimental biology and medicine (2019), Vol. 167, No. 5, pp. 663-666	Relevant for all uses	x		

Study summary and a detailed assessment of reliability for the paper of Garcia-Torres T. et al. 2014. Bulletin of environmental contamination and toxicology (2014), Vol. 93, No. 2, pp. 209	Relevant for all uses	x		
Summary and an assessment of relevance and reliability for the paper of Ge HuiLin et al., 2014. China Environmental Science (2014), Vol. 34, No. 9, pp. 2413-2419	Relevant for all uses	x		
Summary and an assessment of relevance and reliability of the paper of Gomes M. P. et al. 2017. Environmental pollution (2017), Vol. 220, No. Pt A, pp. 452-459	Relevant for all uses	x		
Summary and an assessment of relevance and reliability of the paper of Gutierrez M. F. et al 2017. Chemosphere (2017), Vol. 171, pp. 644-653	Relevant for all uses	x		
Summary and assessment of relevance and reliability for the paper of Hansen L. R. et al. 2016. Aquatic toxicology (2016),Vol. 179, pp. 36	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of Hasan F. et al. 2016. Chemosphere (2016), Vol. 154, pp. 398-407	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of Helander M. et al. 2019. Scientific reports (2019), Vol. 9, No. 1, pp. 19653	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of Herbert L. T. et al. 2014. The Journal of experimental biology (2014), Vol. 217, No. Pt 19, pp. 3457-64	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of Hong Y. et al. 2018. Chemosphere (2018), Vol. 210, pp. 896-906	Relevant for all uses	x		

Study summary and a detailed assessment of reliability for the paper of Houssou A. M. et al. 2017. ISJ-Invertebrate Survival Journal (2017), Vol. 14, pp. 140-148	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of Iori S. et al. 2019. Environmental research (2019), Vol. 182, pp.108984	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of Janben R. et al. 2019. FRONTIERS IN MARINE SCIENCE (2019),Vol. 6, Article 758	Relevant for all uses	x		
Study summary together with assessment of relevance and reliability for the paper of Janssens L. et al. 2017. Aquatic toxicology (2017), Vol. 193, pp. 210-216	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of Jaskulski D. et al. 2011. Progress in Plant Protection (2011), Vol. 51, No. 2, pp. 927-931	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of Jenkins M. B. et al. 2017. Pest management science (2017), Vol. 73, No. 1, pp. 78-86	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of Kennedy E. et al. 2012. Fundamental and Applied Limnology (2012), Vol. 180,No. 4, pp. 309	Relevant for all uses	x		
Study summary and assessment of relevance and reliability for Kielak E. et al. 2011 or a justification that Roundup Ultra contains POEA. Pesticide biochemistry and physiology (2011), Vol. 99, No. 3, pp. 237-243	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of Kostopoulou S. et al. 2020. Chemosphere (2020), Vol. 239, pp. 124582	Relevant for all uses	x		

Study summary and a detailed assessment of reliability for the paper of Liao L-H. et al. 2017. Scientific reports (2017), Vol. 7, No. 1, pp. 15924	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of Lin JingWen et al. 2015. Acta Agriculturae Universitatis Jiangxiensis (2015), Vol. 37, No. 5, pp. 843	Relevant for all uses	x		
Summary and assessment of relevance and reliability of the paper of Li P-L. et al. 2015 or justify that the test was indeed performed with a formulation containing POEA. Nongyao (2015), Vol. 54, No. 2, pp. 108-111	Relevant for all uses	x		
Summary and assessment of relevance and reliability of the paper of Li Y. et al. 2019. Toxin Reviews (2019), Ahead of Print, https://doi.org/10.1080/15569543.2019.1621903	Relevant for all uses	x		
Summary and assessment of relevance and reliability of the paper of Lipok J. et al. 2010. Ecotoxicology and environmental safety (2010), Vol. 73, No. 7, pp. 1681-8	Relevant for all uses	x		
Summary and an assessment of relevance and reliability of the paper of Lopes F. M. et al. 2018. Ecotoxicology and environmental safety (2018), Vol. 162, pp. 201-207	Relevant for all uses	x		
Summary and assessment of relevance and reliability for the paper of Lozano V. L. et al. 2018. Ecotoxicology and Environmental Safety (2018), Vol. 148, pp. 1010-1019	Relevant for all uses	x		
Summary and assessment of relevance and reliability for the paper of Magano D. A. et al. 2013. Pesquisa Agropecuaria Gaucha (2013), Vol. 19, No. 1/2, pp. 69-80	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of Alvarez M. et al. 2012. Acta Toxicologica Argentina (2012), Vol. 20,No. 1, pp. 5-13	Relevant for all uses	x		

Study summary and a detailed assessment of reliability for the paper of Mateos-Naranjo E. et al. 2013. Chemosphere (2013), Vol. 93, No. 10, pp.2631-8	Relevant for all uses	x		
Justification that Roundup PRO used in Meshkini S. et al. 2019 contains POEA. Otherwise relevance of reliability of the paper will have to be assessed. International Journal of Environmental Science and Technology (2019), Vol. 16, No. 11, pp.6847-6856	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of Mottier A. et al. 2015. Marine pollution bulletin (2015), Vol. 95, No. 2, pp. 665-77	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of Murussi C. R. et al. 2016. Fish physiology and biochemistry (2016), Vol. 42, No. 2, pp. 445-55	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of Mysore D. K. et al. 2013. Journal of food science and technology (2013), Vol. 50, No. 6, pp. 1130-6	Relevant for all uses	x		
Study summary and assessment of relevance and reliability of the results related to glyphosate of the paper of Nathan V. K. et al, 2020, in particular for indirect effects and biodiversity assessment. Environmental science and pollution research international (2020), Vol. 27,pp. 4468	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of Niemeyer J. C. et al. 2012. Chemosphere (2018), Vol. 198, pp. 154-160	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of Nocelli R. C. F. et al. 2019. PLANTA DANINHA (2019), Vol. 37, pp. 1	Relevant for all uses	x		
Study together with a summary and a detailed assessment of reliability for the paper of Nunez S. et al. 2015. Biocell (2015), Vol. 39, Suppl.1. Abstract No.: A71.	Relevant for all uses	x		

Study summary and a detailed assessment of reliability for the paper of Nwani C. D. et al. 2013. JAPS, Journal of Animal and Plant Sciences (2013), Vol. 23, No. 3, pp. 888-892	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of Nwani C. D. et al. 2010. International Aquatic Research (2010), Vol. 2,No. 2, pp. 105-111	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of Olszyk D. et al. 2010. Integrated environmental assessment and management (2010), Vol. 6, No. 4, pp. 725-34	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of Pala A. 2019. Environmental science and pollution research international (2019), Vol. 26, No. 36, pp.36869-36877	Relevant for all uses	x		
Summary and assessment of relevance and reliability for use in biodiversity assessment of the paper of Panettieri M. et al. 2013. Soil & tillage research (2013), Vol. 133, pp. 16	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of Pfleeger T. et al. 2010. Environmental Toxicology and Chemistry (2010), Vol. 30, No. 2, pp. 455-468	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of Piotrowicz- Cieslak A. I. et al. 2010. Polish Journal of Environmental Studies (2010),Vol. 19, No. 1, pp. 123	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of Pochron S. et al. 2019. Applied soil ecology (2019), Vol. 139, pp. 32-39	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of Pochron S. et al. 2020. Chemosphere (2020), Vol.241, pp. 125017	Relevant for all uses	x		

Study summary and a detailed assessment of reliability for the paper of Prevot-D'Alvise N. et al. 2013. Cellular and molecular biology (2013), Vol. 59 Suppl, pp. OL1906	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of Romano-Armada N. et al. 2019. The Science of the total environment (2019), Vol. 682, pp. 639-649	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of Rondon Neto R.M. et al. 2011. Revista Brasileira de Herbicidas (2011), Vol. 10, No. 2, 103 p	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of Roy N. M. et al. 2016. Environmental toxicology and pharmacology (2016), Vol. 42, pp. 45-54	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of Salvio C. et al. 2016. Bulletin of environmental contamination and toxicology (2016), Vol. 96, No. 3, pp. 314-9	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of Samal S. et al. 2019. Journal of Environmental Biology (2019), Vol. 40, No.2, pp. 226	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of Santos S. A. et al. 2019. PLANTA DANINHA (2019), Vol. 37	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of Santric L. et al. 2016 Pesticidi i Fitomedicina (2016), Vol. 31, No. 3/4, pp. 121-128	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of Santric L. et al. 2018. PLANTA DANINHA (2018), Vol. 36	Relevant for all uses	x		

Study summary and a detailed assessment of reliability for the paper of Saunders L. E. et al. 2013. Biology (2013), Vol. 2, No. 4, pp. 1488-96	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of Schwan-Stoffel A. V. et al. 2012. Arquivos do Instituto Biologico Sao Paulo (2012), Vol. 79, No. 3, pp. 381	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of Sheehan N. et al. 2018. Bios (2018), Vol. 89, No. 1, pp. 14-22	Relevant for all uses	x		
Study report together with a study summary and a detailed assessment of reliability for the paper of Shimina V. S. et al. 2010. Advances in Plant Sciences (2010), Vol. 23, No. 2, pp. 515-518	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of Shiogiri N. S. et al. 2010. Acta Scientiarum Biological Sciences (2010), Vol. 32, No.3, pp. 285	Relevant for all uses	x		
Justification that the formulation used in the paper of Shiogiri N. S. et al. 2012 contains POEA. Environmental toxicology and pharmacology (2012), Vol. 34, No. 2, pp. 388-396	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of Sikorski L. et al. 2019. Aquatic Toxicology (2019), Vol. 209, pp.70-80	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of Siddhapara M. R. et al. 2012. Journal of Biological Control (2012), Vol. 26, No. 3, pp. 251	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of Sihtmaee M. et al. 2013. Applied soil ecology (2013), Vol. 72, pp. 215	Relevant for all uses	x		
Full-text articles, summaries and assessment of relevance and reliability of the papers cited in Siti Hanisah Zahuri et al. 2014	Relevant for all uses	x		

that may provide information on effects to ant as non taget species. Borneo Journal of Resource Science and Technology (2014), Vol. 4, No. 1, pp. 28-33				
Study summary and a detailed assessment of reliability for the paper of Smedbol E. et al. 2017. Aquatic toxicology (2017), Vol. 192, pp. 265-273	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of Song H. 2010 (Toxic action of acetamiprid, glyphosate and their combined pollution on Hydra magnipapillata) Anhui Nongye Kexue (2010), Vol. 38, No. 20, pp. 10811	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of Song H. et al. 2010 (The Single and Binary-Combined Acute Toxicities of Five Common Pesticides on Hydra Magnipapillata) Journal of Anhui Normal University (Natural Science) (2010), Vol. 33, no. 2, pp. 159	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of Sribanjam S. et al. 2018. Bioscience Research (2018), Vol. 15, No. 2, pp. 1251-1260	Relevant for all uses	x		
Summary and assessment of relevance and reliability of the paper of Stenoien C. et al. 2018, particularly regarding the information that could be used for assessment of impact on biodiversity. Insect Science (2018), Vol. 25, No. 4, pp. 528-541	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of Sun K.-F. et al. 2013 (Ecological risks assessment of organophosphorus pesticides based on response of Scenedesmus quadricanda) China Environmental Science (2013), Vol. 33,No. 5, pp. 868-873	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of Sun K.-F. et al. 2013 (Ecological risks assessment of organophosphorus pesticides on bloom of Microcystis	Relevant for all uses	x		

wesenbergii) International biodeterioration & biodegradation (2013), Vol. 77, pp. 98-105				
Study summary and a detailed assessment of reliability for the paper of Tang Y. et al. 2014. Advanced Materials Research (2014), Vol. 838-841, pp. 2417-2426	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of Tapkir S. D. et al. 2019. Ecotoxicology (2019), Vol. 28, No. 2, pp. 189-200	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of Tome H. V. V. et al. 2020. Environmental pollution (2020), Vol. 256, pp. 113420	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of Topal A. et al. 2015. Ecotoxicology and environmental safety (2015), Vol. 111, pp. 206-14	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of Udeh G. N. et al. 2014. Journal of Aquatic Sciences (2014), Vol. 29, No. 2, pp. 309-315	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of Udeh G. N. et al. 2014. Journal of Aquatic Sciences (2014), Vol. 29, No. 2, pp. 275-283	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of Ujszegi J. et al. 2016. Acta Zoologica Academiae Scientiarum Hungaricae (2016), Vol. 62, No. 4, pp. 355-367	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of Vajargah M. F. et al. 2018. Environmental Health Engineering and Management Journal (2018), Vol. 5, No. 2, pp.61-66	Relevant for all uses	x		

Study summary and a detailed assessment of reliability for the paper of Vannini A. et al. 2016. Chemosphere (2016), Vol. 164, pp. 233-240	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of Veeraiah K. et al. 2015. International Journal of Bioassays (2015), Vol. 4, No. 7, pp. 4139-4144	Relevant for all uses	x		
Summary and assessment of relevance and reliability of the paper of Vera M. S. et al. 2012 (Direct and indirect effects of the glyphosate formulation Glifosato Atanor® on freshwater microbial communities) that may be useful for biodiversity assessment. Ecotoxicology (2012), Vol. 21, No. 7, pp. 1805-16	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of Vera-Candioti J. et al. 2013. Ecotoxicology and environmental safety (2013), Vol. 89, pp. 166-73	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of Vllasaku I. et al. 2018. International Journal of Pharmaceutical Sciences Review and Research (2018), Vol. 48,No. 1, pp. 7/1-7/3	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of Weeks Santos S. et al. 2019. Aquatic toxicology (2019), Vol. 216, pp.105291	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of Wu L.et al. 2016. Aquatic toxicology (2016), Vol. 178, pp. 72-9	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of Xu Y-g. et al. 2015. Journal of Agro-Environment Science (2015), Vol. 34, No.11, pp. 2076	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of Yang Z. et al. 2018. Agricultural Biotechnology (2018), Vol. 7, No.5, pp. 153-155, 158	Relevant for all uses	x		

Study summary including detailed assessment of relevance and reliability for the paper of Ye J. et al.2019. Bulletin of environmental contamination and toxicology (2019), Vol. 103, No. 4, pp. 585-589	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of Yousaf S. et al. 2013. Pakistan Journal of Zoology 2013), Vol. 45, No. 4, pp. 1063-1067	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of Zabaloy M. C. et al. 2016. Pest management science (2016), Vol. 72, No. 4, pp. 684-91	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of Zaller J. G. et al. 2018. Environmental science and pollution research international (2018), Vol. 25, No. 23, pp.23215-23226	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of Zaller J. G. et al. 2014. Scientific reports (2014), Vol. 4, pp. 5634	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of Zhang M. et al. 2018. Biology and fertility of soils (2018), Vol. 54, No. 6, pp. 697-706	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of Zhang Q. et al. 2016. Environmental monitoring and assessment (2016), Vol. 188, No. 11, pp. 632	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of Zhang Q. et al. 2015. Anhui Nongye Kexue (2015), Vol. 43, No. 36,pp. 157-159	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of Zhu X. et al. 2016. Chemosphere (2016), Vol. 162, pp. 243-51	Relevant for all uses	x		

Study together with a study summary including detailed assessment of relevance and reliability for the paper of Gonzalez D. et al.2019. Ecologia Austral, (2019) Vol. 29, No. 1, pp. 20-27	Relevant for all uses	x		
Study summary including detailed assessment of relevance and reliability for the paper of Guo L. et al.2020. Ying yong sheng tai xue bao = The journal of applied ecology, (2020) Vol. 31, No. 2, pp. 524-532	Relevant for all uses	x		
Study summary including detailed assessment of relevance and reliability for the paper of Faita M. R. et al.2020. Journal of Apicultural Research, (2020) Vol. 59, pp. 332-342	Relevant for all uses	x		
Study summary including detailed assessment of relevance and reliability for the paper of Lu T. et al.2020. Environmental pollution, (2020) Vol. 260, Art. No. 114012	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of Pontes J. P. et al. 2020. Journal of Plant Diseases and Protection, (2020) Vol. 127, No. 1, pp. 73-79	Relevant for all uses	x		
Study summary and a detailed assessment of reliability for the paper of Solis-Gonzalez G. et al. 2019. TIP Revista Especializada en Ciencias Quimico-Biologicas, (2019) Vol. 22, pp. 1-8	Relevant for all uses	x		
Study summary including detailed assessment of relevance and reliability for the paper of Vazquez D. E. et al.2020. Environmental pollution, (2020) Vol. 261, Art. No. 114148	Relevant for all uses	x		
Study summary including detailed assessment of relevance and reliability of the paper of Villar S. et al. 2019. Current Topics in Toxicology, (2019) Vol. 15, pp. 133-139	Relevant for all uses	x		
Study summary including detailed assessment of relevance and reliability of the paper of Ye J. et al. 2019. Bulletin of environmental contamination and toxicology (2019) Vol. 103, No. 4, pp. 585-589	Relevant for all uses	x		

Study report and study summary for Quassinti L. et al.2015. Pesticide Biochemistry and Physiology, Vol. 93, pp. 91-95	Relevant for all uses	x		
Study report and study summary for Fanton et al 2020, Ecotoxicology and environmental safety, (2020 Jun 15) Vol. 196, pp. 110501. Electronic Publication Date: 2 Apr 2020 Journal code: 7805381. E-ISSN: 1090-2414. L-ISSN: 0147-6513.	Relevant for all uses	x		
Study report and study summary for Garcia-Ruiz et al 2020. Insect science, (2020 May 27) . Electronic Publication Date: 27 May 2020 Journal code: 101266965. E-ISSN: 1744-7917. L-ISSN: 1672-9609.	Relevant for all uses	x		
Study report and study summary for Huaraca Luis F et al 2020, Journal of environmental science and health. Part. B, Pesticides, food contaminants, and agricultural wastes, (2020) Vol. 55, No. 7, pp. 646-654. Electronic Publication Date: 20 May 2020 Journal code: 7607167. E-ISSN: 1532-4109. L-ISSN: 0360-1234.	Relevant for all uses	x		
Study report and study summary for Juginu, M. S.; Sujila, T. 2019, International Journal of Advanced Research, (2019) Vol. 7, No. 10, pp. 907-914. CODEN: IJARND. ISSN: 2320-5407.	Relevant for all uses	x		
Study report and study summary for Khan Sajida et al , 2020, Chemosphere, (2020 Jun 11) Vol. 258, pp. 127350. Electronic Publication Date: 11 Jun 2020 Journal code: 0320657. E-ISSN: 1879-1298. L-ISSN: 0045-6535.	Relevant for all uses	x		
Study report and study summary for Lanzarin et al 2020, Chemosphere, (2020 Aug) Vol. 253, pp. 126636. Electronic Publication Date: 1 Apr 2020 Journal code: 0320657. E-ISSN: 1879-1298. L-ISSN: 0045-6535.	Relevant for all uses	x		
Study report and study summary for Maderthaner et al, 2020, Environmental science and pollution research international, (2020 May) Vol. 27, No. 14, pp. 17280-17289. Electronic Publication Date: 9 Mar 2020 Journal code: 9441769. E-ISSN: 1614-7499. L-ISSN: 0944-1344. Report No.: PMC-PMC7192858.	Relevant for all uses	x		

Study report and study summary for Matozzo et al, 2020, Journal of Marine Science and Engineering, Vol. 8, No. 6, 20200101 E-ISSN: 2077-1312 DOI: 10.3390/jmse8060399 Published by: MDPI AG, Basel	Relevant for all uses	x		
Study report and study summary for Nuutinen, et al 2020, European journal of soil biology (2020), Volume 96 ISSN: 1164-5563 Published by: Elsevier Masson SAS Source Note: 2020 Jan., Feb., v. 96	Relevant for all uses	x		
Study report and study summary for Sabio et al 2020, Aquatic toxicology (Amsterdam, Netherlands), (2020 May) Vol. 222, pp. 105463. Electronic Publication Date: 3 Mar 2020 Journal code: 8500246. E-ISSN: 1879-1514. L-ISSN: 0166-445X.	Relevant for all uses	x		
Study report and study summary for Vazquez et al, 2020, Scientific reports, (2020 Jun 29) Vol. 10, No. 1, pp. 10516. Electronic Publication Date: 29 Jun 2020 Journal code: 101563288. E-ISSN: 2045-2322. L-ISSN: 2045-2322. Report No.: PMC-PMC7324403.	Relevant for all uses	x		
Study report and study summary for Whitlock, J. R. et al, 2020, INTEGRATIVE AND COMPARATIVE BIOLOGY, (2020 MAR 2020) Vol. 60, pp. E444-E444. ISSN: 1540-7063.	Relevant for all uses	x		
Study report and study summary for Xiang et al, 2020, Environmental science and pollution research international, (2020 Jun) Vol. 27, No. 17, pp. 21939-21952. Electronic Publication Date: 13 Apr 2020 Journal code: 9441769. E-ISSN: 1614-7499. L-ISSN: 0944-1344.	Relevant for all uses	x		
Study report and study summary for Ximenes et al, 2020, Soil & Sediment Contamination, (2020) Vol. 29, No. 5, pp. 545-556. CODEN: SSCOC6. ISSN: 1532-0383.	Relevant for all uses	x		
Study report and study summary for Benamu M. A. et al., 2010, Chemosphere, (2010) Vol. 78, No. 7, pp. 871 6.	Relevant for all uses	x		

Study report and study summary for Pleasants et al, 2013, Insect conservation and diversity (2013), Volume 6, Number 2, pp. 135-144 ISSN: 1752-458X Published by: Blackwell Publishing Ltd Source Note: 2013 Mar., v. 6, no. 2	Relevant for all uses	x		
Study report and study summary for Boutin et al, 2014, Environmental pollution (2014), Vol. 185, pp. 295-306	Relevant for all uses	x		
Study report and study summary for Carpenter D et al, 2020, Toxicol Chem 39(6):1244-1256. doi: 10.1002/etc.4712.	Relevant for all uses	x		
Study report and study summary for Cederlund H. 2017, Environ Toxicol Chem. Nov;36(11):2879-2886. doi: 10.1002/etc.3925.	Relevant for all uses	x		
Study report and study summary for Damgaard C et al, 2014, Journal of Environmental Science and Health, Part B, 49:12, 897-908, DOI: 10.1080/03601234.2014.951571	Relevant for all uses	x		
Study report and study summary for Dupont et al, 2018, Agriculture, Ecosystems & Environment Volume 262, Pages 76-82	Relevant for all uses	x		
Study report and study summary for Schmitz J et al, 2014, Agric. Ecosyst. Environ. 189, 82–91. doi:10.1016/j.agee.2014.03.007	Relevant for all uses	x		
Study report and study summary for Strandberg, S.K 2012, Pesticide Research No 137 Danish Ministry of the Environment, EPA (2012), p. 114	Relevant for all uses	x		
Study report and study summary for Piola L 2013, Chemosphere S0045-6535: 01537-8. doi: 10.1016/j.	Relevant for all uses	x		

3.1.5 Issues that could not be finalised

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

Area of the risk assessment that could not be finalised on the basis of the available data	**Relevance in relation to representative use(s)**
Impact from glyphosate and AMPA residues in rotational crops on the dietary burden calculation and the consumer risk assessment.	Relevant for the 'post-harvest, pre-sowing, pre-planting, pre-emergence outdoor use' and the inter-row use (not relevant for the use on orchards)
Risk assessment for wild mammals: Due to lack of sufficient information related to the kinetic evaluation of the available residue decline data, the higher tier risk assessment for wild mammals could not be finalized.	Relevant for all uses
Risk assessment for amphibians and reptiles (due to lack of guidance however EFSA Scientific Opinion on the state of the science on pesticide risk assessment for amphibians and reptiles (2018) may be useful).	Relevant for all uses
Risk assessment for aquatic organisms	Relevant for all uses

3.1.6 Critical areas of concern

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

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

Critical area of concern identified	Relevance in relation to representative use(s)
None	

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

(If a particular condition proposed to be taken into account to manage an identified risk, as listed in 3.3.1, has been evaluated as being effective, then 'risk identified' is not indicated in this table.)
All columns are grey as the material tested in the toxicological studies has not been demonstrated to be representative of the technical specification.

Representative use		Use "A" (X[1])
Operator risk	Risk identified	
	Assessment not finalised	
Worker risk	Risk identified	
	Assessment not finalised	
Bystander risk	Risk identified	X (risk identified for resident child in use against invasive species in agricultural and non-agricultural areas)
	Assessment not finalised	
Consumer risk	Risk identified	
	Assessment not finalised	X ('post-harvest, pre-sowing, pre-planting, pre-emergence outdoor use' and the inter-row use)
Risk to wild non target terrestrial vertebrates	Risk identified	
	Assessment not finalised	X all uses
Risk to wild non target terrestrial organisms other than vertebrates	Risk identified	
	Assessment not finalised	
Risk to aquatic organisms	Risk identified	
	Assessment not finalised	X all uses
Groundwater exposure active substance	Legal parametric value breached	
	Assessment not finalised	
Groundwater exposure metabolites	Legal parametric value breached	
	Parametric value of 10µg/L[(a)] breached	
	Assessment not finalised	
Comments/Remarks		

The superscript numbers in this table relate to the numbered points indicated within chapter 3.1.5 and 3.1.6. Where there is no superscript number, see level 2 for more explanation.
(a): Value for non relevant metabolites prescribed in SANCO/221/2000-rev 10-final, European Commission, 2003

3.1.8 Area(s) where expert consultation is considered necessary

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

Area(s) where expert consultation is considered necessary	Justification
Assessment of effects on salivary glands	The approach is based on a precautionary principle and is conservative. This assessment may need particular consideration during peer review. The RMS considers the observed histopathological effects on salivary glands a treatment-related effect for which human relevance cannot be excluded (refer to Vol 1 section 2.6.8.2). However, for the interpretation of effects on salivary glands several aspects are considered in order to decide if the effect is adverse or not. The RMS considers salivary gland weight changes and histopathology (severity grade and incidence) along with dose-response and statistical significance. A histopathological finding which is statistically significant is not considered adverse if the severity grade of the finding is minor and there are no salivary gland weight changes. In the case there are no data for salivary gland weights, the effect on histopathology might be considered as potentially adverse in the absence of such data as a precautionary approach. As for the 90-day rat study no data is available on the parotid gland weight, the RMS proposes to set the LOAEL at the lowest dose level of 30 mg/kg bw/day as a precautionary approach although the severity grade of findings observed at this dose level was minimal (very mild). Based on this approach, the LOAEL of 30 mg/kg bw/day is the most critical value relevant for reference dose setting (AOEL). In the 2-year study in rats, both histopathological changes and organ weight changes in the salivary glands were observed at 100 mg/kg bw/day and above. Based on these observations, the dose level of 100 mg/kg bw/day is considered the LOAEL. Then, NOAEL of the study was set at 10 mg/kg bw/day, which is the most critical value relevant for reference dose setting (ADI).
Ecotoxicology: Risk to biodiversity	An assessment of biodiversity via indirect effects and trophic interaction is proposed. As no guidance exist on how to assess this, an expert meeting is considered necessary.

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

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

Issue on which Co-RMS disagrees with RMS	Opinion of Co-RMS	Opinion of RMS
Not relevant		

3.2 PROPOSED DECISION

Based on the the current assessment, glyphosate does meet the approval criteria set in Regulation (EC) N° 1107/2009.

RMS considers that authorisation in at least one Member State is expected to be possible for at least one plant protection product containing the active substance for at least one of the representative uses.

It is considered that the following is specified in Part A of the Commission Implementing Regulation for the approval of the active substance:

Only uses as herbicide may be authorised.

It is considered that the following be specified in Part B of the Commission Implementing Regulation as areas requiring particular attention from Member States when evaluating applications for product authorisation(s):

The risk for residents in uses on invasive species

It is considered that it should be specified that conditions of use shall include risk mitigation measures, where appropriate.

It is proposed that the Member States concerned shall request the submission of confirmatory information:
(a) where new data requirements are established during the evaluation process, or
(b) as a result of new scientific and technical knowledge, or
(c) to increase confidence in the decision.

3.3 RATIONAL FOR THE CONDITIONS AND RESTRICTIONS TO BE ASSOCIATED WITH THE APPROVAL OR AUTHORISATION(S), AS APPROPRIATE

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

Proposed condition/risk mitigation measure	Relevance in relation to representative use(s)
Unsprayed buffer zones and/or drift reduction techniques to protect non-target plants	Relevant for all uses.

3.4 APPENDICES

GUIDANCE DOCUMENTS USED IN THIS ASSESSEMENT

General

SANCO/2012/11251 rev. 5 [Guidance Document on the renewal of approval of active substances to be assessed in compliance with Regulation (EU) No 844/2012 (the Renewal Regulation)]

EFSA (European Food Safety Authority), 2019. Administrative guidance on submission of dossiers and assessment reports for the peer-review of pesticide active substances, EFSA supporting publication 2019:EN-1612. 49 pp. doi:10.2903/sp.efsa.2019.EN-1612

Section identity, physical chemical and analytical methods

Section physico chemical properties
Manual on development and used of FAO and WHO specifications for pesticides, First Edition – third revision-March 2016

Guidance on the application of the CLP Criteria, version 5.0, July 2017

Section analytical methods
Technical Active Substance and Plant protection products: Guidance for generating and reporting methods of analysis in support of pre- and post-registration data requirements for Annex (Section 4) of Regulation (EU) No 283/2013 and Annex (Section 5) of Regulation (EU) No 284/2013., Guidance document SANCO/3030/99 rev.5

Technical Guideline on the Evaluation of Extraction Efficiency of Residue Analytical Methods, SANTE 2017/10632 Rev. 3

Guidance document on pesticide residue analytical methods, SANCO/825/00 rev. 8.1

Residues: Guidance for generating and reporting methods of analysis in support of pre-registration data requirements for Annex II (part A, Section 4) and Annex III (part A, Section 5) of Directive 91/414, SANCO/3029/99 rev.4

Section Data on application and efficacy

None

Section Toxicology

ECHA (European Chemicals Agency), 2017. Guidance on the Application of the CLP Criteria; Guidance to Regulation (EC) No 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures. Version 5.0, July 2017. Reference: ECHA-17-G-21-EN; ISBN: 978-92-9020-050-5

ECHA and EFSA (European Chemicals Agency and European Food Safety Authority), with the technical support of the Joint Research Centre (JRC), 2018. Guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009. EFSA Journal 2018;16(6):5311, 135 pp.

EFSA (European Food Safety Authority), 2011. Submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) No 1107/2009. EFSA Journal 2011;9(2):2092, 49 pp.

EFSA (European Food Safety Authority), 2011, Scientific Opinion. Scientific opinion on genotoxicity testing strategies applicable to food and feed safety assessment. EFSA Journal 2011;9(9):2379.

EFSA (European Food Safety Authority), 2012, Guidance on selected default values to be used by the EFSA Scientific Committee, Scientific Panels and Units in the absence of actual measured data. EFSA Journal 2012;10(3):2579

EFSA (European Food Safety Authority), 2014c. Guidance on the assessment of exposure of operators, workers, residents and bystanders in risk assessment for plant protection products. EFSA Journal 2014;12(10):3874, 55pp.

EFSA (European Food Safety Authority), 2017, Guidance on dermal absorption. EFSA Journal 2017;15(6):4873.

EFSA (European Food Safety Authority), 2017, Scientific Opinion of the PPR Panel on the follow-up of the findings of the External Scientific Report 'Literature review of epidemiological studies linking exposure to pesticides and health effects'. EFSA Journal 2017;15(12):5113.

EFSA (European Food Safety Authority), 2017, Scientific Opinion. Draft for internal testing Scientific Committee guidance on appraising and integrating evidence from epidemiological studies for use in EFSA's scientific assessments. EFSA Journal 2020;18(8):6221.

EFSA (European Food Safety Authority), 2020, Scientific Opinion. Scientific opinion on genotoxicity testing strategies applicable to food and feed safety assessment. EFSA Journal 2011;9(9):2379.

European Commission, 2012. Guidance document on the assessment of the equivalence of technical materials of substances regulated under Regulation (EC) No 1107/2009. SANCO/10597/2003-rev. 10.1, 13 July 2012.

WHO, 2015. JMPR Guidance document for WHO monographers and reviewers. WHO/HSE/FOS/2015.1

Section Residue and consumer risk assessment

OECD (Organisation for Economic Co-operation and Development), 2009. Guidance document on overview of residue chemistry studies. ENV/JM/MONO(2009)31, 28 July 2009.

OECD (Organisation for Economic Co-operation and Development), 2011. OECD MRL calculator: spreadsheet for single data set and spreadsheet for multiple data set, 2 March 2011. In: Pesticide Publications/Publications on Pesticide Residues. Available online: www.oecd.org

European Commission, 2017. Guidelines on comparability, extrapolation, group tolerances and data requirements for setting MRLs. SANCO 7525/VI/95-rev. 10.3. June 2017.

European Commission, 2018. Technical guidelines for determining the magnitude of pesticide residues in honey and setting Maximum Residue Levels in honey. SANTE/11956/2016 rev. 9. September 2018.

JMPR (Joint Meeting on Pesticide Residues), 2004. Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group on Pesticide Residues, Rome, Italy, 20–29 September 2004, 383 pp.

JMPR (Joint Meeting on Pesticide Residues), 2007. Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group on Pesticide Residues, Geneva, Switzerland, 18–27 September 2007, 164 pp.

Section Fate and behavior in environment

European Commission (2014) - Assessing Potential for Movement of Active Substances and their Metabolites to Ground Water in the EU" Report of the FOCUS Ground Water Work Group, EC Document Reference Sanco/13144/2010 version 3, 613 pp.

EFSA (European Food Safety Authority), 2014. EFSA Guidance Document for evaluating laboratory and field dissipation studies to obtain DegT50 values of active substances of plant protection products and transformation products of these active substances in soil. EFSA Journal 2014;12(5):3662, 37 pp., doi:10.2903/j.efsa.2014.3662

FOCUS (1997) - Soil persistence models and EU Registration - The Final Report of the Soil Modelling Workgroup of FOCUS (Forum for the Co-ordination of Pesticide Fate Models and their Use) – 29 February 1997.

FOCUS (2001) - FOCUS Surface Water Scenarios in the EU Evaluation Process under 91/414/EEC. Report of the FOCUS Working Group on Surface Water Scenarios, EC Document Reference SANCO/4802/2001-rev.1. 221 pp.

FOCUS (2006) - Guidance document on estimating persistence and degradation kinetics from environmental fate studies on pesticides in EU registration. Report of the FOCUS Work Group on Degradation Kinetics, EC Document Reference Sanco/10058/2005, version 2.0, 434 pp.

FOCUS (2008) - Pesticides in Air: Considerations for Exposure Assessment. Report of the FOCUS Working Group on Pesticides in Air, EC Document Reference SANCO/10553/2006 Rev 2 June 2008. 327 pp.

FOCUS (2014a) - Generic guidance for Tier 1 FOCUS groundwater assessments. Version 2.2, May 2014.

FOCUS (2014b) - Generic Guidance for Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration, version 1.1

FOCUS (2015) - Generic guidance for FOCUS surface water Scenarios, Version: 1.4, Date: May 2015

SANCO (2003) Guidance document on the assessment of the relevance of metabolites in groundwater of substances regulated under Council directive 91/414/EEC. Sanco/221/2000-rev.10-final, 25 February 2003.

Section ecotoxicology

EFSA (European Food Safety Authority), 2009. Guidance on the Risk Assessment for Birds and Mammals: EFSA Journal 2009; 7(12):1438

EFSA (European Food Safety Authority), 2013. Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters: EFSA Journal 2013;11(7):3290

Guidance document on regulatory testing and risk assessment procedures for plant protection products with non-target arthropods ESCORT II (2000)

EFSA (European Food Safety Authority) Guidance Document on the risk assessment of plant protection products on bees (Apis mellifera, Bombus spp. and solitary bees)

Guidance Document on Terrestrial Ecotoxicology Under Council Directive 91/414/EEC: SANCO/10329/2002

EFSA (European Food Safety Authority), 2011. Guidance Submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) No 1107/2009: EFSA Journal 2011;9(2):2092

ECHA and EFSA (European Chemicals Agency and European Food Safety Authority), 2018. Guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009 (EFSA/ECHA, 2018). EFSA Journal, Vol 16, Issue 6, June 2018, e05311 https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2018.5311.

3.5 REFERENCE LIST

Across sections

EFSA (2015) Conclusion on the peer review of the pesticide risk assessment of the active substance glyphosate. EFSA Journal 2015;13(11):4302

Section identity, physical chemical and analytical methods

None

Section data on application and efficacy

None

Section toxicology

BAuA, Federal Institute for Occupational Safety and Health, 2016. CLH report for glyphosate, May 2016.

ECHA (European Chemicals Agency), 2017. RAC opinion for glyphosate, March 2017.

WHO and FAO, 2016. Joint FAO/WHO Meeting on Pesticide Residues. Evaluations 2016, Part II toxicological evaluation. Toxicological monograph on glyphosate.

Section residue and consumer risk assessment

European Food Safety Authority (EFSA), 2019. Review of the existing maximum residue levels for glyphosate according to Article 12 of Regulation (EC) No 396/2005 – revised version to take into account omitted data. EFSA Journal 2019;17(10):5862, 211 pp. https://doi.org/10.2903/j.efsa. 2019.5862

SCPAFF Residues, 15-16 June 2020, summary report,
https://ec.europa.eu/food/sites/food/files/plant/docs/sc_phyto_20200615_ppr_sum.pdf

Section fate and behavior in environment

DG SANCO Working Document on "Evidence Needed to Identify POP, PBT and vPvB Properties for Pesticides", 25.09.2012 – rev. 3

Section ecotoxicology

EFSA (European Food Safety Authority) Scientific Committee, 2016. Guidance to develop specific protection goals options for environmental risk assessment at EFSA, in relation to biodiversity and ecosystem services. EFSA Journal 2016;14(6):4499, 50 pp. doi:10.2903/j.efsa.2016.4499

EFSA (European Food Safety Authority), 2015. Scientific Opinion addressing the state of the science on risk assessment of plant protection products for non-target arthropods. EFSA Journal 2015;13(2):3996, 212 pp. doi:10.2903/j.efsa.2015.3996

EFSA (European Food Safety Authority), 2017. Scientific Opinion addressing the state of the science on risk assessment of plant protection products for in-soil organisms. EFSA Journal 2017;15(2):4690, 225 pp. doi:10.2903/j.

EFSA (European Food Safety Authority), 2014. Scientific Opinion addressing the state of the science on risk assessment of plant protection products for non-target terrestrial plants. EFSA Journal 2014;12(7):3800, 163 pp. doi:10.2903/j.efsa.2014.3800

"Science for Environment Policy": European Commission DG Environment News Alert Service, edited by SCU,

The University of the West of England, Bristol. https://ec.europa.eu/environment/integration/research/newsalert/pdf/maximum_benefit_aem_target_specific_environmental_pressures_germany_526na5_en.pdf

Communication "Science for Environment Policy" based on Früh-Müller, A., Bach, M., Breuer, L., Hotes, S., Koellner, T., Krippes, C. and Wolters, V. (2018). The use of agri-environmental measures to address environmental pressures in Germany: Spatial mismatches and options for improvement. Land Use Policy, 84, pp. 347–362.

Maes, J., et al., 2020. Mapping and Assessment of Ecosystems and their Services: An EU ecosystem assessment, EUR 30161 EN, Publications Office of the European Union, Ispra, 2020, ISBN 978-92-76-17833-0, doi:10.2760/757183, JRC120383.