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Conclusion on Pesticides Peer Review

Peer review of the pesticide risk assessment of the potential endocrine disrupting properties of glyphosate

European Food Safety Authority (EFSA)

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EFSA was requested by the European Commission to consider information on potential endocrine activity of the pesticide active substance glyphosate in accordance with Article 31 of Regulation (EC) No 178/2002. In this context, the conclusions of EFSA following the peer review of the initial risk assessment carried out by the competent authority of the rapporteur Member State, Germany, are reported, following the submission and evaluation of pertinent data made available by the applicants. The current conclusion presents a follow-up assessment to the existing EFSA Conclusion on the peer review for the renewal of the approval of glyphosate (EFSA Journal 2015;13(11):4302) focussed on the outstanding issues identified in relation to the potential endocrine activity of glyphosate. The current assessment concluded that the weight of evidence indicates that glyphosate does not have endocrine disrupting properties through oestrogen, androgen, thyroid or steroidogenesis mode of action based on a comprehensive database available in the toxicology area. The available ecotox studies did not contradict this conclusion.

Summary

On 12 November 2015, the European Food Safety Authority (EFSA) published its Conclusion on the peer review of the pesticide risk assessment of the active substance glyphosate in the framework of the renewal of the approval under Commission Regulation (EU) No 1141/2010 (EFSA Journal 2015;13(11):4302). Based on the assessment of the representative uses evaluated during the peer review, EFSA noted that for certain effects observed in one study at parental toxic doses, signs of endocrine activity could not be completely ruled out and a data gap was identified.

While pertinent data became available which could not be included in the renewal procedure, it was considered by the European Commission desirable to address this issue through a focussed scientific assessment.

On 27 September 2016, EFSA received a mandate from the European Commission to consider information on potential endocrine activity of glyphosate in accordance with Article 31 of Regulation (EC) No 178/2002. In particular, EFSA has been requested to assess the available information on potential endocrine activity of glyphosate, and conclude whether the data gap set in the EFSA Conclusion published on 12 November 2015 (EFSA Journal 2015;13(11):4302) is addressed.

On 4 October 2016, EFSA has requested relevant data related to potential endocrine activity of glyphosate from the applicant for the renewal of the approval of glyphosate, i.e. the Glyphosate Task Force. The initial assessment of the data submitted was carried out by the competent authority of the rapporteur Member State, Germany, in the format of an addendum to the renewal assessment report, which was received by EFSA on 31 March 2017. Subsequently, the addendum was distributed to Member States, the applicant and EFSA for comments on 3 April 2017. In addition, an expert consultation was conducted in the areas of mammalian toxicology and ecotoxicology.

The current conclusion presents a follow-up assessment to the existing EFSA Conclusion on the peer review for the renewal of the approval of glyphosate (EFSA Journal 2015;13(11):4302) focussed on the data gap identified in relation to the endocrine activity of the substance.

The current assessment concluded that glyphosate does not have oestrogen, androgen, thyroid and steroidogenesis (EATS)-mediated endocrine disrupting properties based on the facts that no endocrine-mediated adverse effects were identified in apical studies; the weak evidence seen in a limited number of

5 studies; and no EATS-mediated endocrine mode of action was identified. Since the database available to reach this conclusion was quite comprehensive, it was concluded that the data gap identified in the previous EFSA conclusion (EFSA Journal 2015;13(11):4302) was adequately addressed.

Glyphosate effects on reproductive parameters were observed in some ecotoxicology studies. However, these effects were not consistently observed and no indication was found that the effects are related to an androgenic, estrogenic, steroidogenic or thyroidal mode of action. No evidence was found in the available ecotoxicology studies which would contradict the conclusion of mammalian toxicology that there is no evidence of endocrine mode of action of glyphosate.

Background

The active substance glyphosate was included in Annex I to Directive 91/414/EEC[1] on 1 July 2002 by Commission Directive 2001/99/EC,[2] and has been deemed to be approved under Regulation (EC) No 1107/2009[3], in accordance with Commission Implementing Regulation (EU) No 540/2011[4], as amended by Commission Implementing Regulations (EU) No 541/2011[5], 2016/1056[6] and 2016/1313[7].

On 12 November 2015, the European Food Safety Authority (EFSA) published its Conclusion on the peer review of the pesticide risk assessment of the active substance glyphosate in the framework of the renewal of the approval of the substance under Commission Regulation (EU) No 1141/2010[8] (EFSA, **2015**). Based on the assessment of the representative uses evaluated during the peer review, it was concluded that glyphosate does not meet the interim criteria of Annex II, Point 3.6.5 of Regulation (EC) No 1107/2009 for endocrine disrupting properties concerning human health, and that apical studies in the area of mammalian toxicology did not show adverse effects on the reproduction. However, EFSA noted that for certain effects observed in one study at parental toxic doses, signs of endocrine activity could not be completely ruled out and the full battery of the Tier I screening assays according to the Endocrine Disruptor Screening Programme (EDSP) of the US Environmental Protection Agency, or the Level 2 and 3 tests currently indicated in the Organisation for Economic Co-operation and Development (OECD) Conceptual Framework would be needed to address this point conclusively. EFSA identified a data gap for this information.

While pertinent data became available which could not be included in the renewal procedure, it was considered by the European Commission desirable to address this issue through a focussed scientific assessment.

By means of a mandate received on 27 September 2016, EFSA has been requested by the European Commission to consider information on potential endocrine activity of glyphosate in accordance with Article 31 of Regulation (EC) No 178/2002[9]. In particular, EFSA has been requested to assess the available information on potential endocrine activity of glyphosate, and conclude whether the data gap set in the EFSA Conclusion published on 12 November 2015 (EFSA, **2015**) is addressed. For this purpose, EFSA is producing a focussed EFSA Conclusion as a follow-up assessment to the previous EFSA Conclusion on the peer review for the renewal of the approval of glyphosate, to be delivered by 31 August 2017.

As invited in the mandate, on 4 October 2016, EFSA has requested relevant data related to potential endocrine activity of glyphosate from the applicant for the renewal of the approval of glyphosate, i.e. the Glyphosate Task Force. In particular, the following data not yet considered under the renewal procedure were requested:

Conceptual Framework, as outlined in the EFSA Conclusion;

- Any other study that may be suitable to address the data gap regarding potential endocrine activity set in the EFSA Conclusion, in particular with regard to the studies evaluated by the EDSP;
- An update on the scientific peer-reviewed open literature in accordance with the EFSA guidance on the submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) No 1107/2009 (EFSA, **2011**), to take into account any publications relevant for the data gap, in particular those published after the most recent submission of scientific peer-reviewed open literature in the context of the renewal procedure.

The initial evaluation of the data submitted was carried out by the competent authority of the rapporteur Member State (RMS), Germany, in the format of an addendum to the renewal assessment report, which was received by EFSA on 31 March 2017 (Germany, **2017a**). The peer review was initiated on 3 April 2017 by dispatching the addendum to the Member States and the applicant, the Glyphosate Task Force, for consultation and comments. EFSA also provided comments. The comments received were collated by EFSA and forwarded to the RMS for consideration during the revision of the addendum. A revised addendum was made available by the RMS on 26 May 2017 (Germany, **2017b**).

Considering the complexity of the assessment in view of the nature and extent of data submitted, further discussions took place at the Pesticides Peer Review Experts' Meeting 159 on mammalian toxicology and at the Pesticides Peer Review Experts' Meeting 160 on ecotoxicology in June 2017. Details of the issues discussed, together with the outcome of these discussions were recorded in the respective meeting reports. In addition, a further revision of the addendum was produced by the RMS in line with the outcome of the expert consultations.

A final consultation on the conclusions arising from the peer review of the focussed risk assessment took place with Member States via a written procedure in July 2017.

The conclusions laid down in this report were reached on the basis of the peer review of the RMS's evaluation of the pertinent data submitted in relation to the potential endocrine activity of glyphosate. A key supporting document to this conclusion is the peer review report, which is a compilation of the documentation developed to evaluate and address all issues raised in the peer review, from the commenting on the RMS addendum to the conclusion. The peer review report (EFSA, **2017**) comprises the following documents, in which all views expressed during the course of the peer review, including minority views where applicable, can be found:

- the comments received on the RMS addendum together with the RMS response;
- the reports of the scientific consultation with Member State experts;
- the comments received on the draft EFSA conclusion.

Given the importance of the RMS addendum including its revisions (Germany, **2017b**) and the peer review report, these documents are considered as background documents to this conclusion.

It is recommended that this conclusion report and its background documents would not be accepted to support any registration outside the European Union (EU) for which the applicant has not demonstrated to have regulatory access to the information on which this conclusion report is based.

Glyphosate is the ISO common name for *N*-(phosphonomethyl)glycine (IUPAC).

It should be mentioned that the salts glyphosate-isopropylammonium, glyphosate-potassium, glyphosate-monoammonium, glyphosate-dimethylammonium are the modified ISO common names for iso-propylammonium *N*-(phosphonomethyl)glycinate, potassium *N*-[(hydroxyphosphinato)methyl]glycine, ammonium *N*-[(hydroxyphosphinato)methyl]glycine and dimethylammonium *N*-(phosphonomethyl)glycinate (IUPAC), respectively. These salts are derivatives of the active substance glyphosate.

The representative formulated product for the evaluation in the framework of the renewal of the approval of glyphosate and considered in the current peer review was 'MON 52276', a soluble concentrate (SL) containing 360 g/L glyphosate as isopropylammonium salt (486 g/L).

The representative uses considered are spraying applications against emerged annual, perennial and biennial weeds in all crops (crops including but not restricted to root and tuber vegetables, bulb vegetables, stem vegetables, field vegetables (fruiting vegetables, brassica vegetables, leaf vegetables and fresh herbs, legume vegetables), pulses, oil seeds, potatoes, cereals, and sugar- and fodder beet; orchard crops and vine, before planting fruit crops, ornamentals, trees, nursery plants, etc.) and foliar spraying for desiccation in cereals and oilseeds (pre-harvest). Full details of the good agricultural practices (GAPs) can be found in Appendix A.

Conclusions of the evaluation

Mammalian toxicology

The endocrine disruption potential of glyphosate was discussed during the Pesticides Peer Review Experts' Meeting 159 in June 2017.

As already concluded in the EFSA conclusion (EFSA, **2015**), glyphosate is not classified or proposed to be classified as carcinogenic or toxic for reproduction category 2 in accordance with the provisions of Regulation (EC) No 1272/2008[10] (harmonised classification confirmed in 2017 by the Risk Assessment Committee of the European Chemical Agency (ECHA, **2017**)), and therefore, the conditions of the interim provisions of Annex II, Point 3.6.5 of Regulation (EC) No 1107/2009 concerning human health for the consideration of endocrine disrupting properties are not met.

The scientific assessment of the endocrine disruption potential of glyphosate was based on the EFSA Scientific Committee opinion on the hazard assessment of endocrine disruptors (EFSA Scientific Committee, **2013**) and the testing strategy indicated in the OECD Conceptual Framework (OECD, **2012**).

The only effect that could be related to a possible endocrine-mediated mode of action in apical studies (level 4 and 5 of the OECD Conceptual Framework) is an isolated marginal (but statistically significant) delay in preputial separation (PPS), observed in males at the limit dose of ca. 1000 mg/kg body weight (bw) per day in the first generation (F1 generation) of a two-generation reproductive toxicity study in rats. This effect was not reproduced in the second generation (F2 generation) of the same study or in another study investigating the same endpoint, and general toxicity has been shown at this dose level in other studies (reduced parental and offspring's body weight). In addition, studies on short- and long-term toxicity, carcinogenicity, developmental toxicity, one-generation range-finding and five other two-generation reproductive toxicity studies did not show any evidence of endocrine disruption potential. On this basis, it was concluded that glyphosate shows no endocrine-mediated adverse effects.

test guidelines were negative except for one published study showing a weak oestrogenic activity.

Since the database for glyphosate is quite comprehensive and includes studies performed according to the current state-of-art, all experts agreed that a firm conclusion can be reached regarding the endocrine disruption potential of glyphosate for the oestrogen, androgen, steroidogenesis and thyroid (EATS) modalities.

Glyphosate shows no endocrine-mediated adverse effects in apical studies; the weak evidence in a limited number of supplementary *in vitro* studies was inconsistent with the findings of the acceptable OECD tests and it was not expressed *in vivo* in the OECD level 4 and 5 studies, and no EATS-mediated endocrine mode of action was identified.

All the experts agreed that the weight of evidence indicates that glyphosate does not have EATS-mediated endocrine disrupting properties and that the data gap identified in the previous EFSA conclusion (EFSA, **2015**) has been adequately addressed.

Ecotoxicology

Effects observed in some of the studies submitted were discussed at the Pesticides Peer Review Experts' Meeting 160 in June 2017, in view of underlying potential endocrine mechanisms.

Effects on gonadosomatic index (GSI), egg production and ovarian abnormalities observed in one published study with zebrafish (*Danio rerio*) were considered as unlikely to be linked to an endocrine activity. The reason is that an endocrine activity would be expected to trigger positive responses in the *in vitro* studies testing battery (see above mammalian toxicology section). It is noted that the tested concentration of glyphosate of 10 mg a.s./L was relatively high to test for reproductive effects in zebrafish as in another study significant mortality was already observed at the concentration of 10 mg a.s./L. In addition, no effects on reproduction were detected in a standard test guideline fish reproduction study with fathead minnow (*Pimephales promelas*) with concentrations tested up to 30 mg a.s./L. An endocrine mode of action would be expected to have led to reproductive effects in the standard test guideline study.

Effects on hatching of larvae, larvae morphology and GSI were observed in a study with the estuarine crab (*Neohelice granulate)* . However, the effects on larvae hatching were statistically significant only for the test with the formulation 'Roundup' and the effects on larvae morphology did not show a dose response relationship for glyphosate. An increase in GSI was statistically significant only for glyphosate but not for 'Roundup'. It is difficult to attribute the observed effects to a specific mode of action. The observed increase in GSI (without concurrent hepatosomatic index increases) is likely, as the authors supposed, due to increased egg resorption, but the reason/mechanism for this is unclear and could be the result of general toxicity. Overall, it was concluded that it is not possible to relate the observed effects to an endocrine mode of action.

In the fish short-term reproduction study, reduced vitellogenin levels were observed. These differences were not statistically significant. None of the reproductive parameters (fecundity, fertilisation 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 addition, no effects on vitellogenin or spiggin levels were observed in a study with stickleback (*Gasterosteus aculeatus*) and no effect on vitellogenin production was found in a study with rainbow trout (*Oncorhynchus mykiss*). Therefore, it was concluded that the available information does not provide evidence for endocrine effects on reproduction of fish.

an increase in growth should never solely be relied on to determine thyroidal effects. No significant effects were observed on developmental stage, morphometry (hind limb length normalised to snout vent length) and thyroid histology. Therefore, it was concluded that the study does not provide an indication of thyroidal activity.

The available ecotoxicology studies suggest that glyphosate has no androgenic, estrogenic, steroidogenic or thyroidal effects.

In the mammalian toxicology section, it was concluded that glyphosate does not have endocrine disrupting properties based on the available information. No evidence was found in the ecotoxicological studies which would contradict that conclusion.

Data gaps

This is list of data gaps identified in the context of the current focussed peer review. The data gaps identified in the course of the previous peer review in the framework of the renewal of approval of glyphosate and not related to the scope of the current assessment remain unchanged.

- No data gaps have been identified in the context of this evaluation. The data gap identified in the framework of the EFSA, **2015** Conclusion regarding the endocrine disrupting properties of glyphosate is considered addressed.

Concerns

1 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 in accordance with Article 29(6) of Regulation (EC) No 1107/2009 and as set out in Commission Regulation (EU) No 546/2011[11], 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).

None identified. The endocrine disrupting properties of glyphosate have been addressed, finalising the issue identified in Section 9.1 of the EFSA, **2015** Conclusion.

2 Critical areas of concern

An issue is listed as a critical area of concern where there is enough information available to perform an assessment for the representative uses in line with the Uniform Principles in accordance with Article 29(6) of Regulation (EC) No 1107/2009 and as set out in Commission Regulation (EU) No 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.

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.

No critical areas of concerns were identified in the context of the current focussed peer review on endocrine disrupting properties.

Notes

1 Council Directive 91/414/EEC of 15 July 1991 concerning the placing of plant protection products on the market. OJ L 230, 19.8.1991, p. 1–32, as last amended. Repealed by Regulation (EC) No 1107/2009.

2 Commission Directive 2001/99/EC of 20 November 2001 amending Annex I to Council Directive 91/414/EEC concerning the placing of plant protection products on the market to include glyphosate and thifensulfuron-methyl as active substances. OJ L 304, 21.11.2001, p. 14–16.

3 Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC. OJ L 309, 24.11.2009, p. 1–50.

4 Commission Implementing Regulation (EU) No 540/2011 of 25 May 2011 implementing Regulation (EC) No 1107/2009 of the European Parliament and of the Council as regards the list of approved active substances. OJ L 153, 11.6.2011, p. 1–186.

5 Commission Implementing Regulation (EU) No 541/2011 of 1 June 2011 amending Implementing Regulation (EU) No 540/2011 implementing Regulation (EC) No 1107/2009 of the European Parliament and of the Council as regards the list of approved active substances. OJ L 153, 11.6.2011, p. 187–188.

6 Commission Implementing Regulation (EU) 2016/1056 of 29 June 2016 amending Implementing Regulation (EU) No 540/2011 as regards the extension of the approval period of the active substance glyphosate. OJ L 173, 30.6.2016, p. 52–54.

7 Commission Implementing Regulation (EU) 2016/1313 of 1 August 2016 amending Implementation Regulation (EU) No 540/2011 as regards the conditions of approval of the active substance glyphosate. OJ L 208, 2.8.2016, p. 1–3.

8 Commission Regulation (EU) No 1141/2010 of 7 December 2010 laying down the procedure for the renewal of the inclusion of a second group of active substances in Annex I to Council Directive 91/414/EEC and establishing the list of those substances. OJ L 322, 8.12.2010, p. 10-19.

9 Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJ L 31, 1.2.2002, p. 1–24.

10 Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006. OJ L 353, 31.12.2008, p. 1–1355.

11 Commission Regulation (EU) No 546/2011 of 10 June 2011 implementing Regulation (EC) No 1107/2009 of the European Parliament and of the Council as regards uniform principles for

Abbreviations

a.s.
active substance

AMPA
aminomethylphosphonic acid

AR
androgen receptor

bw
body weight

E2
17β-estradiol

E_bC_{50}
effective concentration (biomass)

E_rC_{50}
effective concentration (growth rate)

EATS
oestrogen, androgen, thyroid and steroidogenesis (modalities)

EC_{50}
effective concentration

ECHA
European Chemicals Agency

EEC
European Economic Community

ED
endocrine disruptor

EDSP
Endocrine Disruptor Screening Programme

EPA
(US) Environmental Protection Agency

ER_{50}
emergence rate/effective rate, median

ERα
oestrogen receptor subtype α

ERβ
oestrogen receptor subtype β

GAP
Good Agricultural Practice

GM
genetically modified

IPA
isopropylammonium

ISO
International Organization for Standardization

IUPAC
International Union of Pure and Applied Chemistry

LD_{50}
lethal dose, median; dosis letalis media

M&K
Magnusson–Kligman maximisation test

mm
mean measured concentrations

NOAEC
no observed adverse effect concentration

NOAEL
no observed adverse effect level

NOEC
no observed effect concentration

NOErC
no observed effect concentration growth rate

nom
Nominal concentrations

OECD
Organisation for Economic Co-operation and Development

PHI
preharvest interval

PPS
preputial separation

RAR
renewal assessment report

RMS
rapporteur Member State

SD
Sprague–Dawley

SL
Soluble concentrate

SMILES
simplified molecular-input line-entry system

UDS
unscheduled DNA synthesis

and the representative formulation

Summary of representative uses evaluated in the framework of the renewal of approval and considered in the current focussed peer review (*Glyphosate*)

Crop and/or situation[a]	Member State or Country	Product name	F G or I[b]	Pests or Group of pests controlled[c]	Formulation		Application		
					Type (d–f)	Conc. a.s.[i]	Method kind (f–h)	Growth stage & season[j]	Number min–max[k]
All crops ** (all seeded or transplanted crops)	EU	MON 52276	F	Emerged annual, perennial and biennial weeds	SL	360 g/L	Spray	Preplanting of crop	1–2
All crops ** (all seeded crops)	EU	MON 52276	F	Emerged annual, perennial and biennial weeds	SL	360 g/L	Spray	Post-planting/pre-emergence of crop	1
Cereals (pre-harvest) wheat, rye, triticale	EU	MON 52276	F	Emerged annual, perennial and biennial weeds	SL	360 g/L	Spray	Crop maturity < 30% grain moisture	1
Cereals (pre-harvest) barley and oats	EU	MON 52276	F	Emerged annual, perennial and biennial weeds	SL	360 g/L	Spray	Crop maturity < 30% grain moisture	1
Oilseeds (pre-harvest) rapeseed, mustard seed, linseed	EU	MON 52276	F	Emerged annual, perennial and biennial weeds	SL	360 g/L	Spray	Crop maturity < 30% grain moisture	1

Crop and/or situation	Member State or Country	Product name	F G or I	Pests or Group of pests controlled	Type (d–f)	Conc. a.s.	Method kind (f–h)	Growth stage & season	Number min–max
Orchard crops, vines, including citrus and tree nuts	EU	MON 52276	F	Emerged annual, perennial and biennial weeds	SL	360 g/L	Spray	Post-emergence of weeds	1–3
Orchard crops, vines, including citrus and tree nuts	EU	MON 52276	F	Emerged annual, perennial and biennial weeds	SL	360 g/L	(ULV) Sprayer or Knapsack use (spot treatment)	Post-emergence of weeds	1–3

Crop and/or situation	Member State or Country	Product name	F G or I	Pests or Group of pests controlled	Type (d–f)	Conc. a.s.	Method kind (f–h)	Growth stage & season	Number min–max
N/A: not applicable; SL: soluble concentrate; a.s.: active substance.									
** Crops including but not restricted to: root & tuber vegetables, bulb vegetables, stem vegetables, field vegetables (fruiting potatoes, cereals, and sugar & fodder beet; before planting fruit crops, ornamentals, trees, nursery plants, etc.									
[a] For crops, the EU and Codex classifications (both) should be taken into account; where relevant, the use situation should b									
[b] Outdoor or field use (F), greenhouse application (G) or indoor application (I).									
[c] e.g. biting and suckling insects, soil born insects, foliar fungi, weeds.									
[d] e.g. wettable powder (WP), emulsifiable concentrate (EC), granule (GR).									
[e] GCPF Codes – GIFAP Technical Monograph No 2, 1989.									
[f] All abbreviations used must be explained.									
[g] Method, e.g. high volume spraying, low volume spraying, spreading, dusting, drench.									
[h] Kind, e.g. overall, broadcast, aerial spraying, row, individual plant, between the plant- type of equipment used must be indi									
[i] g/kg or g/L. Normally, the rate should be given for the active substance (according to ISO) and not for the variant in order to **where only one variant is synthesised, it is more appropriate to give the rate for the variant (e.g. benthiavalicarb-isc**									
[j] Growth stage at last treatment (BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4), including									
[k] Indicate the minimum and maximum number of application possible under practical conditions of use.									
[l] PHI: minimum preharvest interval.									

Impact on human and animal health

Other toxicological studies (Annex IIA, point 5.8)

		cavity but an adrenergic mechanism may be also involved No evidence of immunotoxicity (humoral immune response, thymus and spleen weights) in mice Pharmacological effects: No haematological, electrocardiographic or behavioural/functional changes after oral administration; contractile response similar to that seen with known parasympatho mimetic agents in isolated guinea pig ileum; no neuromuscular blocking activity on innervated rat gastrocnemius muscle Toxicity studies on farm animals: Goat LD_{50} oral = 3530 mg/kg bw (glyphosate acid) Goat LD_{50} oral = 5700 mg/kg bw (IPA salt) 7-day, cow: NOAEL 540 mg/kg bw per day, based on diarrhoea, decreased feed intake (IPA salt)	
Endocrine disrupting properties	OECD Level	Study type & acceptability	Effects observed
	Level 5 (*in vivo*)	2-generation reproductive toxicity (addendum 2 on glyphosate ED properties; Germany, 2017b); study acceptable	Delayed preputial separation in one of seven two-generation studies at the limit 1000 mg/kg bw per day (2 of which performed according to current standards, i.e. investigating oestrus cycles, sperm parameters, sexual maturation)
		(Germany, 2015)	6 other two-generation studies: Negative Overall conclusion for Level 5: negative
	Level 4 (*in vivo*)	(Germany, 2015)	Studies on short-term toxicity, chronic toxicity, developmental toxicity, one-generation range-finding and carcinogenicity: negative

	assay in female rats; acceptable even though not OECD agreed guideline	regularly cycling at the end of the study based on a limited number of animals but study not appropriate for addressing this endpoint (sexual immaturity of animals at end of study)
	A pubertal development and thyroid function assay in male rats – acceptable even though not OECD agreed guideline	Overall, the study is considered negative because isolated effects were either not significant or within the performance standards set in respective EPA guideline
	Hershberger assay; acceptable	Negative
	Uterotrophic assay; acceptable	Negative
	Effect of glyphosate on reproductive organs in male SD rat; supplementary non-guideline study	Significantly decreased the absolute but not relative weight of seminal vesicle gland and coagulating gland. Total sperm count was significantly decreased at a dose of 500 mg/kg bw, the highest dose tested. No significant effects were detected on immuno histochemistry of androgen receptor (AR), testosterone-, oestradiol- or progesterone-concentration and oxidative stress parameters
Level 2 (*in vitro*)	Oestrogen receptor transcriptional activation (human cell Line (HeLa-9903)) screening assay; acceptable	Negative
	Oestrogen receptor binding (rat uterine cytosol) screening assay; acceptable	Negative

	cytosol) screening assay; acceptable	
	Human recombinant aromatase assay; acceptable	Negative
	H295R steroidogenesis assay; acceptable	Negative
Level 2 (*in vitro*) non-guideline studies	Differential effects of glyphosate and roundup on human placental cells and aromatase; study supplementary	For the active substance, no effects were described giving evidence for endocrine disruption. As in several other published papers, however, the pesticide formulation roundup seemed to have an array of toxic effects
	Glyphosate-based herbicides are toxic and endocrine disruptors in human cell lines; study supplementary	The data confirm that formulations are more toxic than the active substance. Some of them seem to have anti-androgenic properties. This cannot be confirmed to the same extent for the active substance, however, a non-dose-dependent reduction of transcriptional activity at the androgen receptor was observed
	BLTK1 murine Leydig cells: a novel steroidogenic model for evaluating the effects of reproductive and developmental toxicants; study supplementary	Glyphosate was negative in this non-guideline steroidogenesis assay
	Glyphosate induces human breast cancer cells growth via oestrogen receptors; study supplementary	Glyphosate showed some oestrogenic activity in T47D cells under the conditions of this test

	ovarian (BG1) cell line containing oestrogen receptor alpha and beta for improved detection of oestrogenic/ antioestrogenic chemicals; study supplementary	*in vitro* under the conditions of this test
	Coformulants in glyphosate-based herbicides disrupt aromatase activity in human cells below toxic levels; study supplementary	The reported data showed that glyphosate did not significantly inhibit aromatase activity at non-cytotoxic concentrations
	Evidence for direct effects of glyphosate on ovarian function: glyphosate influences steroidogenesis and proliferation of bovine granulosa but not theca cells *in vitro*; study supplementary	Proliferation of granulosa cells was impaired and at the same time E2 production inhibited in a non-dose-dependent manner by an unknown mode of action
Conclusion	The weight of evidence indicates that glyphosate does not have EATS-mediated endocrine disrupting properties	
Studies performed on metabolites or impurities	<u>Aminomethylphosphonic acid (AMPA, metabolite in glyphosate-tolerant GM plants and in soil and water):</u> Rat and mice LD_{50} oral > 5000 mg/kg bw Rat LD_{50} dermal > 2000 mg/kg bw Skin sensitisation: negative (M&K test) 90-day, rat: NOAEL: 400 mg/kg bw per day based on bw gain↓, urothelial hyperplasia (bladder) and gastro intestinal clinical signs 90-day, dog: NOAEL 263 mg/kg bw per day, the highest dose tested Genotoxicity: consistently negative in Ames tests, mammalian cell gene mutation and UDS tests *in vitro* and in micronucleus assays *in vivo* Rat developmental toxicity: No evidence of teratogenicity, maternal NOAEL 150 mg/kg bw per day, based on clinical signs, bw gain/food consumption↓, developmental NOAEL 400 mg/kg bw per day, based on mean foetal wt↓ AMPA presents a similar toxicological profile as glyphosate and the reference values of the latter apply to its metabolite AMPA Data gaps were identified for toxicological data on the <u>metabolites *N*-acetylglyphosate and *N*-acetyl-AMPA</u> as they were included in the residue definition for plants with glyphosate-tolerant GM plant varieties	

Effects on terrestrial vertebrates (Annex IIA, point 8.1, Annex IIIA, points 10.1 and 10.3)

Species	Test substance	Time scale	End point (mg/kg body weight per day)	End point (mg/kg feed)
Birds				
Bobwhite quail	Glyphosate acid	Acute	4334 (extrapolated with factor 2.167)	–
Bobwhite quail	AMPA	Acute	> 2250	–
Bobwhite quail	Glyphosate acid	Short-term	> 5200	–
Bobwhite quail	AMPA	Short-term	> 5620	–
Bobwhite quail	Glyphosate acid	Long-term	96.3	1000
Mallard duck	Glyphosate acid	Long-term	125.3	1000
Mammals				
Rat	Glyphosate acid	Acute	> 2000	–
Rat	Glyphosate acid	Long-term	197	–
Rabbit	Glyphosate acid	Long-term	50	–
Additional higher tier studies				
Amphibian metamorphosis assay/glyphosate acid/no effects indicating thyroidal activity AMPA: aminomethylphosphonic acid.				

Toxicity data for aquatic species (most sensitive species of each group) (Annex IIA, point 8.2, Annex IIIA, point 10.2)

Group	Test substance	Time-scale (Test type)	End point	Toxicity[a] (mg/L)
Laboratory tests				

Fish				
Oncorhynchus mykiss	Glyphosate acid	96 h (static)	Mortality, EC_{50}	38 (nom.)
Lepomis macrochirus	Glyphosate acid	96 h (static)	Mortality, EC_{50}	47 (nom.)
Danio rerio	Glyphosate acid	96 h (semistatic)	Mortality, EC_{50}	123 (nom.)
Cyprinus carpio	Glyphosate acid	96 h (semistatic)	Mortality, EC_{50}	> 100 (nom.)
Oncorhynchus mykiss	MON 52276	96 h (static)	Mortality, EC_{50}	> 989 (mm.) > 306 a.e. [b]
Cyprinus carpio	MON 52276	96 h (static)	Mortality, EC_{50}	> 895 (mm.) > 277 a.e. [b]
Oncorhynchus mykiss	AMPA	96 h (static)	Mortality, EC_{50}	520 (mm.)
Pimephales promelas	Glyphosate acid	255 days	Growth NOEC	25.7 (mm.)
Brachydanio rerio	Glyphosate acid	168 h	Growth NOEC	1 (nom.)
Pimephales promelas	Glyphosate acid	21 days	Reproduction NOEC	> 33 (mm)
Pimephales promelas	AMPA	33 days	Growth NOEC	12 (mm.)
Aquatic invertebrate				
Daphnia magna	Glyphosate acid	48 h (static)	Mortality, EC_{50}	40 (nom.)
Daphnia magna	AMPA	48 h (static)	Mortality, EC_{50}	690 (nom.)
Daphnia magna	HMPA	48 h (static)	Mortality, EC_{50}	> 100 (nom.)
Daphnia magna	MON 52276	48 h (static)	Mortality, EC_{50}	676 (nom.) 209 a.e.
Daphnia magna	Glyphosate acid	21 days (semi-static)	Reproduction, NOEC	12.5 (nom.)

Daphnia magna	AMPA	21 days (semi-static)	Reproduction, NOEC	15 (nom.)
Sediment dwelling organisms				
Chironomus riparius	Glyphosate acid	28 days (static)	NOEC	–
Algae				
Anabaena flos-aquae	Glyphosate acid	72 h (static)	Biomass: E_bC_{50} Growth rate: E_rC_{50} NOErC	8.5 (nom.) 22 (nom.) 12 (nom.)
Skeletonema costatum	Glyphosate acid	72 h (static)	Biomass: E_bC_{50} Growth rate: E_rC_{50} NOErC	11 (nom.) 18 (nom.) 1.82 (nom.)
Pseudokirchneriella subcapitata	Glyphosate acid	72 h (static)	Biomass: E_bC_{50} Growth rate: E_rC_{50} NOErC	18 (nom.) 19 (nom.) 10 (nom.)
Desmodesmus subspicatus	AMPA	72 h (static)	Biomass: E_bC_{50} Growth rate: E_rC_{50} NOErC NOEC	89.8 (nom.) 452 (nom.) 0.96 (nom.) 24 (nom.)
Pseudokirchneriella subcapitata	AMPA	72 h (static)	Biomass: E_bC_{50} Growth rate: E_rC_{50} NOErC	110 (nom.) 200 (nom.) 46 (nom.)
Pseudokirchneriella subcapitata	HMPA	72 h (static)	Biomass: E_bC_{50} Growth rate: E_rC_{50} NOAEC	> 115 (nom.) > 115 (nom.) 60 (nom.)
Pseudokirchneriella subcapitata	MON 52276	72 h (static)	Biomass: E_bC_{50} Growth rate: E_rC_{50} NOEC	178 (55 a.e.) [b] (nom.) 284 (88 a.e.) (nom.) 90 (28 a.e.)
Higher plant				
Lemna gibba	Glyphosate acid	14 days (semistatic)	Fronds, EC_{50} $NOEC_{empiric}$	12 (nom.) 1.5 (nom.)

Lemna gibba	HMPA	7 days (semistatic)	Fronds, EC_{50} NOEC	> 123 (nom.) 123 (nom.)
Lemna gibba	MON 52276	7 days (semistatic)	Fronds, EC_{50} NOEC	67 (nom.) 21 (a.e.) 0.9 (nom.) 0.3 (a.e.)
Myriophyllum aquaticum	Glyphosate acid (MON 77973)	14 days (static)	Fresh weight, relative increase, EC_{50} NOEC	12.3(nom.) << 5 (nom.)
Myriophyllum aquaticum	AMPA	14 days (static)	Fresh weight, relative increase, EC_{50} dry weight, relative increase, EC_{50} for root length NOEC	70.8 (mm.) 63.2 (mm.) 31.1 (mm) << 5.4 (nom.)
Myriophyllum aquaticum	MON 52276	14 days (static)	Fresh weight, relative increase, EC_{50} NOEC	4.44 a.e. [b] (mm.) < 0.3 a.e. [b] (mm.)

Microcosm or mesocosm tests -/-

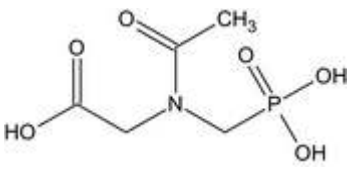
Indicate if not required -/-

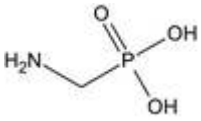
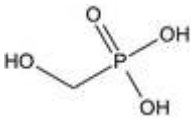
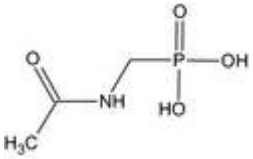
EC_{50}: effective concentration; AMPA: aminomethylphosphonic acid; NOEC: no observed effect concentration; HMPA: hydroxymethylphosphonic acid; E_bC_{50}: effective concentration (biomass); E_rC_{50}: effective concentration (growth rate); NOErC: no observed effect concentration growth rate.

[a] Indicate whether based on nominal ($_{nom}$) or mean measured concentrations ($_{mm}$). In the case of preparations indicate whether end points are presented as units of preparation or a.s.

[b] a.e.: acid equivalents.

Appendix B – Used compound codes

Code/trivial name[a]	Chemical name/SMILES notation[b]	Structural formula
N -Acetyl-glyphosate	*N* -Acetyl- *N* -(phosphonomethyl)glycine OC(=O)CN(CP(=O)(O)O)C(C)=O	

AMPA	(Aminomethyl)phosphonic acid NCP(=O)(O)O	
HMPA	(Hydroxymethyl)phosphonic acid OCP(=O)(O)O	
N -Acetyl-AMPA	(Acetamidomethyl)phosphonic acid CC(=O)NCP(=O)(O)O	

[a] ACD/ChemSketch, Advanced Chemistry Development, Inc., ACD/Labs Release: 12.00 Product version: 12.00 (Build 29305, 25 Nov 2008).

[b] SMILES: simplified molecular-input line-entry system.

References

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