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

European Food Safety Authority (EFSA)

Abstract

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.

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Keywords: glyphosate, peer review, potential endocrine activity, risk assessment, pesticide, herbicide

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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 supplementary in vitro studies was inconsistent with the findings of the acceptable OECD (Organisation for Economic Co-operation and Development) 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. 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,\(^5\) 540/2011\(^6\), 541/2011\(^7\), 2016/1056\(^8\), and 2016/1313\(^9\).

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:

- Data according to the Endocrine Disruptor Screening Programme (EDSP) or the Level 2 and 3 tests indicated in the Organisation for Economic Co-operation and Development (OECD) Conceptual Framework, as outlined in the EFSA Conclusion;

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\(^1\) 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.
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.

The active substance and the formulated product

Glyphosate is the ISO common name for \( N-(\text{phosphonomethyl})\text{glycine} \) (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 \( N-(\text{phosphonomethyl})\text{glycinate} \), potassium \( N-[(\text{hydroxyphosphinato})\text{methyl}]\text{glycine} \), ammonium \( N-[(\text{hydroxyphosphinato})\text{methyl}]\text{glycine} \) and dimethylammonium \( N-(\text{phosphonomethyl})\text{glycinate} \) (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 (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\(^\text{10}\) (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.

The slight delay in PPS observed in one level 5 study was not confirmed in mechanistic in vivo studies (OECD Conceptual Framework level 3 studies); all available in vitro studies performed according to OECD 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

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\(^{10}\) 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.
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 amphibian metamorphosis assay was submitted. Slightly larger body sizes of tadpoles were observed with some of the glyphosate concentrations tested. However, according to technical guideline OECD 231, 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 focused 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, 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.

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 evaluation and authorisation of plant protection products. OJ L 155, 11.6.2011, p. 127-175.
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.

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 lack of information, and where the assessment performed at a 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.

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

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Abbreviations

| Abbreviation | Description |
|--------------|-------------|
| a.s. | active substance |
| AMPA | aminomethylphosphonic acid |
| AR | androgen receptor |
| bw | body weight |
| E2 | 17\beta-estradiol |
| E\textsubscript{b}C\textsubscript{50} | effective concentration (biomass) |
| E\textsubscript{C}C\textsubscript{50} | effective concentration (growth rate) |
| EATS | oestrogen, androgen, thyroid and steroidogenesis (modalities) |
| E\textsubscript{C50} | effective concentration |
| ECHA | European Chemicals Agency |
| EEC | European Economic Community |
| ED | endocrine disruptor |
| EDSP | Endocrine Disruptor Screening Programme |
ER$_{50}$ emergence rate/effective rate, median
ER$^\alpha$ oestrogen receptor subtype $\alpha$
ER$^\beta$ oestrogen receptor subtype $\beta$
GAP Good Agricultural Practice
GM genetically modified
GSI gonadosomatic index
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
Appendix A – List of end points for the active substance 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 or I (b) | Pests or Group of pests controlled(c) | Formulation | Application | Application rate per treatment | Remarks |
|--------------------------|-------------------------|--------------|------------|---------------------------------------|-------------|------------|-------------------------------|---------|
| 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 | 1-6 | 100-400 | 0.36-2.16 | Spring and autumn after harvest (incl. stubble and/or seedbed prep.) Max. application rate 4.32 kg/ha glyphosate in any 12-month period across use categories, equivalent to the sum of preplant, preharvest and post-harvest stubble applications. The interval between applications is dependent on new weed emergence after the first treatment, relative to the time of planting the crop. |
| 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 | 1-3 | 100-400 | 0.36-1.08 |
| Crop and/or situation<sup>a</sup> | Member State or Country | Product name | FG or I<sup>b</sup> | Pests or Group of pests controlled<sup>c</sup> | Formulation | Application | Application rate per treatment | PHI<sup>(d) (l)</sup> | Remarks |
|----------------------|--------------------------|--------------|----------------|-----------------------------------------------|--------------|-----------------|-------------------------------|----------------|--------|
| 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 | 2-6 | 100-400 | 0.72-2.16 | 7 | Max. application rate 4.32 kg/ha glyphosate in any 12-month period across use categories, equivalent to the sum of preplant, preharvest and post-harvest stubble applications. Preharvest uses in all crops include uses for weed control (higher doses) and harvest aid, sometimes referred to as desiccation (lower doses). The critical GAP is the high dose recommended used for weed control. |
| 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 | 2-6 | 100-400 | 0.72-2.16 | 7 | |
| 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 | 2-6 | 100-400 | 0.72-2.16 | 14 | |
| 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 | 28 days | 2-8 | 100-400 | 0.72-2.88 | N/A | Stone and pome fruit, olives. Applications to avoid contact with tree branches. Maximum cumulative application rate 4.32 kg/ha glyphosate in any 12-month period. Note: Because applications are made to the intrarows (inner strips between the trees within a row), application rates per ha are expressed per ‘unit of treated surface area’ the actual application rate per ha orchard or vineyard will roughly only be 33% |
| Crop and/or situation(a) | Member State or Country | Product name | Pests or Group of pests controlled(b) | Formulation | Application | Application rate per treatment | Remarks |
|-------------------------|-------------------------|--------------|--------------------------------------|-------------|------------|-------------------------------|---------|
| 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) | 28 days | 2.0 - 8.0 | 0.72 - 2.88 |

Stone and pome fruit, olives Applications made round base of trunk (0.0 L/ha water addresses ULV application of the undiluted product) Max. cumulative application rate 4.32 kg/ha glyphosate in any 12-month period. Note: Because applications are made round base of trunk and to the intra-rows, (inner strips between two trees within a row), application rates per ha are expressed per ‘unit of treated surface area’ the actual application rate per ha orchard or vineyard will roughly only be 33 - 50%

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 vegetables, brassica vegetables, leaf vegetables and fresh herbs, legume vegetables), pulses, oil seeds, 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 be described (e.g. fumigation of a structure).

(b): Outdoor or field use (F), greenhouse application (G) or indoor application (I).

(c): e.g. biting and sucking 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 indicated.

(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 compare the rate for same active substances used in different variants (e.g. fluoroxypyr). **In certain cases, where only one variant is synthesised, it is more appropriate to give the rate for the variant (e.g. benthiavalicarb-isopropyl).**

(j): Growth stage at 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.

(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)

Supplementary studies on the active substance

Severity of salivary gland findings is strain-specific in rats; effects are likely due to low pH in oral 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    | 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)       | Studies on short-term toxicity, chronic toxicity, developmental toxicity, one-generation range-finding and carcinogenicity: negative |
|            | (in vivo)       | (Germany, 2015) |
| Level 3 (in vivo) | A pubertal development and thyroid function assay in female rats; acceptable even though not OECD agreed guideline | Significantly lower percentage of females 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 |
| Level 2 (in vitro) non-guideline studies | Androgen receptor binding (rat prostate cytosol) screening assay; acceptable | Negative |
|----------------------------------------|--------------------------------------------------------------------------|----------|
|                                        | Human recombinant aromatase assay; acceptable                            | Negative |
|                                        | H295R steroidogenesis assay; acceptable                                  | Negative |
|                                        | 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 |
Development of a recombinant human ovarian (BG1) cell line containing oestrogen receptor alpha and beta for improved detection of oestrogenic/antioestrogenic chemicals; study supplementary

| Conclusion | The weight of evidence indicates that glyphosate does not have EATS-mediated endocrine disrupting properties |

| Studies performed on metabolites or impurities | Aminomethylphosphonic acid (AMPA, metabolite in glyphosate-tolerant GM plants and in soil and water): |
| | 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$_{\uparrow}$, 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$_{\downarrow}$, developmental NOAEL 400 mg/kg bw per day, based on mean foetal wt$_{\downarrow}$ |
| | 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 metabolites N-acetylglyphosate and N-acetyl-AMPA as they were included in the residue definition for plants with glyphosate-tolerant GM plant varieties |

Glyphosate has no ER$_{\alpha}$, ER$_{\beta}$ agonistic activities, 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
**Ecotoxicology**

**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.)        |
| Cyprinus carpio  | MON 52276     | 96 h (static)          | Mortality, EC$_{50}$ | > 895 (mm.)        |
| 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.)         |
| Daphnia magna    | Glyphosate acid | 21 days               | Reproduction, NOEC | 12.5 (nom.)        |
| Daphnia magna    | AMPA           | 21 days               | Reproduction, NOEC | 15 (nom.)          |
| Group                          | Test substance       | Time-scale (Test type) | End point       | Toxicity (a) (mg/L) |
|-------------------------------|----------------------|------------------------|-----------------|---------------------|
| **Sediment dwelling organisms** |                      |                        |                 |                     |
| Chironomus riparius          | Glyphosate acid      | 28 days (static)       | NOEC            | –                   |
| **Algae**                    |                      |                        |                 |                     |
| Anabaena flos-aquae          | Glyphosate acid      | 72 h (static)          | Biomass: $E_{50}$  | 8.5 (nom.)          |
|                              |                      |                        | Growth rate: $E_{50}$ | 22 (nom.)          |
|                              |                      |                        | NOErC           | 12 (nom.)           |
| Skeletonema costatum         | Glyphosate acid      | 72 h (static)          | Biomass: $E_{50}$  | 11 (nom.)           |
|                              |                      |                        | Growth rate: $E_{50}$ | 18 (nom.)          |
|                              |                      |                        | NOErC           | 1.82 (nom.)         |
| Pseudokirchneriella subcapitata | Glyphosate acid         | 72 h (static)          | Biomass: $E_{50}$  | 18 (nom.)           |
|                              |                      |                        | Growth rate: $E_{50}$ | 19 (nom.)          |
|                              |                      |                        | NOErC           | 10 (nom.)           |
| Desmodesmus subspicatus      | AMPA                 | 72 h (static)          | Biomass: $E_{50}$  | 89.8 (nom.)         |
|                              |                      |                        | Growth rate: $E_{50}$ | 452 (nom.)         |
|                              |                      |                        | NOErC           | 0.96 (nom.)         |
|                              |                      |                        | NOEC            | 24 (nom.)           |
| Pseudokirchneriella subcapitata | AMPA             | 72 h (static)          | Biomass: $E_{50}$  | 110 (nom.)          |
|                              |                      |                        | Growth rate: $E_{50}$ | 200 (nom.)         |
|                              |                      |                        | NOErC           | 46 (nom.)           |
| Pseudokirchneriella subcapitata | HMPA            | 72 h (static)          | Biomass: $E_{50}$  | > 115 (nom.)        |
|                              |                      |                        | Growth rate: $E_{50}$ | > 115 (nom.)       |
|                              |                      |                        | NOAEC           | 60 (nom.)           |
| Pseudokirchneriella subcapitata | MON 52276        | 72 h (static)          | Biomass: $E_{50}$  | 178 (55 a.e.) (b) (nom.) |
|                              |                      |                        | Growth rate: $E_{50}$ | 284 (88 a.e.) (nom.) |
|                              |                      |                        | NOEC            | 90 (28 a.e.)        |
| **Higher plant**             |                      |                        |                 |                     |
| Lemna gibba                  | Glyphosate acid      | 14 days (semistatic)   | Fronds: $EC_{50}$ | 12 (nom.)           |
|                              |                      |                        | NOCEmpiric      | 1.5 (nom.)          |
| Lemna gibba                  | HMPA                 | 7 days (semistatic)    | Fronds: $EC_{50}$ | > 123 (nom.)        |
|                              |                      |                        | NOEC            | 123 (nom.)          |
| Lemna gibba                  | MON 52276           | 7 days (semistatic)    | Fronds: $EC_{50}$ | 67 (nom.) 21 (a.e.) |
|                              |                      |                        | NOEC            | 0.9 (nom.) 0.3 (a.e.) |
| Myriophyllum aquaticum       | Glyphosate acid (MON 77973) | 14 days (static) | Fresh weight, relative increase, $EC_{50}$ | 12.3 (nom.) |
|                              |                      |                        | NOEC            | <= 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.) |
|                              |                      |                        | NOEC            | 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; $E_{50}$: effective concentration (biomass); $E_{50}$: effective concentration (growth rate); NOErC: no observed effect concentration growth rate.

(a): Indicate whether based on nominal ($c_{nom}$) or mean measured concentrations ($c_{mm}$). 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<sup>(a)</sup> | Chemical name/SMILES notation<sup>(b)</sup> | Structural formula |
|---------------------------------|---------------------------------------------|--------------------|
| N-Acetyl-glyphosate             | N-Acetyl-N-(phosphonomethyl)glycine          | ![Structural formula](image1) |
|                                 | OC(-O)CN(CP(-O)(O)O)C(C)=O                  |                    |
| AMPA                            | (Aminomethyl)phosphonic acid NCP(-O)(O)O     | ![Structural formula](image2) |
| HMPA                            | (Hydroxymethyl)phosphonic acid OCP(-O)(O)O   | ![Structural formula](image3) |
| N-Acetyl-AMPA                   | (Acetamidomethyl)phosphonic acid CC(-O)NCP(-O)(O)O | ![Structural formula](image4) |

(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.