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Evaluation of the impact of glyphosate and its residues in feed on animal health

European Food Safety Authority (EFSA)

Abstract

EFSA was requested by the European Commission to consider the impact of glyphosate residues in feed on animal health in accordance with Article 31 of Regulation (EC) No 178/2002. The current report presents the assessment of the available information on glyphosate residues in feed, including feed imported from outside the European Union (i.e. third countries) and the conclusions on the possible impact of those residues on animal health. The conclusions of this scientific report were reached on the basis of the initial evaluation carried out by the competent authority of the rapporteur Member State, Germany, using data made available by industry, on the existing EFSA Conclusion on glyphosate as well as on the basis of a literature review conducted by EFSA. The current report is delivered at the same time as the EFSA Reasoned Opinion on the review of existing maximum residue levels for glyphosate according to Article 12 of Regulation (EC) No 396/2005. With regard to the assessment of the impact of glyphosate and its residues on animal health, considering the available data, glyphosate is not expected to have a major impact, if any, on animal health.

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Keywords: glyphosate, herbicide, residues, feed, animal health, genetically modified crops, risk assessment

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Summary

On 12 November 2015, 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 as amended by Commission Implementing Regulation (EU) No 380/2013 (EFSA Journal 2015;13(11):4302). Based on the assessment of the representative uses evaluated during the peer review, EFSA did not raise concerns as regards harmful effects on animal health. According to the current regulatory framework for pesticides, the absence of harmful effects on animal health is part of the authorisation criteria for plant protection products and thus assessed at national level.

A significant amount of food and feed is imported into the European Union (EU) from third countries, including food and feed produced from glyphosate-tolerant genetically modified (GM) crops. Provided that these products (or parts thereof) are not used exclusively as ingredients for animal feed, the maximum residue levels (MRLs) set under Regulation (EC) No 396/2005 do apply. Whilst this Regulation has a strong focus on consumer protection, animal health aspects are considered for MRL setting only as regards their impact on human health, and data related to animal health only is not required. Therefore, it was considered by the European Commission desirable to address this issue through a risk assessment focused on animal health.

On 25 February 2016, EFSA received a mandate from the European Commission to consider the impact of glyphosate residues in feed on animal health in accordance with Article 31 of Regulation (EC) No 178/2002. In particular, EFSA has been requested to assess the available information on glyphosate residues in feed, including feed imported from outside the EU (i.e. third countries), e.g. glyphosate-tolerant GM crops, and conclude on the possible impact of those residues on animal health.

On 13 March 2016, EFSA requested relevant data related to animal health from companies involved in the renewal of the approval of glyphosate, in MRL applications and companies authorised to place feed produced from GM crops on the EU market. The initial assessment of the data submitted was carried out by the competent authority of the rapporteur Member State (RMS), Germany, in the format of a draft addendum to the renewal assessment report, which was submitted to EFSA on 7 April 2017. Subsequently, the addendum on the considerations of data on animal health was distributed to Member States, the applicants and EFSA for written comments. In addition, an expert consultation was conducted in the area of mammalian toxicology with regard to the hazard assessment aspects of the evaluation.

The current report aims to assess the health risk for farm animals, in relation to the presence of glyphosate and its residues in feed, including GM feed. The conclusions were reached on the basis of the initial assessment carried out by the RMS; relevant data from the EFSA peer review carried out in the framework of the renewal of glyphosate and the EU review of MRLs for glyphosate under Article 12 of Regulation (EC) No 396/2005 were also taken into account in the current evaluation. In addition, a systematic literature review has been conducted by EFSA to complement the available information.

Before finalisation of the report, a consultation with Member States was conducted via a written procedure in October 2017. The European Medicines Agency has been involved as additional reviewer of this scientific report to ensure methodological consistency with other EU agencies, in particular for interspecies extrapolation. In addition, during the finalisation of the assessment, additional information available to the RMS but not submitted to EFSA was identified. The European Commission asked EFSA to request that information and consider it in the final assessment. Therefore, further amendments have become necessary at the final stage. More specifically, the toxicological profile of the metabolites *N*-acetyl-aminomethylphosphonic acid (AMPA) and *N*-acetyl-glyphosate was further considered during the Pesticides Peer Review Experts' Teleconference 175 (27 February 2018) on the basis of the assessment of toxicological data provided in the context of the Art 10 MRL Opinion (EFSA, Journal 2009;7(9):1310), taking into account also the raw studies made available to EFSA in January 2018 and other international evaluations.

The current scientific report is delivered simultaneously with the Reasoned Opinion on the review of the existing MRLs for glyphosate according to Article 12 of Regulation (EC) No 396/2005. The following conclusions are reached.

For cattle and sheep (bovine and ovine species), equine, porcine and selected avian (poultry) species, glyphosate (and its metabolite AMPA) are not expected to have an impact on the health of these animals on the basis of the available data (resulting in margins of exposure between 4 and 44).

For cattle and sheep (bovine and ovine species), the absence of adverse effects on the ruminal microflora based on *in vitro* data was demonstrated up to a dose of 292 ppm in the diet (dry matter).

This level covers the maximum dietary burden for all authorised uses except the use of glyphosate on grass forage. Minor effects on the microbial communities of the rumen have not been investigated for the use of glyphosate on grass forage but can be excluded for all other uses.

The maximum dietary burden covering all uses including grass forage (342 ppm for cattle and 530 ppm for sheep) has been investigated in *in vivo* animal studies. Considering the results of the toxicological studies on different species and in particular the absence of adverse effect in dairy cows fed during 28 days with a diet containing 400 ppm of glyphosate, glyphosate is not expected to have, even at the maximum dietary burden, effects on the microbial communities in the rumen impacting on the health of bovine and ovine species.

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1. Introduction

1.1. Background and Terms of Reference as provided by the European Commission

The active substance glyphosate was included in Annex I to Directive 91/414/EEC¹ on 1 July 2002 by Commission Directive 2001/99/EC,² and was deemed to be approved under Regulation (EC) No 1107/2009³, in accordance with Commission Implementing Regulation (EU) No 540/2011⁴, as amended by Commission Implementing Regulations (EU) No 541/2011⁵, 2016/1056⁶ and 2016/1313⁷.

On 12 November 2015, 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 1141/2010⁸ as amended by Commission Implementing Regulation (EU) No 380/2013⁹ (EFSA, 2015b). Based on the assessment of the representative uses evaluated during the peer review, EFSA did not raise concerns as regards harmful effects on animal health.

A significant amount of food and feed is imported into the EU from third countries, including food and feed produced from glyphosate-tolerant genetically modified (GM) crops. Provided that these products (or parts thereof) are not used exclusively as ingredients for animal feed, the maximum residue levels (MRLs) set under Regulation (EC) No 396/2005¹⁰ do apply. This Regulation has a strong focus on consumer protection, while data related to animal health only are not required. Therefore, it was considered by the European Commission desirable to address this issue through a risk assessment focused on animal health.

By means of a mandate received on 25 February 2016, EFSA was requested by the European Commission to consider the impact of glyphosate residues in feed on animal health in accordance with Article 31 of Regulation (EC) No 178/2002¹¹. In particular, EFSA has been requested to assess the available information on glyphosate residues in feed, including feed imported from outside the EU (i.e. third countries), e.g. glyphosate-tolerant GM crops, and conclude on the possible impact of those residues on animal health.

EFSA was invited within the mandate to request relevant information from companies involved in the renewal of the approval of glyphosate, in MRL applications and companies authorised to place feed produced from GM crops on the EU market.

For purpose of the evaluation of the impact of glyphosate and its residues in feed on animal health, EFSA is producing a scientific report which is delivered simultaneously with the Reasoned Opinion on the review of the existing MRLs for glyphosate according to Article 12 of Regulation (EC) No 396/2005.

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 Commission Implementing Regulation (EU) No 380/2013 of 25 April 2013 amending Regulation (EU) No 1141/2010 as regards the submission of the supplementary complete dossier to the Authority, the other Member States and the Commission, OJ L 116, 26.4.2013, p. 4–4.

¹⁰ Regulation (EC) No 396/2005 of the European Parliament and of the Council of 23 February 2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EEC. OJ L 70, 16.3.2005, p. 1–16.

¹¹ 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.

1.2. Additional information

This scientific assessment was chosen as a case study to test the four-step (plan/carry out/verify/report) approach for data collection, appraisal and integration, as described in the European Food Safety Authority (EFSA) report on 'Principles and process for dealing with data and evidence in scientific assessments' (EFSA, 2015a, deliverable 1 of PROMETHEUS project – PROMoting METHods for Evidence Use in Scientific assessments). In this context, a Protocol was drafted by EFSA defining the strategy for the assessment and submitted for written comments to Member States (see Annex A).

2. Data and methodologies

2.1. Data

As invited within the mandate, on 13 March 2016, EFSA requested information relevant for the assessment on animal health from the companies involved in the renewal of the approval of glyphosate, in MRL applications and companies authorised to place feed produced from GM crops on the EU market. In particular, the following data were requested:

- Toxicological studies on livestock (mammals and birds) with glyphosate,
- Toxicological studies with the metabolites, relevant for uses on conventional crops and for the uses on glyphosate tolerant GM varieties,
- Metabolism studies in primary crops including GM crops, rotational crops and livestock,
- Any additional information relevant for the identification and assessment of potential direct or secondary adverse effects of glyphosate residues in feed on animal health.

Germany, as rapporteur Member State (RMS), agreed to provide an evaluation taking into account the previously peer-reviewed endpoints, new data from the literature and any new information provided by industry related to potential adverse effects on animal health.

The initial assessment of the animal health aspects by Germany was provided in the form of a draft addendum to the renewal assessment report, which was received by EFSA on 7 April 2017 (Germany, 2017a). Subsequently, the draft addendum with the RMS initial considerations of the data submitted was dispatched to the Member States and the relevant companies for consultation and comments. EFSA also provided comments. Comments received were collated by EFSA and forwarded to the RMS for consideration during the revision of the addendum. Furthermore, a systematic literature review was conducted by EFSA to complement the available data set, in particular as regards *in vivo* studies on farm animals (see Appendix A). The additional studies identified were also assessed in the revised addendum made available by the RMS on 23 June 2017 (Germany, 2017b).

With regard to the considerations of the hazard assessment aspects of the evaluation, further discussions took place at the Pesticides Peer Review Experts' Teleconference 148 on mammalian toxicology on 29 June 2017. Details of the issues discussed, together with the outcome of these discussions were recorded in the respective meeting report. In addition, further revisions of the addendum were produced by the RMS following the expert consultation (Germany, 2017b).

Data including feed consumption data and studies assessing levels of glyphosate residues in feed items taken from the Reasoned Opinion on the review of the existing MRLs for glyphosate according to Article 12 of Regulation (EC) No 396/2005 (EFSA, 2018b) were taken into consideration for the exposure part of the risk assessment.

A final consultation on the conclusions arising from the evaluation of the impact of glyphosate and its residues in feed on animal health took place with Member States via a written procedure in October 2017. The European Medicines Agency has been involved as an additional reviewer of this scientific report to ensure methodological consistency with other EU agencies, in particular for interspecies extrapolation. In addition, during the finalisation of the assessment, additional information available to the RMS but not submitted to EFSA was identified. The European Commission asked EFSA to request that information and consider it in the final assessment. Therefore, further amendments have become necessary at the final stage. More specifically, further considerations on the toxicological profile of the *N*-acetyl compounds were made on the basis of the assessment of toxicological data provided in the context of the Art 10 MRL Opinion (EFSA, 2009), following consideration of the raw studies made available to EFSA in January 2018 and taking into account other international evaluations. Furthermore, following late changes occurred in the authorised uses on grass, amendments were triggered in the livestock exposure assessment in the Art 12 MRL review, and the impact on animal health was reconsidered accordingly.

The conclusions were reached on the basis of the assessment carried out by the RMS, with the complementary results of the literature review undertaken by EFSA as well as taking into account relevant data from the EFSA peer review carried out in the framework of the renewal of glyphosate (EFSA, 2015b) and the EU review of MRLs for glyphosate under Article 12 of Regulation (EC) No 396/2005 (EFSA, 2018b).

Furthermore, a key-supporting document to this scientific report is the peer review report (EFSA, 2018a), which is a compilation of the documentation developed to evaluate and address all issues raised in the course of this assessment, comprising of the following documents:

- the comments received on the draft Protocol for risk assessment on glyphosate residues and animal health;
- the comments received on the initial addendum provided by Germany (RMS);
- the reports of the scientific consultation with Member State experts;
- the comments received on the draft scientific report of EFSA.

2.2. Methodologies

The Terms of Reference are addressed by assessing the health risk for farm animals, in relation to the presence of residues of glyphosate and its metabolites in feed (including GM feed). This implies conducting hazard identification and characterisation, exposure and risk assessment, as detailed in the sections below. The strategy followed to perform this assessment was defined beforehand by EFSA in a Protocol submitted to Member States for comments in November 2016 and finalised at the end of the peer review (see Annex A).

The overall objective of the assessment is to establish whether there is an association between any possible adverse effect on animal health and glyphosate (and its residues) when ingested via feed of plant origin (including GM feed). If an association is demonstrated, a qualitative and, if possible, a quantitative description of the relationship between the exposure and the identified adverse effect is performed. This includes an assessment of dose–response and potentially an evaluation of possible uncertainties where relevant. However, due to the draft status of the EFSA guidance on uncertainty (EFSA, 2016) and to time constraints, an uncertainty assessment applying the proposed methods was not carried out.

The extensive literature review undertaken by EFSA followed a structured, stepwise process (see Appendix A). The evidence from the literature (also collected by the RMS) was combined together with the evidence from the EFSA conclusion (EFSA, 2015b) and from the MRL Reasoned Opinion (EFSA, 2018b), permitting to draw risk assessment conclusions.

For hazard identification and characterisation, direct evidence of adverse effects from *in vivo* studies on farm animals is taken into account together with indirect evidence from laboratory animals and *in vitro* studies. For risk characterisation, the relevant no observed adverse effect levels (NOAEL (s)) are used as point of departure to derive a margin of exposure for farm animals. Where evidence of adverse effect in farm animals supports NOAELs lower than those identified during the peer review of glyphosate, these new NOAELs should be used for risk characterisation. Otherwise, the risk assessment will rely upon the existing NOAELs from the peer review (EFSA, 2015b).

In the final step of the assessment, the results of the exposure estimates taken from the Reasoned Opinion on the MRL review are considered to identify a margin of exposure for farm animals or any potential dietary exposure exceeding levels of no concern for animal health.

A cooperation with the European Medicines Agency following the experts' consultation and as additional reviewer has been sought for this scientific report to ensure methodological consistency with other EU agencies, in particular for interspecies extrapolation.

3. Assessment

The effects of glyphosate (and its residues) on animal health were discussed by the experts in the Pesticides Peer Review Experts' Teleconference 148 (29 June 2017).

During the assessment, the exposure to glyphosate, aminomethylphosphonic acid (AMPA), *N*-acetyl-AMPA and *N*-acetyl-glyphosate was considered and respective NOAELs were derived for consideration in the impact assessment for livestock species including bovine, equine, ovine, porcine and selected avian species.

3.1. Hazard identification and characterisation

3.1.1. Bovine and ovine species

Metabolism studies in lactating goats revealed a higher conversion to the metabolite AMPA (compared to rats). Published data in lactating cows also suggest that metabolism to AMPA may be more pronounced in ruminants. Oral absorption in goats accounted for ~20% based on urinary excretion, whereas in lactating cows, a lower urinary excretion of 8% of the daily glyphosate intake was observed.

Single and repeated administration of glyphosate in goats and cattle at high-dose levels (more than 1,000 mg/kg body weight (bw) (per day)) demonstrated that systemic intoxication in these animals was mainly characterised by gastrointestinal and neurological signs; the kidneys and gastrointestinal tract (mucosal irritation) were identified as target organs in ruminants by histopathological examination. In **goats**, the acute oral toxicity of glyphosate was low, with the isopropylamine salt of glyphosate being less toxic ($LD_{50} = 5,700$ mg/kg bw) than glyphosate acid ($LD_{50} = 3,530$ mg/kg bw). In **cows**, clinical signs at 830 mg/kg bw triggered a NOAEL of 540 mg/kg bw per day in a 7-day oral toxicity study with the isopropylamine salt of glyphosate. In a supplementary 7-day study with cows, a NOAEL of 400 mg/kg bw per day resulted from the administration of glyphosate within a Roundup formulation.

In a 28-day feeding study in lactating dairy cows, no treatment-related impact on animal health was observed up to the highest dose of 11.2–13.6 mg/kg bw per day (equivalent to 400 ppm in the diet). Milk production was not compromised.

Several issues of potential concern were found in articles of the open literature related to glyphosate.

With regard to a new disease in cattle mainly occurring in the northern parts of Germany and of unknown aetiology, high growth of *Clostridium botulinum* in the rumen and formation of Clostridium neurotoxins due to possible glyphosate-induced microbial imbalance have been hypothesised. *In vitro* data from the 'Rumen Simulation Technique' (Riede et al., 2016) showed that, in general, microbial communities of the rumen were not affected by the administration of glyphosate acid, and the herbicide had no effect on the growth of *Clostridium sporogenes* (surrogate for *Clostridium botulinum*) up to a dose level of 292 ppm in the diet (dry matter), as recalculated by EFSA considering comments provided during the written procedure on the draft scientific report and taking into account the actual analytical determination of the high dose administered. It was also noted that this exploratory study had limitations (e.g. use of only one donor cow, low number of replicates, no positive control, no consideration of other microorganisms than bacteria), resulting in considerable uncertainty. To conclude, a link between the presence of glyphosate residues in the diet and this new disease in cattle could not be established.

With regard to potential chelating properties, a study was reported as possibly demonstrating a relationship between urinary excretion of glyphosate and changes in clinical chemistry parameters (Krüger et al., 2013). During the current assessment, it was concluded that the small amount of ingested glyphosate (~40 mg per animal, equivalent to ~0.07 mg/kg bw per day) is not expected to bind trace elements to such an extent that clinical signs might occur. Furthermore, this study presented major methodological deficiencies (e.g. the absence of a control group) severely affecting its reliability.

With regard to a presumed higher sensitivity of livestock species at different life stages, no evidence was available, either from the available experimental data or from the literature review.

For bovine and ovine species, the experts agreed to use the NOAEL of 50 mg/kg bw per day from the rabbit developmental toxicity studies (also the basis for the derivation of reference values in the framework of the renewal procedure for glyphosate (EFSA, 2015b)). This is supported by the available experimental data with cows (7-day NOAEL of 540 mg/kg bw per day), considering an additional factor (10) to compensate for the limited investigations.

3.1.2. Equine and porcine species

In a 28-day feeding study in cross-bred swine, no health effects could be assigned to treatment up to the highest dose of 11.3–13.1 mg/kg bw per day.

In a feeding study with Danish pigs (6 months and 2.5 years), a decrease in piglet survival rate was observed when the feed included barley treated with Cerone (containing ethephon) and Roundup

(containing glyphosate). This effect could not be attributed to glyphosate since it was not observed with barley treated only with Roundup. Furthermore, a possible impact of the co-formulants could not be excluded.

Malformations in newborn piglets on a Danish farm have been attributed to glyphosate simply because residues of this active substance were found at extremely variable concentrations in different organs and tissues of these animals. In the absence of any control group and taking into account the rather low dietary exposure of the sows and the results of a multitude of developmental studies (Germany, 2015), this assumption is not sufficiently substantiated to be relied upon.

It is noted that possible effects on the microflora of the digestive tract were not investigated in equine and porcine species. Based on the available data and on the review of the literature, no indication of such effects could be identified.

For equine and porcine species, the use of the NOAEL from the rabbit developmental studies was considered appropriate.

3.1.3. Avian species

In acute toxicity studies with Northern bobwhite quail, Japanese quail and Mallard duck, no mortality and no abnormalities were observed after oral administration of up to 2,000 mg/kg bw. In one more study with Northern bobwhite, clinical signs were noted from a dose of 484 mg/kg bw onwards (with the lowest dose being 291 mg/kg bw).

In valid reproductive studies with Northern bobwhite quail and Mallard duck, the lowest NOAEL identified was 96.3 mg/kg bw per day.

In an additional study on male duck fertility, alterations of the testis and epididymal region were observed, together with changes in testosterone and estradiol serum levels, but it could not be concluded whether the effects are caused by the active substance glyphosate or by the co-formulant POE-tallowamine (EFSA, 2015c).

In a 28-day feeding study in laying hens, no health effects or lowering of laying rate was reported up to the highest dose of ca 25.4 mg/kg bw per day.

In a published feeding study in broilers (Kubena et al., 1980), chicken were fed a diet containing the isopropylamine salt of glyphosate for 21 days. Calcium content and Ca/P ratio in the tibiotarsal bones were affected at 21 days for all treated groups. Considering the limitations of this study (no measurements of variability are reported, the use of a statistical analysis having a greater risk of false-positive errors and a lack of consistency of this pattern when the calcium content is considered on an ash basis instead of a dry basis), together with the magnitude of the changes and the lack of effects in valid reproductive studies with birds (see above), this is not expected to be a relevant adverse effect for animal health.

It is noted that possible effects on the microflora of the digestive tract were not investigated in avian species. Based on the available data and on the review of the literature, no indication of such effects could be identified.

For avian species, it was considered appropriate to rely on regulatory studies with birds (EFSA, 2015b), establishing a NOAEL of 100 mg/kg bw per day.

3.1.4. Metabolite AMPA

For the metabolite AMPA, following the previous agreement (EFSA, 2015b) that it was toxicologically similar to glyphosate, the NOAELs of 50 and 100 mg/kg bw per day were also considered as valid for the different categories of livestock animals and for poultry, respectively.

3.1.5. *N*-acetyl-AMPA and *N*-acetyl-glyphosate

The toxicological profile of the metabolites *N*-acetyl-AMPA and *N*-acetyl-glyphosate was further considered during the Pesticides Peer Review Experts' Teleconference 175 (27 February 2018) on the basis of the raw studies made available to EFSA in January 2018 and taking into account other international evaluations. These data included investigations of toxicokinetics and metabolism, acute and subacute oral toxicity and genotoxicity for *N*-acetyl-glyphosate; acute oral toxicity and genotoxicity for *N*-acetyl-AMPA. On this basis, it was concluded that the toxicological profile of glyphosate would cover those of the *N*-acetyl metabolites (and therefore, the same NOAELs for the livestock species and the same reference values for consumers would be applicable).

3.2. Exposure assessment

In the Reasoned Opinion on the review of the existing MRLs according to Article 12 of Regulation 396/2005 (EFSA, 2018b), considerations are given to the nature and magnitude of residues in plants (conventional or GM).

For conventional plants and GM crops containing the CP4-EPSPS modification, the major component of the residues is unchanged glyphosate, while in crops containing the glucose oxidase (GOX) modification, AMPA is present at equal or higher amounts compared to glyphosate. For GM plants containing the glycine *N*-phenylacetyltransferase (GAT) modification, the main component of the residues is represented by *N*-acetyl-glyphosate, a metabolite formed by the action of the GAT enzyme. In addition, *N*-acetyl-AMPA, not observed in conventional crops, was also identified as a major metabolite. While glyphosate is authorised on a wide range of conventional crops within the EU, only few import tolerances for GM glyphosate tolerant crops are currently authorised in the EU; in particular GAT-modified crops are currently not present on the EU market (EFSA, 2018b).

In the framework of the MRL review, the exposure to the different groups of livestock (livestock dietary burden) was calculated according to pertinent OECD guidance (OECD, 2013). Considering that livestock may be exposed to residues originating from conventional and GM crops, the calculation of the livestock dietary burden was performed combining the residues originating from the uses authorised on conventional crops and on GM crops. The results from this calculation are reported in Table 1 below. Considering that GAT-modified crops (the only crops forming the *N*-acetyl compounds) are currently not present on the EU market (see EFSA, 2018b), the exposure calculation was only based on the levels on glyphosate and AMPA present in the crops fed to livestock.

It is also noted that, according to the results of the exposure calculation, the main contributors of the livestock exposure to glyphosate and AMPA were represented by conventional (not GM) grass forage in cattle, sheep and swine and wheat straw in poultry. As additional information and a possible option for risk managers' consideration, when excluding the use of glyphosate on grass forage, the maximum dietary burden for cattle would be 58.2 ppm on the basis of barley straw as main contributor (see Table 1).

Table 1: Residues in livestock (EFSA, 2018b)

Relevant groups	Maximum dietary burden expressed in		Most critical diet	Most critical commodity
	mg/kg bw per day	mg/kg DM		
Cattle (all diets)	13.17 2.24 ^(b)	342 ^(a) 58.2 ^(b)	Cattle (dairy)	Grass, forage (fresh)
Cattle (dairy only)	13.17 2.24 ^(b)	342 58.2 ^(b)	Cattle (dairy)	Grass, forage (fresh)
Sheep (all diets)	17.67 4.33 ^(b)	530 107 ^(b)	Sheep (ram/ewe)	Grass, forage (fresh)
Sheep (ewe only)	17.67 3.57 ^(b)	530 107 ^(b)	Sheep (ram/ewe)	Grass, forage (fresh)
Swine (all diets)	2.85 0.41 ^(b)	123 13.8 ^(b)	Swine (breeding)	Grass, forage (fresh)
Poultry (all diets)	2.28	33.4	Poultry (layer)	Wheat, straw
Poultry (layer only)	2.28	33.4	Poultry (layer)	Wheat, straw

DM: dry matter.

(a): The highest intakes for cattle are considered applicable to equine species (OECD, 2013).

(b): Excluding grass forage and taking into account the limit of quantification (LOQ).

3.3. Risk assessment

Considering the maximum dietary burden for the different livestock categories and the respective NOAELs applicable, the resulting margins of exposure are presented in Table 2.

Table 2: Margin of exposure for livestock categories

Livestock categories	Relevant NOAEL (mg/kg bw per day)	Maximum dietary burden (mg/kg bw per day)	Margin of exposure
Cattle (bovine species) ^(a)	50	13.2	4
Sheep and goats (ovine species)	50	17.7	3
Swine (porcine species)	50	2.85	18
Poultry (avian species)	100	2.28	44

(a): The highest intakes for cattle are considered applicable to equine species (OECD, 2013).

For equine, porcine and selected avian species, the margins of exposure are 4, 18 and 44, respectively, demonstrating that the dietary burden is not expected to exceed the relevant NOAEL for these species.

For bovine and ovine species (e.g. cattle and sheep), the margin of exposure ranges from 3 to 4. It is noted that the potential effect on the microbial communities of the rumen were not observed *in vitro* up to a dose level of 292 ppm in the diet (dry matter), while the maximum dietary burden could be up to 342 ppm in cattle and 530 ppm in sheep (see Table 1). Nevertheless, based on the available evidence from *in vivo* animal studies on different species, and in particular the lack of adverse effects in lactating cows after 28 days of exposure to 400 ppm in the diet, glyphosate is not expected to have, even at the maximum dietary exposure, effects on the ruminal microflora impacting on the health of bovine and ovine species.

4. Conclusions

For cattle and sheep (bovine and ovine species), equine, porcine and selected avian (poultry) species, glyphosate (and its metabolite AMPA) are not expected to have an impact on the health of these animals on the basis of the available data, resulting in margins of exposure between 4 and 44.

For cattle and sheep (bovine and ovine species), the absence of adverse effects on the ruminal microflora based on *in vitro* data was demonstrated up to a dose of 292 ppm in the diet (dry matter). This level covers the maximum dietary burden for all authorised uses except the use of glyphosate on grass forage. Minor effects on the microbial communities of the rumen have not been investigated for the use of glyphosate on grass forage but can be excluded for all other uses.

The maximum dietary burden covering all uses including grass forage (342 ppm for cattle and 530 ppm for sheep) has been investigated in *in vivo* animal studies. Considering the results of the toxicological studies on different species and in particular the absence of adverse effect in dairy cows fed during 28 days with a diet containing 400 ppm of glyphosate, glyphosate is not expected to have, even at the maximum dietary burden, effects on the microbial communities in the rumen impacting on the health of bovine and ovine species.

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Abbreviations

ADME	absorption, distribution, metabolism and excretion
AMPA	aminomethylphosphonic acid
bw	body weight
DM	dry matter
EEC	European Economic Community
EPSPS	5-enolpyruvylshikimate-3-phosphate (EPSP) synthase
GAT	glycine <i>N</i> -phenylacetyltransferase
GM	genetically modified
GOX	glucose oxidase
LD ₅₀	lethal dose, median; dosis letalis media
LOQ	limit of quantification
MRL	maximum residue level
NOAEL	no observed adverse effect level
OECD	Organisation for Economic Co-operation and Development
PROMETHEUS	PROMoting METHods for Evidence Use in Scientific assessments
RMS	rappporteur Member State
SMILES	simplified molecular-input line-entry system

Annex A – Protocol for the risk assessment on the impact of glyphosate residues in feed on animal health

Annex A can be found in the online version of this output ('Supporting information' section):
<https://doi.org/10.2903/j.efsa.2018.5283>

Appendix A – Results of the systematic literature review on glyphosate (animal health) conducted by EFSA

A.1. Search strategy and results

The search strategy was composed of two key elements:

- Glyphosate residues
- Target population: farm animals including bovine, equine, ovine, swine and domestic birds and poultry

The outcomes were not included in the search, since there can be great variability in the language used to describe them, and studies do not report outcomes in the title or abstract.

The literature searches were performed in the following databases: Web of Science (platform encompassing many databases), PubMed and Toxline.

The searches were limited to studies published from 1970 onwards, and, when possible, a language limit was applied to retrieve articles published in English, French, Italian, Spanish and Portuguese. Limits to study design were not applied.

The search strategies were based on the preliminary search strategies to address subquestions 1 and 2 (hazard identification and characterisation) reported in the protocol. These were further refined to obtain the final search strings. The searches were run for all the databases on 15 February 2017. The full search strings included the following:

A.1.1. Pubmed

Filters applied: published from 1970, language English, French, Italian, Portuguese, Spanish

Set	Query	Results
#12	Search #10 AND #3 Filters: Publication date from 1970/01/01; English; French; Italian; Portuguese; Spanish	176
#11	Search #10 AND #3	179
#10	Search #9 OR #8 OR #7 OR #6 OR #5 OR #4	1,240,880
#9	Search "Poultry"[Mesh] OR poultry[tiab] OR Poultries[tiab] OR "domestic bird"[tiab] OR "domestic birds"[tiab] OR (domesticated[tiab] AND (bird[tiab] OR birds[tiab])) OR fowl [tiab] OR fowls[tiab] OR galliform*[tiab] OR wildfowl*[tiab] OR "gallinaceous bird"[tiab] OR "gallinaceous birds"[tiab] OR landfowl*[tiab] OR chicken*[tiab] OR "Gallus gallus"[tiab] OR "Gallus domesticus"[tiab] OR broiler*[tiab] OR capon[tiab] OR capons[tiab] OR cockerel* [tiab] OR hen[tiab] OR hens[tiab] OR pullet[tiab] OR pullets[tiab] OR rooster[tiab] OR roosters[tiab] OR waterfowl*[tiab] OR Anatidae[tiab] OR duck[tiab] OR ducks[tiab] OR mallard*[tiab] OR "Anas platyrhynchos"[tiab] OR Geese[tiab] OR goose[tiab] OR anser [tiab] OR branta[tiab] OR coturnix[tiab] OR quail*[tiab] OR turkey*[tiab] OR meleagris [tiab] OR pigeon*[tiab] OR dove[tiab] OR doves[tiab] OR columb*[tiab] OR ostrich[tiab] OR ostriches[tiab] OR struthio[tiab]	245,909
#8	Search "Sheep"[Mesh] OR ovis[tiab] OR ovine*[tiab] OR ewe[tiab] OR ewes[tiab] OR lamb [tiab] OR lambs[tiab] OR sheep*[tiab] OR mouflon*[tiab] OR hogget*[tiab] OR ram[tiab] OR rams[tiab] OR tup[tiab] OR tups[tiab] OR "Goats"[Mesh] OR goat[tiab] OR goats[tiab] OR capra[tiab] OR capras[tiab] OR caprin*[tiab] OR buck[tiab] OR bucks[tiab] OR geep [tiab] OR geeps[tiab] OR dam[tiab] OR dams[tiab] OR wether[tiab] OR wethers[tiab]	193,797
#7	Search "Equidae"[Mesh] OR equidae*[tiab] OR equus[tiab] OR horse*[tiab] OR equine* [tiab] OR colt[tiab] OR colts[tiab] OR foal[tiab] OR foals[tiab] OR yearling*[tiab] OR gelding*[tiab] OR mare[tiab] OR mares[tiab] OR pony[tiab] OR ponies[tiab] OR stallion* [tiab] OR filly[tiab] OR fillies[tiab] OR ass[tiab] OR asses[tiab] OR mule[tiab] OR mules [tiab] OR donkey*[tiab] OR hinny[tiab] OR hinnies[tiab]	120,212
#6	Search "Swine"[Mesh] OR swine[tiab] OR "sus scrofa" [tiab] OR "sus domestica"[tiab] OR "sus domesticus"[tiab] OR pork[tiab] OR porks[tiab] OR porcine[tiab] OR suidae[tiab] OR pig[tiab] OR pigs[tiab] OR piglet*[tiab] OR sow[tiab] OR sows[tiab] OR barrow*[tiab] OR boar[tiab] OR boars[tiab] OR hog[tiab] OR hogs[tiab] OR gilt[tiab] OR gilts[tiab]	335,839

Set	Query	Results
#5	Search "Cattle"[Mesh] OR cattle[tiab] OR bovin*[tiab] OR cow[tiab] OR cows[tiab] OR bull [tiab] OR bulls[tiab] OR calf[tiab] OR calves[tiab] OR heifer*[tiab] OR bullock*[tiab] OR steer[tiab] OR steers[tiab] OR ox[tiab] OR oxen[tiab] OR (Bos[tiab] AND Taurus[tiab]) OR dairy herd*[tiab] OR dairy breed*[tiab] OR weaner*[tiab] OR yearling*[tiab] OR stirk[tiab] OR stirks[tiab] OR springer*[tiab] OR beef*[tiab] OR veal[tiab] OR veals[tiab] OR feeder*[tiab])	452,144
#4	Search "Livestock"[Mesh] OR "Ruminants"[Mesh:noexp] OR Livestock*[tiab] OR (Farm*[tiab] AND animal*[tiab]) OR ruminant*[tiab] OR food producing animal*[tiab]	46,943
#3	Search ("glyphosate" [Supplementary Concept] OR 1071-83-6[rn] OR 1071-83-6[tiab] OR 1071836[tiab] OR glyphosate*[tiab] OR glyfosate*[tiab] OR gliphosate*[tiab] OR roundup*[tiab] OR "round up"[tiab] OR "n phosphonomethyl glycine"[tiab] OR "N phosphonomethylglycine"[tiab] OR ((mon[tiab] OR Monsanto[tiab]) AND 2139[tiab]) OR mon2139[tiab] OR Monsanto2139[tiab] OR yerbimat*[tiab])) OR ("1-aminomethylphosphonic acid" [Supplementary Concept] OR "aminomethylphosphonic acid"[tiab] OR "aminomethyl phosphonic acid"[tiab] OR 1066-51-9[rn] OR 1066-51-9[tiab] OR 1066519[tiab] OR amep[tiab] OR aminomethylphosphonate[tiab] OR (AMPA[tiab] AND (metabolit*[tiab] OR pesticide*[tiab] OR herbicide*[tiab] OR "N-acetyl"[tiab])) OR acetamidomethylphosphonic[tiab] OR "acetamidomethyl phosphonic"[tiab] OR acetylglyphosate[tiab])	3,554
#2	Search "1-aminomethylphosphonic acid" [Supplementary Concept] OR "aminomethylphosphonic acid"[tiab] OR "aminomethyl phosphonic acid"[tiab] OR 1066-51-9[rn] OR 1066-51-9[tiab] OR 1066519[tiab] OR amep[tiab] OR aminomethylphosphonate[tiab] OR (AMPA[tiab] AND (metabolit*[tiab] OR pesticide*[tiab] OR herbicide*[tiab] OR "N-acetyl"[tiab])) OR acetamidomethylphosphonic[tiab] OR "acetamidomethyl phosphonic"[tiab] OR acetylglyphosate[tiab]	451
#1	Search "glyphosate" [Supplementary Concept] OR 1071-83-6[rn] OR 1071-83-6[tiab] OR 1071836[tiab] OR glyphosate*[tiab] OR glyfosate*[tiab] OR gliphosate*[tiab] OR roundup*[tiab] OR "round up"[tiab] OR "n phosphonomethyl glycine"[tiab] OR "N phosphonomethylglycine"[tiab] OR ((mon[tiab] OR Monsanto[tiab]) AND 2139[tiab]) OR mon2139[tiab] OR Monsanto2139[tiab] OR yerbimat*[tiab]	3,358

A.1.2. Web of Science. All databases

Filters applied: published from 1970, language English, French, Italian, Portuguese, Spanish

Set	Query	Results
# 13	#11 Refined by: LANGUAGES: (ENGLISH OR FRENCH OR SPANISH OR ITALIAN OR PORTUGUESE) <i>Timespan=1970-2017</i> <i>Search language=Auto</i>	1,382
# 12	#11 <i>Timespan=1970-2017</i> <i>Search language=Auto</i>	1,418
# 11	#10 AND #3 <i>Timespan=All years</i> <i>Search language=Auto</i>	1,453
# 10	#9 OR #8 OR #7 OR #6 OR #5 OR #4 <i>Timespan=All years</i> <i>Search language=Auto</i>	4,627,283

Set	Query	Results
# 9	TOPIC: (poultry OR Poultry OR (domestic* NEAR/5 (bird OR birds)) OR fowl OR fowls OR galliform* OR wildfowl OR wildfowls OR "gallinaceous bird" OR landfowl OR landfowls OR chicken* OR "Gallus gallus" OR "Gallus domesticus" OR broiler* OR capon OR capons OR cockerel* OR hen OR hens OR pullet OR pullets OR rooster OR roosters OR waterfowl OR waterfowls OR anatidae OR duck OR ducks OR mallard* OR "Anas platyrhynchos" OR Geese OR goose OR anser OR branta OR coturnix OR quail* OR turkey* OR meleagris OR pigeon* OR dove OR doves OR columb* OR ostrich OR ostriches OR struthio) <i>Timespan=All years</i> <i>Search language=Auto</i>	1,171,495
# 8	TOPIC: (ovis OR ovine* OR ewe OR ewes OR lamb OR lambs OR sheep* OR mouflon* OR hogget* OR ram OR rams OR tup OR tups OR goat OR goats OR capra OR capras OR caprin* OR buck OR bucks OR geep OR geeps OR dam OR dams OR wether OR wethers) <i>Timespan=All years</i> <i>Search language=Auto</i>	762,437
# 7	TOPIC: (equidae* OR equus OR horse OR horses OR equine* OR colt OR colts OR foal OR foals OR yearling* OR gelding* OR mare OR mares OR pony OR ponies OR stallion* OR filly OR fillies OR ass OR asses OR mule OR mules OR donkey* OR hinny OR hinnies) <i>Timespan=All years</i> <i>Search language=Auto</i>	392,341
# 6	TOPIC: (swine OR "sus scrofa" OR "sus domestica" OR "sus domesticus" OR pork OR porks OR porcine OR suidae OR pig OR pigs OR piglet* OR "sow" OR "sows" OR barrow* OR boar OR boars OR hog OR hogs OR gilt OR gilts) <i>Timespan=All years</i> <i>Search language=Auto</i>	1,037,609
# 5	TOPIC: (cattle OR bovin* OR cow OR cows OR bull OR bulls OR calf OR calves OR heifer* OR bullock* OR steer OR steers OR ox OR oxen OR "Bos Taurus" OR "dairy herd*" OR "dairy breed*" OR weaner* OR yearling* OR stirk OR stirks OR springer* OR beef* OR veal OR veals OR feeder*) <i>Timespan=All years</i> <i>Search language=Auto</i>	1,687,364
# 4	TOPIC: ((Livestock* OR ((Farm OR farms OR farmed OR farming OR "food producing") NEAR/5 animal*) OR ruminant*)) <i>Timespan=All years</i> <i>Search language=Auto</i>	1,014,447
# 3	#2 OR #1 <i>Timespan=All years</i> <i>Search language=Auto</i>	28,372
# 2	TOPIC: ("1 aminomethylphosphonic acid" OR "aminomethylphosphonic acid" OR "aminomethyl phosphonic acid" OR amep OR aminomethylphosphonate OR "1066 51 9" OR 1066519 OR (AMPA AND (metabolit* OR pesticide* OR herbicide* OR "N acetyl")) OR acetamidomethylphosphonic OR "acetamidomethyl phosphonic" OR acetylglyphosate) <i>Timespan=All years</i> <i>Search language=Auto</i>	1,362
# 1	TOPIC: ((glyphosate OR 1071-83-6 OR "1071 83 6" OR 1071836 OR glyphosate OR glyfosate OR roundup* OR "round up" OR "n phosphonomethyl glycine" OR "n phosphonomethylglycine" OR ((mon OR monsanto) NEAR/3 2139) OR mon2139 OR monsanto2139 OR yerbimat*)) <i>Timespan=All years</i> <i>Search language=Auto</i>	27,813

A.1.3. Toxline

Filters applied: published from 1970

Set	Query	Results
# 14	(#13) AND 1970:2017 [yr]	193
# 13	(#11 AND #12)	194

Set	Query	Results
# 12	(#5 OR #6 OR #7 OR #8 OR #9 OR #10)	324,636
# 11	(#1 OR #2 OR #3 OR #4)	3,998
# 10	((("poultry" [kw] OR poultry [na] OR poultries [na] OR "domestic bird" [na] OR "domestic birds" [na] OR "domesticated bird" [na] OR "domesticated birds" [na] OR fowl [na] OR fowls [na] OR galliform* [na] OR wildfowl* [na] OR gallinaceous bird [na] OR landfowl [na] OR chicken* [na] OR "gallus gallus" [na] OR "gallus domesticus" [na] OR broiler* [na] OR capon [na] OR capons [na] OR cockerel* [na] OR hen [na] OR hens [na] OR pullet [na] OR pullets [na] OR rooster [na] OR roosters [na] OR waterfowl* [na] OR anatidae [na] OR duck [na] OR ducks [na] OR mallard* [na] OR "anas platyrhynchos" [na] OR geese [na] OR goose [na] OR anser [na] OR branta [na] OR coturnix [na] OR quail* [na] OR turkey* [na] OR meleagris [na] OR pigeon* [na] OR dove [na] OR doves [na] OR columb* [na] OR ostrich [na] OR ostriches [na] OR struthio [na] OR poultry [ab] OR poultries [ab] OR "domestic bird" [ab] OR "domestic birds" [ab] OR "domesticated bird" [ab] OR "domesticated birds" [ab] OR fowl [ab] OR fowls [ab] OR galliform* [ab] OR wildfowl* [ab] OR gallinaceous bird [ab] OR landfowl [ab] OR chicken* [ab] OR "gallus gallus" [ab] OR "gallus domesticus" [ab] OR broiler* [ab] OR capon [ab] OR capons [ab] OR cockerel* [ab] OR hen [ab] OR hens [ab] OR pullet [ab] OR pullets [ab] OR rooster [ab] OR roosters [ab] OR waterfowl* [ab] OR anatidae [ab] OR duck [ab] OR ducks [ab] OR mallard* [ab] OR "anas platyrhynchos" [ab] OR geese [ab] OR goose [ab] OR anser [ab] OR branta [ab] OR coturnix [ab] OR quail* [ab] OR turkey* [ab] OR meleagris [ab] OR pigeon* [ab] OR dove [ab] OR doves [ab] OR columb* [ab] OR ostrich [ab] OR ostriches [ab] OR struthio [ab]))	143,119
# 9	((("sheep" [kw] OR ovine [na] OR ovine* [na] OR ewe [na] OR ewes [na] OR lamb [na] OR lambs [na] OR sheep* [na] OR mouflon* [na] OR hogget* [na] OR ram [na] OR rams [na] OR tup [na] OR tups [na] OR "goats" [kw] OR goat [na] OR goats [na] OR capra [na] OR capras [na] OR caprin* [na] OR buck [na] OR bucks [na] OR geep [na] OR geeps [na] OR dam [na] OR dams [na] OR wether [na] OR wethers [na] OR ovis [ab] OR ovine* [ab] OR ewe [ab] OR ewes [ab] OR lamb [ab] OR lambs [ab] OR sheep* [ab] OR mouflon* [ab] OR hogget* [ab] OR ram [ab] OR rams [ab] OR tup [ab] OR tups [ab] OR goat [ab] OR goats [ab] OR capra [ab] OR capras [ab] OR caprin* [ab] OR buck [ab] OR bucks [ab] OR geep [ab] OR geeps [ab] OR dam [ab] OR dams [ab] OR wether [ab] OR wethers [ab]))	40,275
# 8	((("equidae" [kw] OR horses [kw] OR equidae* [na] OR equus [na] OR horse [na] OR horses [na] OR equine* [na] OR colt [na] OR colts [na] OR foal [na] OR foals [na] OR yearling* [na] OR gelding* [na] OR mare [na] OR mares [na] OR pony [na] OR ponies [na] OR stallion* [na] OR filly [na] OR fillies [na] OR ass [na] OR asses [na] OR mule [na] OR mules [na] OR donkey* [na] OR hinny [na] OR hinnies [na] OR equidae* [ab] OR equus [ab] OR horse [ab] OR horses [ab] OR equine* [ab] OR colt [ab] OR colts [ab] OR foal [ab] OR foals [ab] OR yearling* [ab] OR gelding* [ab] OR mare [ab] OR mares [ab] OR pony [ab] OR ponies [ab] OR stallion* [ab] OR filly [ab] OR fillies [ab] OR ass [ab] OR asses [ab] OR mule [ab] OR mules [ab] OR donkey* [ab] OR hinny [ab] OR hinnies [ab]))	15,408
# 7	((("swine" [kw] OR swine [na] OR "sus scrofa" [kw] OR "sus scrofa" [na] OR "sus domestica" [na] OR "sus domesticus" [na] OR pork [na] OR porks [na] OR porcine [na] OR suidae [na] OR pig [na] OR pigs [na] OR piglet* [na] OR sow [na] OR sows [na] OR barrow* [na] OR boar [na] OR boars [na] OR hog [na] OR hogs [na] OR gilt [na] OR gilts [na] OR swine [ab] OR "sus scrofa" [ab] OR "sus domestica" [ab] OR "sus domesticus" [ab] OR pork [ab] OR porks [ab] OR porcine [ab] OR suidae [ab] OR pig [ab] OR pigs [ab] OR piglet* [ab] OR sow [ab] OR sows [ab] OR barrow* [ab] OR boar [ab] OR boars [ab] OR hog [ab] OR hogs [ab] OR gilt [ab] OR gilts [ab]))	65,755
# 6	((("cattle" [kw] OR cattle [na] OR bovin* [na] OR cow [na] OR cows [na] OR bull [na] OR bulls [na] OR calf [na] OR calves [na] OR heifer* [na] OR bullock* [na] OR steer [na] OR steers [na] OR ox [na] OR oxen [na] OR (bos [na] AND taurus [na]) OR dairy herd* [na] OR dairy breed* [na] OR weaner* [na] OR yearling* [na] OR stirk [na] OR stirks [na] OR springer* [na] OR beef* [na] OR veal [na] OR veals [na] OR feeder* [na] OR cattle [ab] OR bovin* [ab] OR cow [ab] OR cows [ab] OR bull [ab] OR bulls [ab] OR calf [ab] OR calves [ab] OR heifer* [ab] OR bullock* [ab] OR steer [ab] OR steers [ab] OR ox [ab] OR oxen [ab] OR (bos [ab] AND taurus [ab]) OR dairy herd* [ab] OR dairy breed* [ab] OR weaner* [ab] OR yearling* [ab] OR stirk [ab] OR stirks [ab] OR springer* [ab] OR beef* [ab] OR veal [ab] OR veals [ab] OR feeder* [ab]))	71,973

Set	Query	Results
# 5	(("livestock" [kw] OR "ruminants" [kw] OR livestock* [na] OR "farm animal" [na] OR "farmed animal" [na] OR "farm animals" [na] OR "farmed animals" [na] OR "animal farming" [na] OR "animals farming" [na] OR "food producing animal" [na] OR "food producing animals" [na] OR ruminant* [na] OR livestock* [ab] OR "farm animal" [ab] OR "farmed animal" [ab] OR "farm animals" [ab] OR "farmed animals" [ab] OR "animal farming" [ab] OR "animals farming" [ab] OR ruminant* [ab] OR "food producing animal" [ab] OR "food producing animals" [ab]))	9,207
# 4	(((((ampa [ab] AND (metabolite* [ab] OR pesticide* [ab] OR herbicide* [ab] OR "n-acetyl" [ab])))))	54
# 3	(((((ampa [na] AND (metabolite* [na] OR pesticide* [na] OR herbicide* [na] OR "n-acetyl" [na])))))	35
# 2	("1-aminomethylphosphonic acid" [kw] OR 1066-51-9 [rn] OR "aminomethylphosphonic acid" [na] OR "aminomethyl phosphonic acid" [na] OR 1066-51-9 [na] OR 1066519 [na] OR [na] (amep OR "1 aminomethylphosphonic acid" OR "1 aminomethylphosphonate") OR aminomethylphosphonate [na] OR acetamidomethylphosphonic [na] OR "acetamidomethyl phosphonic" [na] OR acetylglyphosate [na] OR "aminomethylphosphonic acid" [ab] OR "aminomethyl phosphonic acid" [ab] OR 1066-51-9 [ab] OR 1066519 [ab] OR [ab] (amep OR "1 aminomethylphosphonic acid" OR "1 aminomethylphosphonate") OR aminomethylphosphonate [ab] OR acetamidomethylphosphonic [ab] OR "acetamidomethyl phosphonic" [ab] OR acetylglyphosate [ab])	179
# 1	((glyphosate [kw] OR 1071-83-6 [rn] OR glyphosate [na] OR 1071-83-6 [na] OR 1071836 [na] OR glyphosate [na] OR roundup [na] OR "round up" [na] OR "n phosphonomethyl glycine" [na] OR "n phosphonomethylglycine" [na] OR "mon 2139" [na] OR mon2139 [na] OR monsanto2139 [na] OR "monsanto 2139" [na] OR yerbimat [na] OR glyphosate [ab] OR 1071-83-6 [ab] OR 1071836 [ab] OR glyphosate [ab] OR roundup [ab] OR "round up" [ab] OR "n phosphonomethyl glycine" [ab] OR "n phosphonomethylglycine" [ab] OR "mon 2139" [ab] OR mon2139 [ab] OR monsanto2139 [ab] OR "monsanto 2139" [ab] OR yerbimat [ab]))	3,964

The output from the searched databases was exported to separate Endnote X8 files, allowing a count of the individual hits per database, and duplicated records within the same platforms were removed. Files were then combined and duplicate records were removed.

A total of 1,443 articles were identified by this search strategy.

A.2. Study selection

The study selection was performed in DistillerSR[®] (Evidence Partners, Ottawa, Canada).

The screening procedure was done in two steps: at the title/abstract level, and at the full text level, by applying a set of predefined questions (implementing the eligibility criteria) (see Tables A.1 and A.2).

Table A.1: Form of Step 1 – Title/Abstract Screening

Question Text	Possible Answer	Implication
1) Is it a primary toxicological study?	yes	Include
	no	Exclude
	unclear	Include
2) Was the exposure to glyphosate and/or its metabolites?	yes	Include
	no	Exclude
	unclear	Include
3) Is an animal reported in the study, and is it belonging to one of these species: Bovine (beef and dairy cows); Equine (i.e. horses, asses, mules, hinnies); Ovine (sheep) and caprine (goat); Swine (breeding and finishing); Domestic birds and poultry (i.e. chicken (broiler, layer), turkeys, geese, ducks, ostriches, pigeons).	yes	Include
	no	Exclude
	unclear	Include
4) Were health-related parameters/effects investigated during the study?	yes	Include
	no	Exclude
	unclear	Include

Table A.2: Form of Step 2 – Screening of full-text

Question Text	Answer Text	Implication
1) Is it a primary experimental/toxicological study?	Yes	Include
	No	Exclude
2) Is an animal reported in the study, and is it belonging to one of these species: Bovine (beef and dairy cows); Equine (i.e. horses, asses, mules, hinnies); Ovine (sheep) and caprine (goat); Swine (breeding and finishing); Domestic birds and poultry (i.e. chicken (broiler, layer), turkeys, geese, ducks, ostriches, pigeons).	yes	Include
	no	Include
3) Were the animals exposed to glyphosate and/or its metabolites?	yes	Include
	no	Exclude
4) Were health-related parameters/toxicological effects investigated during the study?	yes	Include
	no	Exclude
5) Is the study related to genetically modified organisms (e.g plants resistant to glyphosate)?	yes	Grouping
	no	
6) Was a control group included in the study?	yes	Include
	no	Exclude
7) Was the level of exposure to glyphosate and/or its metabolites known (measured or calculated/estimated)?	yes	Include
	no	Exclude
8) Were potential adverse effects on health-related parameters detected?	yes	Include
	no	Exclude
9) Were ADME parameters investigated during the study?	yes	Grouping
	no	
10) Were <i>in vitro</i> investigations performed with biological material from farm animals?	yes	Grouping
	no	

The next step of the screening aimed to identify potential articles not yet considered during the peer review process (i.e. in the revised RAR (Germany, 2015), in the Conclusion from the renewal procedure (EFSA, 2015b) and in the draft addendum (Germany, 2017a).

Seven potentially relevant articles were identified (12 May 2017) and their assessment was included in the revised addendum (Germany, 2017b) for the follow on step of the peer review process:

- 1) The effects of glyphosate herbicide on chicken egg hatchability
Bruce D.J. Batt and James A. Black
Can J Zool 58: 1940-1942
- 2) Influence of glyphosate (...) on performance and selected parameters in broilers
Kubena L.F., Smalley H.E., Farr F.M.
1981 Poultry Science 60: 132-136
- 3) Evaluation of potential embryotoxicity and teratogenicity of 42 herbicides, insecticides and petroleum contaminants to mallards
David J. Hoffman and Peter H. Albers
Arch. Environ. Contam. Toxicol. 13, 15-27 (1984)
- 4) Biological activity of new aminophosphonates
Bonarska D., Sarapuk J., Kleszczynska H.
Pol. J. Food Nutr. Sci. 2003, Vol. 12/53, SI 2, pp.17-19
- 5) Effects of the herbicide Roundup on the epididymal region of drakes *Anas platyrhynchos*
Oliveira A.G., Telles L.F., Hess R.A., Mahecha G.A.B., Oliveira C.A.
Reproductive Toxicology 23 (2007) 182-191

6) Evidence for direct effects of glyphosate on ovarian functions: glyphosate influences steroidogenesis and proliferation of bovine granulosa but not theca cells *in vitro*

M.C. Perego, Schutz L.F., Caloni F., Cortinovis C., Albonico M., Spicer L.J.
Journal of Applied Toxicology 2016 - (wileyonlinelibrary.com) DOI 10.1002/jat.3417

7) Synthesis, cytotoxicity and clastogenicity of novel alpha-aminophosphonic acids

Naydenova E., Troev K., Topashka-Ancheva M., Hägele G., Ivanov I., Kril A.
Amino Acids (2007) 33: 695-702

The overall flow of the articles (named 'records' in DistillerSR) is described in the following graph.

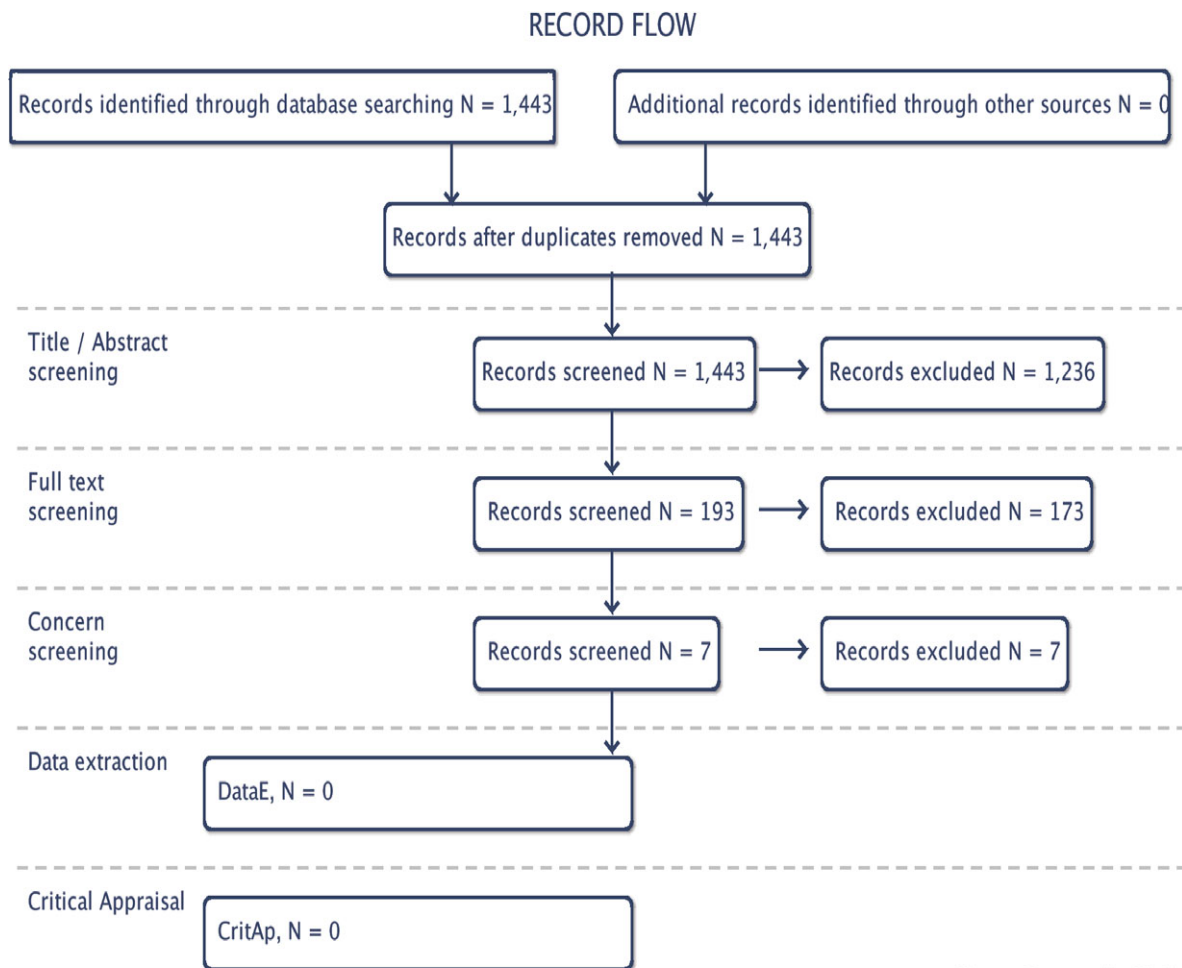
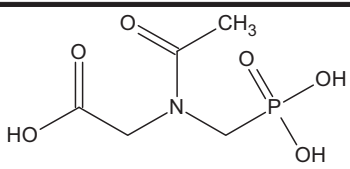
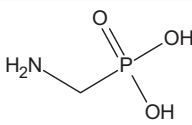
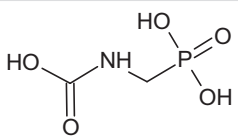


Diagram Generated by DistillerSR

It is noted that the three steps of concern screening, data extraction and critical appraisal were not conducted with DistillerSR.

Appendix B – Used compound codes

Code/trivial name	Chemical name/SMILES notation	Structural formula
<i>N</i> -acetyl-glyphosate	<i>N</i> -acetyl- <i>N</i> -(phosphonomethyl)glycine <chem>OC(=O)CN(CP(=O)(O)O)C(C)=O</chem>	
AMPA	(aminomethyl)phosphonic acid <chem>NCP(=O)(O)O</chem>	
<i>N</i> -acetyl-AMPA	(acetamidomethyl)phosphonic acid <chem>CC(=O)NCP(=O)(O)O</chem>	

SMILES: simplified molecular-input line-entry system.