EFSA explains the carcinogenicity assessment of glyphosate

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Background

During the EFSA peer-review process for the renewal of the approval of the pesticide active substance glyphosate, EFSA received a complementary mandate from the European Commission to consider the findings by the International Agency for Research on Cancer (IARC) regarding the potential carcinogenicity of glyphosate or glyphosate containing plant protection products. Following the request, EFSA has incorporated its scientific assessment in the on-going peer review of the active substance (EFSA, 2015a).

The EFSA Conclusions on Pesticides have a complex structure, designed to support the European Commission and Member States in the approval/renewal process, the subsequent assessments of the Plant Protection Products (PPPs) by the Member States (MSs), and the review of the Maximum Residue Levels (MRLs) of pesticides.

The EFSA Conclusions summarise the main outputs of the scientific assessment of the active substance(Sections 1 to 6), and then focus on the identification of data gaps and studies to be generated (Section 7), recommendations to manage the identified risks (Section 8) and concerns to be considered by risk managers (Section 9). An appendix presents the List of Endpoints recommended by EFSA for the hazard and the risk assessment of the active substance. EFSA also publish supporting background documents providing the scientific justifications.

EFSA's assessment on pesticide active substances is based on original studies (mandatory regulatory Good Laboratory Practice (GLP) studies, other relevant studies and the outcome of the search of peer-reviewed scientific studies published within the last 10 years before the submission of the dossier). Those are summarised by the rapporteur Member State (RMS) in the draft renewal assessment report (RAR). The peer-review includes a public consultation of the draft RAR and several commenting phases by EFSA scientists and MSs experts, the possibility for requiring additional information from the applicants, and a set of experts' meetings covering different scientific areas; this is reflected in a further development of the RMS RAR during the EFSA peer review.

Due to the length and complexity of the background documents, the peer review report supporting the EFSA Conclusion (EFSA, 2015b) and the RAR (Germany, 2015), EFSA has considered important to clarify some key elements of its scientific evaluation in this complementary document, in particular, the assessment of the carcinogenicity of the active substance glyphosate.

Assessment of the carcinogenic potential of the active substance glyphosate

EFSA has assessed the carcinogenicity and genotoxicity potential of the active substance glyphosate according to the principles and criteria applicable for the classification and labelling of chemical substances in the EU under Regulation (EC) No 1272/2008 (CLP

Regulation)[1]. This regulation implements in the EU the Globally Harmonized System developed by the United Nations for the classification and labelling of hazardous chemical substances. The European Chemicals Agency (ECHA) has developed guidance on the application of these criteria (available on the ECHA web page); ECHA also submitted specific comments regarding the Rapporteur Member State (RMS) assessment (EFSA, 2015b) that were considered by EFSA and the MSs during the peer review. Glyphosate carcinogenicity was discussed in two expert meetings, first during the overall assessment of the mammalian toxicity and second during a dedicated experts' teleconference, involving a large number of experts.

Genotoxicity

The genotoxicity data package available for the peer review is comprehensive. A large number of studies provided in the draft RAR (over 100 studies all together; Germany, 2013) was complemented by additional studies identified during the commenting period and the public consultation that took place in 2014, and which were included in the revised RAR (Germany, 2015).

a) glyphosate

EFSA assessed the genotoxic potential of the active substance, glyphosate, and focused on the studies performed on well characterised test substances according to defined technical specifications. Glyphosate is produced by many different companies, each manufacturing a technical material presenting different impurity profiles than the others. Where considered necessary, data gaps have been set for each individual company to complete the toxicological information on the impurities present in their technical material (EFSA, 2015a).

All required genotoxicity endpoints, which consist of gene mutation in bacterial and mammalian cells, structural and numerical chromosome aberrations *in vitro* and *in vivo* have been investigated in validated OECD guideline-compliant studies following the principles of GLP, as well as published studies often following non-GLP protocols designed by the study authors.

In vitro tests

Bacterial and mammalian cells mutagenicity studies gave consistently negative results; even studies that were considered less reliable and of lower quality did not reveal any indication of genotoxicity. With regards to *in vitro* mammalian chromosome aberration (CA), all tests that were performed under GLP conditions, as well as a number of published studies gave negative results at concentrations up to 1250 µg/L. In contrast positive results were obtained in some published studies on CA at lower dose levels, and on other endpoints considered as indicator tests, such as sister chromatid exchange (SCE) and induction of DNA strand breaks *in vitro*. The positive indications observed in some *in vitro* tests were not confirmed by *in vivo* studies covering the appropriate end-points, such as *in vivo* micronucleus tests.

In vivo tests

Sixteen *in vivo* studies in somatic cells were reported on rodents treated orally with dose levels up to 5000 mg/kg bw or via intraperitoneal injections. All studies conducted according to internationally validated guidelines and some non-GLP published studies gave negative results, while two non-GLP studies were positive in mice treated intraperitoneally with dose levels in the range of the intraperitoneal LD_{50} for mice, one study presenting major flaws. Conflicting results were obtained regarding DNA adduct

[1] 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

formation; induction of DNA strand breaks was observed in mice treated intraperitoneally with doses close to or in excess of the LD_{50}, this induction may be caused by secondary effects of cytotoxicity. No genotoxic effects on germ cells were detected in rats or mice treated orally at dose levels up to 2000 mg/kg bw.

Considering a weight of evidence approach, taking into account the quality and reliability of all available data, the EFSA peer review concluded that glyphosate is unlikely to be genotoxic *in vivo* and does not require hazard classification regarding mutagenicity according to the CLP Regulation. It is noted that unpublished studies that were the core basis of the peer review evaluation were not available to the IARC experts as reported in the IARC monograph 112 on glyphosate (IARC, 2015).

b) glyphosate-based formulations

A number of published studies performed with glyphosate based formulations of unknown composition gave positive results when tested *in vitro* and *in vivo*. Some of the test systems are not validated and/or of difficult interpretation due to possible confounding effects, such as cytotoxicity, specific organ toxicity or unclear relevance to human health when tested in fish, amphibians or invertebrates according to the current knowledge. POE-tallowamine is one of the co-formulants that is known to be used in some glyphosate-based formulations. This co-formulant has been shown to be more toxic than the active substance glyphosate on several toxicological endpoints, namely acute, short term, reproductive and developmental toxicity, further to equivocal evidence of DNA damage *in vitro* at high doses (EFSA, 2015c). Although POE-tallowamine is not present in the representative formulation, for which data have been submitted under the European re-approval procedure and which were assessed by EFSA, the peer review concluded that the toxicity of formulations and in particular their genotoxic potential should be further considered and addressed.

It is noted that a number of human studies were not evaluated, since the exposure was linked to glyphosate-based formulations of unknown composition. Therefore their assessment would not have changed the overall conclusion.

Carcinogenicity

a) Studies in animals

Long term toxicity and carcinogenicity potential of glyphosate was assessed in five mouse studies and nine rat studies; one further study in mice and one in rats were considered inadequate for the evaluation of glyphosate carcinogenicity.

In the nine long term rat studies, the EFSA peer review concluded that no significant increase in tumour incidence was observed in any of the treated groups of animals. Three of these studies were not evaluated by the IARC experts. In one study, the IARC reported a statistically significant increased incidence of pancreatic islet cell adenomas in males treated with the low dose, which was not reproduced at higher dose levels. In another study, the IARC reported significant increases in pancreatic islet adenomas in males at two dose levels (not dose-related), a significant positive trend for hepatocellular adenomas in males without progression to malignancy and a significant trend for C-cell adenomas in females. The two evaluations differ primarily regarding the statistical evaluation: according to a pair-wise comparison [Fisher´s exact test (one-tailed) as well as in combination with Bonferroni inequality procedure for incidences of non-neoplastic (at $p \leq 0.01$) and neoplastic lesions (at $p \leq 0.01$ and ≤ 0.05) and Peto Analysis for evaluation of histopathological data as planned in the study protocol] no significant change is observed, while a trend analysis (Cochran-Armitage trend test) performed by the IARC experts identified significant changes. EFSA is of the opinion that the planning of a study before the initiation of the experimentation itself as established in the respective protocol –that includes the statistical analysis - is a key element in assessing

the quality of a study, therefore deviations from the statistical analysis used by the study authors should be limited and properly justified. Furthermore, clear dose-response was not always observed and when observed, it affected only the highest dose level of 940 to 1183 mg/kg bw per day in males and females respectively, a dose eliciting other adverse effects on body weight, liver, stomach mucosa and eyes (cataracts).

In mice, the EU peer review evaluated five studies; another study was considered inadequate for the evaluation of glyphosate carcinogenicity. From these studies, only one study in Swiss albino mice presented a carcinogenic effect characterised by a statistically significant increased incidence of malignant lymphomas at the top dose level of 1460 mg/kg bw per day. However, the validity of the study was questioned due to the occurrence of viral infection that could influence survival as well as tumour incidence – especially lymphomas. No other carcinogenic effects were observed up to the highest dose levels of each of the other studies as tumour incidences remained within valid historical control data from the respective performing laboratory and/or did not attain the level of statistical significance. The IARC evaluated two out of these five studies and identified a positive trend in males for renal tubule adenomas and carcinomas in one study and a positive trend for haemangiosarcoma in the other study according to Cochran-Armitage trend test. EFSA adopted a weight of evidence approach which was agreed by the peer review taking into account all available data: the statistical significance found in trend analysis (but not in pair-wise comparison) was balanced against the lack of consistency in multiple animal studies, slightly increased incidences only at dose levels at or above the limit dose of 1000 mg/kg bw per day recommended for the oral route of exposure in chronic toxicity and carcinogenicity studies (OECD, 2012a), doses at which confounding of concomitant toxicity is expected, incidences within valid historical control range from the performing laboratory and lack of pre-neoplastic lesions.

Furthermore no genotoxic potential is attributed to the active substance glyphosate. Other mechanisms of action were reported by IARC, such as inflammation, immunosuppression, endocrine disrupting (ED) activity and oxidative stress. Although no robust information is available to conclude on the immunomodulatory potential of glyphosate, indications of such effects were limited to inflammatory responses of the respiratory tract. No interaction of glyphosate with the oestrogen, androgen or thyroid endocrine pathways were identified by the US EPA Endocrine Disruptor Screening Program (EDSP) according to a full battery of Tier I screening assays (data gaps were however identified in the EFSA conclusion for the submission of the respective studies for confirmation); and no significant ED effects were identified in published studies reported by IARC, except for a higher activity of formulations in comparison to the active substance. Some indications of increased oxidative stress were observed in combination with cytotoxic or degenerative effects of the target organs; however, even if these indications are confirmed, the observation of a plausible mechanism of action *per se* is insufficient to assume a carcinogenic potential in humans according to the current knowledge. On this basis, EFSA concluded that glyphosate is unlikely to pose a carcinogenic hazard to humans and no classification regarding carcinogenicity is proposed according to CLP Regulation.

b) Epidemiological studies

Overall thirty epidemiological studies are reported in the IARC monograph, including cohort, case-control studies and meta-analyses. A few of these studies were not reported in the RAR (Germany, 2013), but were considered by the RMS in an addendum on the assessment of the IARC monograph (Germany, 2015). In ten cohort studies, that include the Agricultural Health Study (AHS) (Alavanja *et al*., 1996), the largest prospective cohort study undertaken until today and used in many other publications, glyphosate did not cause different types of cancer and did not increase the risk of all cancers. Nine case-control studies did not indicate an increased risk of carcinogenicity by glyphosate, or presented limited power. Further five case-control and one prospective

cohort studies were considered to assess the strength of evidence linking glyphosate to non-Hodgkin lymphoma (NHL), a statistical significant association was observed in a small number of cases that was considered insufficient to conclude on the causality, due to the poor consistency of the results. Epidemiological studies face several problems linked to the small number of cancer cases and difficult identification/separation of confounders: glyphosate is generally analysed together with several other pesticides, exposure cannot be easily measured as it is generally based on interviews and questionnaires that have several intrinsic recall bias, furthermore, the classification of the type of cancer is not consistent, the adverse outcomes are not always obtained from medical records, and finally, the contribution of co-formulants' toxicity cannot be assessed. Taking into consideration the weight of evidence, EFSA concluded that there is very limited evidence of association between glyphosate exposure and the occurrence of NHL, which would not alter the classification proposal derived from animal studies that glyphosate is unlikely to pose a carcinogenic hazard to humans.

Recognising the value that epidemiological studies can potentially provide to pesticide risk assessments, EFSA is doing a number of initiatives in this field. First, EFSA granted an external review on epidemiological studies on pesticides which identified some limitations (Ntzani *et al*., 2013). As a follow up, EFSA launched a project including the preparation of two Scientific Opinions, one to facilitate the use of epidemiological studies in the risk assessment of pesticides, and another investigating the potential link of pesticides toxicity with Parkinson's disease and childhood leukaemia by using criteria within the Adverse Outcome Pathway framework. The two scientific Opinions are expected to be launched for public consultation in November 2016 and June 2016 respectively.

Assessment of Plant Protection Products and co-Formulants

In line with Article 12 of Regulation (EC) No 1107/2009[2] the EFSA Conclusion presents the assessment and properties of the active substance glyphosate, considering the technical specifications provided by the applicants. The dossier includes a representative formulated product. The information on this representative product has been considered by EFSA during the evaluation of the active substance according to the relevant guidance documents for the different scientific sections. Studies on the representative product or other formulated products, either included in the dossier or made available during the peer-review process, have been considered by EFSA when relevant for the assessment of the active substance. The EFSA assessment would be relevant for the assessment of the hazard properties, including classification and labelling, and risk assessment of PPPs by the MSs, however, EFSA has not evaluated the hazard and risk of the PPPs as this is out of EFSA remit and would require a specific mandate and submission of additional information, including the PPP dossiers.

EFSA has been mandated by the European Commission to assess a co-formulant, POE-tallowamine, that although not present in the representative formulation, is reported as co-formulant for other glyphosate containing PPPs. EFSA does not support the health-based reference values proposed by the RMS, and considers that the genotoxicity, long term toxicity/carcinogenicity, reproductive/developmental toxicity and endocrine disrupting potential of this co-formulant should be clarified before setting health-based reference values and conducting the risk assessment (EFSA, 2015c).

Consumer Risk Assessment

[2] 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 No L 309, 24.11.2009, p. 1-50

Following previous EU and JMPR assessments, the RMS in its initial assessment (Germany, 2013) did not propose setting an Acute Reference Dose (ARfD). However, following the re-evaluation during the EFSA peer-review, EFSA has concluded that the toxicity of glyphosate requires setting an ARfD of 0.5 mg/kg bw. It should be noted that this proposal has been agreed by the RMS.

This recommendation triggers the evaluation of acute exposure during the consumer risk assessment. EFSA will consider this conclusion during the review of the MRLs for glyphosate under Art. 12 of Regulation (EC) No 396/2005, to be conducted by EFSA in cooperation with the MSs during 2016.

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