Renewal Assessment Report

31 August 2015

Glyphosate
Addendum 1 to RAR

Assessment of
IARC Monographs
Volume 112 (2015):
Glyphosate

RMS: Germany

Version history (Addendum 1)

Date	**Reason for revision**
31 August 2015	First Draft of Addendum 1

Preface

In February 2015 a revised health risk assessment report on glyphosate prepared by the Federal Institute for Risk Assessment (BfR) was discussed at the expert meeting of the European Food Safety Authority (EFSA). Subsequently, the report was amended by the BfR. This revision comprised additional evaluation tables as well as additional amendments for more clarification on some factual matters. On 1 April 2015 BfR sent this supplemented and revised version of the report to the Federal Office of Consumer Protection and Food Safety (BVL) for forwarding to EFSA.

The International Agency for Cancer Research (IARC) of the World Health Organization (WHO) evaluated glyphosate as "probably carcinogenic to humans (Group 2A)", based on the available and evaluated studies by IARC. The full report on glyphosate from the IARC monograph (Volume 112) has been publicly available since 29 July 2015.

As Rapporteur Member State (RMS) for the European renewal of approval of glyphosate, Germany was commissioned by EFSA to evaluate the IARC Monographs Volume 112 on glyphosate by 31 August 2015, so that this scientific analysis could be included in the renewal process of the active substance glyphosate. Once this addendum has been subjected to a consultation process with the other Member States and a subsequent discussion in a separate Expert Meeting of EFSA at the end of September 2015, the results of this Addendum may be considered in the "EFSA Conclusion on the peer review of the pesticide risk assessment" of glyphosate.

Abstract

Based on the studies on cancer in humans IARC concluded: „*There is limited evidence in humans for the carcinogenicity of glyphosate*". The Rapporteur Member State (RMS) agrees with IARC that the other IARC categories are not suitable for the classification of the evidence from studies in humans. **The evaluation of the epidemiological studies by the RMS is comparable to IARC. However**, RMS adopts a more cautious view since **no consistent positive association was observed**, and the most powerful study showed no effect. The IARC interpretation is more precautionary. It was also noted that **in the epidemiological studies a differentiation between the effects of glyphosate and the co-formulants is not possible.**

Based on carcinogenicity studies in experimental animals IARC concluded that glyphosate induced a positive trend in the incidence of rare renal tumours; a positive trend for haemangiosarcoma in male mice and increased pancreatic islet-cell adenoma in male rats in two studies, and therefore: „*There is sufficient evidence in animals for the carcinogenicity of glyphosate*". A much larger number of animal studies have been performed to evaluate the carcinogenic potential of glyphosate than necessary by the legal requirements. In mice, a total of five long-term carcinogenicity studies using dietary administration of glyphosate were considered. In rats, seven chronic toxicity and carcinogenicity studies using dietary administration of glyphosate and two studies with application via drinking-water were reviewed.

- Renal tumours:

  In two studies in CD-1 mice and one study in Swiss albino mice, the statistical analysis with the Cochran-Armitage test for linear trend yielded a significant result, whereas the analysis by pair-wise comparisons indicated no statistically significant differences between the groups and the incidences were within the historical control range of up to 6% for adenoma and carcinoma combined. A confounding effect of excessive toxicity cannot be excluded at the highest doses of 1460 - 4841 mg/kg bw/d. In both studies in CD-1 mice, but not in Swiss albino mice, the body weight gain was decreased by more than 15% compared to controls, but mortality/survival was not affected.

- Haemangiosarcoma:

  In two studies in CD-1 mice, the incidences of haemangiosarcoma in male mice were reconsidered for statistical evaluation. For both studies, the statistical analysis with the Cochran-Armitage test for linear trend yielded a significant result, whereas the analysis by pair-wise comparisons indicated no statistically significant differences between the groups. The background incidences for haemangiosarcoma in male CD-1 mice were up to 12% if multiple organs were considered. Therefore, the observed incidences for haemangiosarcoma were spontaneous and unrelated to treatment.

- Pancreatic and other tumours:

  The statistically significant increase in pancreatic tumours incidences in the male rats of the low dose groups are considered incidental. With regard to the positive trend for liver cell adenoma in male rats and thyroid C-cell adenoma in female rats for the study of Stout and Ruecker, IARC also noted a lack of evidence for progression.

- Malignant lymphoma:

  IARC also considered a review article containing information on five long-term bioassay feeding studies in mice, in which a statistically significant increase in the incidence of malignant lymphoma was reported, but the Working Group was unable to evaluate this study because of the limited experimental data provided in the review article and supplemental information. In three studies in CD-1 mice, the incidences of malignant lymphoma in male mice were reconsidered for statistical evaluation by the RMS. For two studies, the statistical analysis with the Cochran-Armitage trend test yielded a significant result, whereas the analysis by pair-wise comparisons indicated no statistically significant differences between the groups for all three studies. The incidences observed in the above studies, with a maximum of 12%, were all within the historical control range. Therefore, the observed malignant lymphomas were spontaneous and unrelated to treatment.

For an overall conclusion, the large volume of animal data for glyphosate has been evaluated using a weight of evidence approach. It should be avoided to base any conclusion only on the statistical significance of an increased tumour incidence identified in a single study without consideration of the biological significance of the finding. In summary, based on the data from five carcinogenicity studies in mice and seven chronic toxicity and carcinogenicity studies in rats, **the weight of evidence suggests that there is no carcinogenic risk related to the intended herbicidal uses and, in addition no hazard classification for carcinogenicity is warranted for glyphosate according to the CLP criteria.**

Based on the mechanistic and other studies, IARC concluded: „*There is mechanistic evidence for genotoxicity, oxidative stress, inflammation, immunosuppression, receptor-mediated effects, and cell proliferation or death of glyphosate*". Glyphosate has been tested in a broad spectrum of mutagenicity and genotoxicity tests *in vitro* and *in vivo*. **Taking into account all available data and using a weight of evidence approach, it is concluded that glyphosate does not induce mutations *in vivo* and no hazard classification for mutagenicity is warranted according to the CLP criteria. In the absence of sufficient evidence for a carcinogenic risk related to the intended herbicidal uses the mechanistic and other studies do not provide further evidence for a carcinogenic mechanism.**

AMPA has been tested for mutagenicity and genotoxicity *in vitro* and *in vivo* in an adequate range of assays. Taking into account all available data and using a weight of evidence approach, it is concluded that AMPA does not induce mutations *in vivo* and no hazard classification for mutagenicity is warranted according to the CLP criteria.

Glyphosate-based formulations have been extensively tested for mutagenicity and genotoxicity *in vitro* and *in vivo* in a wide range of assays. However, since formulation compositions are considered proprietary, the specific composition of the formulations tested was not available for the published studies. Positive results from *in vitro* chromosomal damage assays and tests for DNA strand breakage and SCE induction were reported in published studies. For specific glyphosate-based formulations, *in vivo* mammalian chromosomal aberration or micronucleus assays as well as tests for DNA adducts, DNA strand breakage and SCE induction gave positive results in some published studies. However, no

regulatory studies for these endpoints were provided. Thus, **for the different glyphosate-based formulations, no firm conclusions can be drawn with regard to a need for classification according to the CLP criteria.**

Considering the low level of metabolism and the chemical structure of glyphosate, glyphosate radical formation initiating oxidative stress appears unlikely. However, uncoupling or inhibition of mitochondrial oxidative phosphorylation also represents an established mechanism for ROS generation. Notably, uncoupling of oxidative phosphorylation by glyphosate has been reported in rat liver microsomes and a glyphosate formulation. Induction of oxidative stress can provide a mechanistic explanation for any observed cytotoxic/degenerative and indirectly genotoxic effects of substances. However, from the sole observation of oxidative stress and the existence of a plausible mechanism for induction of oxidative stress through uncoupling of mitochondrial oxidative phosphorylation alone, genotoxic or carcinogenic activity in humans cannot be deduced for glyphosate and glyphosate based formulations. Furthermore, the RMS concludes that the evidence from available data does not allow the conclusion that glyphosate caused immunosuppression. However it is to note that due to the small number of studies assessed and the fact that all studies show limitations, no robust information is available to conclude on the immunomodulatory action of glyphosate.

Glyphosate was included into the U.S. EPA Endocrine Disruptor Screening Program's (EDSP). It was concluded that, based on the Tier 1 assays that had been performed at different independent laboratories and taking into account the 'higher tier' regulatory safety studies, glyphosate should not be considered an endocrine disrupter or to have other receptor-mediated effects. Information on apoptosis and proliferation in cell systems from humans and mice was reported, but this was not considered as additional mechanistic evidence for carcinogenicity of glyphosate.

Results of four occupational and two para-occupational studies using various glyphosate-containing plant protection products have been evaluated in the International Agency for Cancer Research (IARC) monograph, which were carried out between 1988 and 2007 in different countries of North America and Europe. The recorded exposure values in these studies were below or in the same order of magnitude as those predicted in the Renewal Assessment Report (RAR). For resources on dietary exposure and for results on biological markers IARC refers to several selected reports from national food- and bio-monitoring programmes as well as to some studies in the public literature. With respect to exposure, no relevant deviating conclusions between the RAR and IARC were identified.

In addition, the RMS strongly recommends further genotoxicity studies in compliance with OECD test guidelines in general and for the representative formulation as confirmatory information for the authorisation of plant protection products.

Contents

1 Exposure Data

1.1 Identification of the agent

The information reported in the sections 1.1.1 - 1.1.4 of the IARC Monographs Volume 112 (2015, ASB2015-8421) is generally summarized in line with the information in the cited references and with the information given in the RAR (2015, ASB2015-1194). Regarding section 1.1.4 it is noted that a different specification was derived by the RMS than by FAO (2000, ASB2015-8587). In summary, these sections appear to be an appropriate summary of the available knowledge on glyphosate.

1.2 Production and use

1.2.1 Production

1.2.1.1 Manufacturing process

In the IARC Monographs Volume 112 (2015, ASB2015-8421) it is stated that: "*To increase the solubility of technical-grade glyphosate acid in water, it is formulated as its isopropylamine, monoammonium, potassium, sodium, or trimesium salts*".

The manufacture and use of different active substance variants is not a glyphosate-specific feature; it is a common issue for many active substances. This circumstance has to be considered in the zonal/national authorisation procedure of the plant protection product. Thus, for the evaluation and assessment of the toxicological properties of active substance variants differently from the representative variant in the Annex I renewal, further studies may therefore be required for a bridging between the different variants of active substances on Member State level.

1.2.2 Uses

1.2.2.1 Agriculture

In the IARC Monographs Volume 112 (2015, ASB2015-8421) it is stated that: "*Common application methods include broadcast, aerial, spot and directed applications (EPA, 1993a).*" It should be noted that within the European Union, applications of plant protection products by aircraft are generally prohibited according to Directive 2009/128/EC (2009, ASB2015-8588). Only very few exceptions, for which it has to be applied particularly, can be granted, if no other effective method of pest control is available, e.g. for applications in the forest or on steep slopes in viticulture in Germany. However, no herbicidal applications by aircraft have ever been authorized. Thus, there is no aerial application of glyphosate-containing plant protection products, at least in Germany.

Within the scope of the European authorization procedure for glyphosate, only downwards directed applications have been intended and have been taken into account for risk assessment.

1.3 Measurement and analysis

Not one of the about 40 studies evaluated in Volume 3 sections B.5.2 - B.5.4 (2015, ASB2015-1194) are mentioned in the IARC Monographs Volume 112 (2015, ASB2015-8421). In section 1.3 of the IARC monograph in total 16 analytical reports from the open literature are cited. Two of them are merely

mentioned in the general introduction. Details of the remaining 14 studies are described in Table 1.1 of the IARC monograph. All details listed in Table 1.1 of the IARC monograph correspond exactly to the data of the cited studies. However, the limit of detection reported in these studies is estimated only and not statistically validated. A revised version of Table 1.1, listed in the Annex as Table A-5.5-1, additionally contains for that reason the limit of quantification, which is the only parameter that allows the evaluation of numerical data in other studies. In addition, Table A-5.5-1 contains the derivatisation agent (if used), a statement on the extent of validation data presented in cited studies and those sections of the IARC monograph, which refer to studies reported in section 1.3.

Due to the fact that quantitative analytical results will be more reliable if stable isotope labelled glyphosate is used as internal standard, it should be mentioned that the methods by Lee et al. (2001, ASB2015-8239) and Botero-Coy et al. (2013, ASB2015-7882) use such special internal standards.

Three of the studies reported in section 1.3 of the IARC monograph are cited in other sections. These are the studies by Acquavella et al. (2004, ASB2012-11528), Chang et al. (2011, ASB2015-7895) and Curwin et al. (2007, ASB2012-11597), which are mentioned in sections 1.4.1, 4.1.2, and 4.1.5. Due to missing analytical validation data in these studies, it is not possible to assess the reliability of results presented in these three studies. All other reports are not cited outside of section 1.3 of the IARC monograph.

In summary, the IARC Monographs Volume 112 (2015, ASB2015-8421) provides an overview on several studies published in scientific journals. About 50% of the methods reported in these studies are considered as sufficiently validated, even if the extent of validation data does not fully correspond to requirements of Regulation (EC) No 1107/2009 (2009, ASB2015-8589) as detailed in SANCO/825/00 rev. 8.1 (2010, ASB2015-8438).

1.4 Occurrence and exposure

1.4.1 Exposure

1.4.1.1 Occupational exposure

In section 1.4.1 of the IARC Monographs Volume 112 (2015, ASB2015-8421) results of four occupational and two para-occupational studies using various glyphosate-containing plant protection products are cited and summarized in Table 1.2. The studies were carried out between 1988 and 2007 in different countries of North America and Europe. Four of these studies (Centre de Toxicology du Québec, 1988, ASB2015-7889; Lavy et al., 1992, TOX9650912; Johnson et al., 2005, ASB2012-11859, and Curwin et al., 2007, ASB2012-11597) have not yet been included in the RAR (2015, ASB2015-1194). Nevertheless, all six exposure studies have been roughly evaluated now (see Table A-5.5-2). A short summary of the evaluation of these studies is given in section 5.1.

1.4.1.2 Community exposure

For residues in food and feed references were made to several food monitoring reports and data from the EU, Denmark, United Kingdom and Brazil. The information are freely available, however, not included in the RAR due to the "safe-use" approach for the assessment of active substances under Regulation (EC) No 1107/2009 (2009, ASB2015-8589). The "safe-use" concept relies on supervised field trial data treated at the maximum application rates for the active substance, resulting in a more conservative exposure scenario compared to food monitoring results.

All studies reported by IARC on biological markers for glyphosate are also included in the RAR (2015, ASB2015-1194).

1.4.2 Exposure assessment

The methodology for the exposure assessment of glyphosate will be described in IARC Monographs Volume 112 for Malathion, which has not yet been published.

2 Studies of Cancer in Humans

In the section on cancer in humans (epidemiological studies) the IARC describes in Tables 2.1. and 2.2 the primary cohort and control studies with the reference, study location, study design, population size, exposure assessment methods, organ site, exposure category, exposed cases, risk estimate (95% confidence intervals) and covariates controlled and comments. Overall, these descriptions reflect the information in the articles (Instead of the cases and the response rates, it would have been helpful to detail the actual cases analysed.) The general discussion of the epidemiological studies was not available since it will appear in the IARC Monographs Volume 112 on Malathion which as of today has not been published. There are small differences in the way the strength of evidence may be judged and the limitations of the studies according to the descriptions in either report (RAR and IARC monograph). For example, the RMS considers it problematic that Hardell et al. (2002, ASB2012-11839) put two studies one on NHL and the other only on HCL together – different types of cancer without inclusion of the other respective cancer group – and analysed them together. Even though IARC does weighting and uses quality criteria it is not always detailed. It is not described in detail how the literature search and the selection of literature were done for the IARC report. Overall, BfR agrees that the relevant studies on NHL-lymphoma are included in the IARC monograph.

The epidemiological studies face several problems: only a small number of cancer cases are observed in all the individual studies, making it difficult to obtain clear results. Also the number of adequate epidemiological studies is limited. There are a lot of problems with confounders: in most studies glyphosate is analysed together with several other pesticides/insecticides so that the effects of each individual substance are difficult if not impossible to disentangle. Farmers who use one chemical substance may also use another. It is not clearly stated in which formulation glyphosate is used that is, it could be different brands with slightly different chemical mixtures and co-formulants, which may have carcinogenic effects. The exposure cannot be easily measured. For example no measures from biomarkers from the blood are used. Exposure is measured through interviews or questionnaires. Here, there is a big recall problem to judge the amount of exposure to the chemicals. Furthermore, there may be a recall biases since individuals with cancer are more likely to think about possible reasons for their cancer than healthy individuals. Moreover, in these studies we find a problem with the classification of the cancers. NHLs are not consistently defined over time. The definition has changed over time due to the use of different diagnostic methods: first morphological methods, than modern immunological methods were applied. Therefore, the NHLs reported do not always comprise the same cancers. For instance, some include, others exclude hairy cell leukaemia. Multiple myelomas may also be considered presently as NHL but not previously. Some studies are thus not comparable and some comparisons are difficult because of the in- and exclusion of certain subtypes which are not the same. This may bias the picture. The same applies to the combination in meta-analyses. IARC notes in quite a number of studies that there is limited power for glyphosate exposure. On the other hand, evidence from epidemiological studies has to be considered with all necessary care since at least uncertainties due to extrapolating from animal to human toxicology is avoided in this approach.

2.1 Cohort studies

12 publications have been reported by IARC in section 2.1. These publications are summarized in Table 2.1-1). The conclusion of most of these studies is that glyphosate did not cause different types of cancer or did not increase the risk of all cancers.

Glyphosate did not significantly increase the risk of prostate cancer, pancreatic cancer, melanoma, lung cancer, colon cancer, rectum cancer, kidney cancer, urinary bladder cancer, breast cancer, childhood cancer and all types of cancer. Cohort studies reported also no increased risk of all lymphohaematopoietic cancers, non-Hodgkin lymphoma (NHL), multiple myeloma, and of monoclonal gammopathy which is considered to be a premalignant disorder that often precedes multiple myeloma. The results on NHL and multiple myeloma are discussed together with the results of case-control studies below (see section 2.2).

Table 2.1-1: Discussion of studies in section 2.1 Cohort studies of the IARC monograph

Study (Author/year)	Subject	Evaluation by IARC	Comment by RMS on IARC evaluation	Study reported in RAR Draft April 2015	Final conclusion of RMS, considering IARC evaluation
Alavanja et al., 1996, ASB2015-7849	The Agricultural Health Study (AHS), large prospective cohort study	The only cohort study to date to have published findings on exposure and the risk of cancer at many different sites.	The data of this study were used in further studies. Conclusions are described there.	The AHS study was described in the RAR as basis for a number of publications.	Data of this publication were used for further studies. Conclusions on glyphosate are presented with these studies.
Alavanja et al., 2003, ASB2012-11535	Use of pesticides and prostate cancer risk (based on AHS)	No significant exposure-response association of glyphosate with cancer of prostate was found.	Agreement	Yes, page 531	No significantly increased risk of prostate cancer.
Andreotti et al., 2009, ASB2012-11544	Pesticide use and risk of pancreatic cancer (based on AHS)	The odds ratio for ever- versus never-exposure to glyphosate was 1.1 (0.6-1.7) while the odds ratio for the highest category of level of intensity-weighted lifetime days was 1.2 (0.6-2.6)	Agreement	Yes, Page 531	No significantly increased risk of pancreatic cancer.
Blair et al., 2011, ASB2015-7868	Impact of pesticide exposure misclassification on estimates of relative risks in the AHS	Nondifferential exposure misclassification biases relative risk estimates towards the null in the AHS and tends to decrease the study power.	Glyphosate was not assessed in this study.	No, no assessment of glyphosate in this study	No assessment of glyphosate in this study
Dennis et al., 2010, ASB2015-8439	Pesticide use and risk of melanoma (based on data of AHS)	Exposure to glyphosate was not associated with cutaneous melanoma within the AHS.	Agreement	No	No increased risk of melanoma.
De Roos et al., 2005a, ASB2012-11605	Cancer incidence among glyphosate-exposed pesticide applicators (based on data of the AHS)	No increased risk of all cancers and of cancers in lung, oral cavity, colon, rectum, pancreas, kidney, bladder, prostate and of melanoma, all lympho-haematopoietic cancers, NHL and leukaemia. For multiple myeloma the relative	Agreement with the reported results and the conclusion on limited power of the study. Further discussion of multiple	Yes, page 539	No increased risk of all cancers and of cancers in lung, oral cavity, colon, rectum, pancreas,

		risk was 1.1 (0.5-2.4) when adjusted for age, but was 2.6 (0.7-9.4), when adjusted for multiple confounders. The study had limited power for the analysis of multiple myeloma. Missing data limit the interpretation of the findings.	myeloma in this study see also re-evaluation by Sorahan (2015, ASB2015-2284), below		kidney, bladder, prostate and of melanoma, all lympho-haematopoietic cancers, NHL and leukaemia. Interpretation of multiple myeloma is limited.
De Roos et al., 2005b, ASB2015-8437	Response in the discussion on the study of De Roos et al., 2005a, ASB2012-11605 (see above)	The study had limited power for the analysis of multiple myeloma. Missing data limit the interpretation of the findings.	Agreement	No, the paper is no study but only a response in the discussion on study of De Roos et al., 2005a, ASB2012-11605 (see above).	See De Roos et al., 2005a, ASB2012-11605
Engel et al., 2005, ASB2012-11613	Pesticide use and breast cancer risk	No difference in incidence of breast cancer for women who reported ever applying glyphosate (odds ratio 0.9 (0.7-1.1); Women who never used glyphosate but whose husband had used (no information on duration of use): odds ratio 1.3 (0.8-1.9)	Agreement	Yes, page 531	No significantly increased risk of breast cancer.
Flower et al., 2004, ASB2012-11620	Parental pesticide application and cancer risk in children; (based on data of AHS)	"For all the children of the pesticide applicators, risk was increased for all childhood cancers combined, for all lymphomas combined, and for Hodgkin lymphoma, compared with the general population." Limited power of the study for glyphosate exposure.	The cited IARC conclusion considers the risk for children of all pesticide applicators. However, this statement is not relevant for the assessment of glyphosate. There was an increased odds ratio in result of application of pesticides aldrin, dichlorvos and ethyl dipropylthiocarbamate. However, the results for glyphosate did not demonstrate any risk for childhood cancer. The odds ratios for maternal use and paternal use of glyphosate are	Yes, page 531	No increased risk of childhood cancer.

			even clearly below 1. Agreement with the limited power of the study.		
Landgren et al., 2009, ASB2012-11875	Pesticide exposure and risk of monoclonal gammopathy (based on data of AHS)	No association between exposure to glyphosate and risk of monoclonal gammopathy of undetermined significance, a premalignant plasma disorder that often precedes multiple myeloma; odds ratio 0.5 (0.2-1.0)	The study authors conclude a nonsignificant decrease of monoclonal gammopathy of undetermined significance (MGUS), on the large data base of the AHS.	Yes, page 531	Nonsignificant decrease of risk of MGUS which usually precedes multiple myeloma
Lee et al., 2007, ASB2015-8228	Pesticide use and risk of colorectal cancer (based on data of AHS)	Most of the 50 pesticides studied were not associated with risk of cancer of the colorectum, and the relative risks with exposure to glyphosate were 1.2 (0.9-1.6), 1.0 (0.7-1.5) and 1.6 (= 0.9-2.9) for cancers of the colorectum, colon and rectum respectively.	Agreement	No	No significantly increased risk of colorectal cancers.
Sorahan, 2015, ASB2015-2284	Glyphosate and multiple myeloma, re-analysis of AHS data; data of the study of De Roos et al., 2005a, ASB2012-11605 (see above) are reanalysed	Sorahan confirmed that the excess risk of multiple myeloma was present only in the subset with no missing information.	The author concluded that "*this secondary analysis of AHS data does not support the hypothesis that glyphosate use is a risk factor for multiple myeloma*".	No, study was published after completion of the RAR.	No significantly increased risk of multiple myeloma based on the AHS data

2.2 Case–control studies on non-Hodgkin lymphoma, multiple myeloma, and leukaemia

16 studies have been reported in section 2.2 of the IARC monograph and are summarized including comments of the RMS in Table 2.2-1.

Two of these 16 studies did not mention glyphosate (Waddell et al., 2001, ASB2015-8037 and Zahm et al., 1990, ASB2013-11501).

Five studies reported no increased risk of non-Hodgkin lymphoma and/or leukaemia or multiple myeloma. (Brown et al., 1990, TOX2003-999; Cantor et al., 1992, ASB2015-7885; Karunanayake et al., 2012, ASB2012-11865; Lee et al., 2004a, ASB2015-8238, and Orsi et al., 2009, ASB2012-11985).

Some of the reported studies had according to the IARC assessment in agreement with the RMS assessment a limited or even very limited power to assess effects of glyphosate. In three studies only 4 exposed cases have been compared with 2, 3 or 5 control subjects (Cocco et al., 2013, ASB2014-7523; Hardell and Eriksson, 1999, ASB2012-11838; and Nordström et al., 1998, TOX1999-687).

Further studies reported different, contradictory results. Depending from the used method of statistical analysis the risk was increased in some cases or not increased in other cases.

The relevant studies on non-Hodgkin lymphoma have been selected by Schinasi and Leon (2014, ASB2014-4819) to perform a meta-analysis. For the analysis of an association between glyphosate and non-Hodgkin lymphoma the following studies have been used: De Roos et al., 2003, ASB2012-11606; De Roos et al., 2005a, ASB2012-11605; Eriksson et al., 2008, ASB2012-11614; Hardell et al., 2002, ASB2012-11839; McDuffie et al., 2001, ASB2011-364, and Orsi et al., 2009, ASB2012-11985.

Furthermore, for the analysis of an association between glyphosate and B cell lymphoma 2 studies have been used: Eriksson et al., 2008, ASB2012-11614 and Cocco et al., 2013, ASB2014-7523.

2 of the 6 studies used for the analysis of non-Hodgkin lymphoma reported no increased risk of non-Hodgkin lymphoma (De Roos et al., 2005a, ASB2012-11605 and Orsi et al., 2009, ASB2012-11985).

3 of the above cited 7 studies were considered by IARC to have limited or even very limited power (Hardell et al., 2002, ASB2012-11839 and Cocco at al., 2013, ASB2014-7523) or a low participation rate (McDuffie et al., 2001, ASB2011-364).

Finally, IARC referred in a publication in Lancet (Guyton et al., 2015, ASB2015-7076) to 3 studies (De Roos et al., 2003, ASB2012-11606; McDuffie et al., 2001, ASB2011-364, and Eriksson et al., 2008, ASB2012-11614) in context with the conclusion that there was limited evidence in humans for carcinogenicity of glyphosate. These 3 studies are discussed by RMS in Table 2.2-2.

Table 2.2-1: Discussion of studies in section 2.2 Case-control studies on non-Hodgkin lymphoma (NHL), multiple myeloma and leukaemia of the IARC monograph

Study (Author/year)	Subject	Evaluation by IARC	Comment by RMS on IARC evaluation	Study reported in RAR Draft April 2015	Final conclusion of RMS, considering IARC evaluation
Brown et al., 1990, TOX2003-999	Pesticide exposure and other agricultural risk for leukaemia	The odds ratio for glyphosate was 0.9 (0.5-1.6). The study had limited power to assess effects of glyphosate.	Agreement	No, because released before 2000	No increased risk of leukaemia, limited power of the study.
Brown et al., 1993, TOX2002-1000	Pesticide exposure and multiple myeloma	The odds ratio for glyphosate was 1.7 (0.8-3.6). The study had limited power to assess effects of glyphosate.	Agreement	No, because released before 2000	Limited power of the study to assess effects of glyphosate.
Cantor et al., 1992, ASB2015-7885	Pesticides and other agricultural risk factors for non-Hodgkin lymphoma	The odds ratio for men who ever handled glyphosate was 1.1 (0.7-1.9), low power of the study to assess risk of NHL associated with glyphosate	Agreement	No, because released before 2000	No significantly increased risk of non-Hodgkin lymphoma, limited power of the study
Cocco et al., 2013, ASB2014-7523	Pesticide exposure and lymphoma risk	Odds ratio for glyphosate exposure was 3.1 (0.6-17.4); the study had a very limited power to assess the effects of glyphosate on risk of NHL	Agreement with the reported results and the conclusion on limited power of the study. Only 4 exposed cases and 2 control subjects have been considered in this study.	Yes, page 532	Very limited power of the study (only 4 exposed cases and 2 control subjects)
De Roos et al., 2003, ASB2012-11606	Pesticide exposure and risk of non-Hodgkin lymphoma	See separate assessment in this addendum	See separate assessment in this addendum	Yes, pages 529 and 537	See Table 2.2-2
Eriksson et al., 2008, ASB2012-11614	Pesticide exposure and risk of non-Hodgkin lymphoma	See separate assessment in this addendum	See separate assessment in this addendum	Yes, pages 531 and 540	See Table 2.2-2
Hardell and Eriksson,	Pesticide exposure and risk of non-Hodgkin	The odds ratio for ever-use of glyphosate was 2.3 (0.4-13.4) in a univariate analysis, and 5.8	Agreement with the reported results and the conclusion on limited power	Yes, pages 530 and 534	no conclusion possible because of

Study (Author/year)	Subject	Evaluation by IARC	Comment by RMS on IARC evaluation	Study reported in RAR Draft April 2015	Final conclusion of RMS, considering IARC evaluation
1999, ASB2012-11838	lymphoma	(0.6-54) in a multivariable analysis. The exposure frequency was low for glyphosate, and the study had limited power to detect an effect.	of the study. Only 4 exposed cases and 3 control subjects have been considered in this study.		limited power of the study (only 4 exposed cases and 3 control subjects)
Hardell et al., 2002, ASB2012-11839	Pesticide exposure and risk of non-Hodgkin lymphoma and hairy cell leukaemia	The study is a pooled analysis of two case-control studies (see Hardell and Eriksson, 1999, TOX1999-686, ASB2012-11838 and Nordström et al., 1998, TOX1999-687 in this addendum). Increased risk was found for glyphosate only in univariate analysis (odds ratio, 3.04 (1.08-8.52)), however, the odds ration decreased in multivariate analysis to 1.85 (0.55-6.20). The exposure frequency for glyphosate was low and the study had limited power.	Agreement with the presented results and the conclusion on limited power of the study. The study is a pooled analysis of two case-control studies (see separate discussion on studies of Hardell and Eriksson, 1999, TOX1999-686, ASB2012-11838 and Nordström et al., 1998, TOX1999-687 in this addendum).	Yes, page 530 and 535	See Table 2.2-2
Kachuri et al., 2013, ASB2014-8030	Pesticide exposure and risk of multiple myeloma	The odds ratio for ever-use of glyphosate was 1.19 (0.76-1.87); no association was found for light users (≤ 2 days per year, odds ratio 0.72 (0.39-1.32), the odds ratio in heavier users (>2 days per year) was 2.04 (0.98-4.23). The study had relatively low response rates.	Agreement	Yes, page 532	No increased risk of multiple myeloma for ever use of glyphosate, higher (not significant) OR if mixing or applying glyphosate >2 days per year, low response rate
Karunanayake et al., 2012, ASB2012-11865	Pesticide exposure and risk of non-Hodgkin lymphoma	Based on 38 cases exposed to glyphosate, the odds ratios were 1.14 (0.74-1.76) adjusted for age and province, and 0.99 (0.62-1.56) when additionally adjusted for medical history variables.	Agreement	Yes, page 531	No increased risk of non-Hodgkin lymphoma
Lee et al., 2004a, ASB2015-	Pesticide exposure and risk of non-Hodgkin Lymphoma among	Subject with a history of asthma had a non-significantly lower risk of NHL than non-asthmatics. The odds ratio associated with	Agreement	No	No significantly increased risk of non-Hodgkin

Study (Author/year)	Subject	Evaluation by IARC	Comment by RMS on IARC evaluation	Study reported in RAR Draft April 2015	Final conclusion of RMS, considering IARC evaluation
8238	asthmatics	glyphosate use was 1.4 (0.98-21.) among non-asthmatics and 1.2 (0.4-3.3) among asthmatics.			lymphoma for asthmatics and non-asthmatics; non-significantly lower risk of NHL for asthmatics than non-asthmatics
McDuffie et al., 2001, ASB2011-364	Pesticide exposure and risk of non-Hodgkin lymphoma	Odds ratio of 1.26 (0.87-1.80) and 1.20 (0.83-1.74, adjusted for age, province, high-risk exposures) were observed for exposure to glyphosate. In an analysis by frequency of exposure to glyphosate, participants with 2+ days of exposure per year had an odds ratio of 2.12 (1.2-3.73) compared with those with some but ≤ 2 days of exposure. The study was large, but had relatively low participation rates.	See separate assessment in this addendum	Yes, pages 529 and 545	See Table 2.2-2
Nordström et al., 1998, TOX1999-687	Occupational exposures, animal exposure and smoking as risk factors for hairy cell leukaemia	An age-adjusted odds ratio of 3.1 (0.8-12) was observed for exposure of glyphosate. However, the study had limited power, only 4 exposed cases and there was no adjustment for other exposures.	Agreement with reported results and conclusions on limited power, only 4 exposed cases and 5 exposed controls are considered in this study	Yes, page 530	Limited power of the study (only 4 exposed cases and 5 exposed controls)
Orsi et al., 2009, ASB2012-11985	Pesticide exposure and risk of lymphoid neoplasms	The odds ratios associated with any exposure to glyphosate were 1.2 (0.6-2.1) for all lymphoid neoplasms, 1.0 (0.5-2.2) for NHL, 0.6 (0.2-2.1) for lymphoproliferative syndrome, 2.4 (0.8-7.3) for multiple myeloma, and 1.7 (0.6-5.0) for Hodgkin lymphoma.	Agreement with reported results. It should be considered in the discussion on an association between glyphosate and NHL that the OR of NHL in this study (12 exposed cases and 24 exposed controls) was 1.0.	No	See Table 2.2-2
Waddell et al., 2001, ASB2015-8037	Use of organophosphate pesticides and risk of non-Hodgkin lymphoma	IARC compared the numbers of cases and controls in this study with the study of De Roos et al., 2003; however, no information on glyphosate in this study	No information on glyphosate	No, no information on glyphosate	no information on glyphosate

Study (Author/year)	Subject	Evaluation by IARC	Comment by RMS on IARC evaluation	Study reported in RAR Draft April 2015	Final conclusion of RMS, considering IARC evaluation
Zahm et al., 1990, ASB2013-11501	Exposure to 2,4-D and risk of non-Hodgkin Lymphoma	The study was mentioned by IARC because data were used in the study of De Roos et al., 2003	No information on glyphosate	No, no information on glyphosate	no information on glyphosate

Table 2.2-2: Summary of the RMS assessment on the strength of evidence and validity of epidemiological studies mentioned by IARC.

Short evaluation of the crucial studies in the draft of the Renewal Assessment Report (RAR) of the RMS	Main RMS comment after IARC publication on strength of evidence (none, low, medium, high) based on study type, internal and external validity and estimated effect size	Internal validity, such as quality aspects of the study, sample size, measurement biases, statistical uncertainty.	External validity & relevance for the RMS assessment: how close is the measured endpoint to the health endpoint of concern
De Roos et al. (2003, ASB2012-11606) had reported an association between NHL and glyphosate use.	No unequivocal evidence for causation of NHL by glyphosate based on a pooled analysis of three case control studies in the Midwestern United States (NHL diagnosed between 1979-1986) and reported exposures with 47 pesticides. Logistic regression and hierarchical model provide significant effect (OR, 2.1 with 95% confidence interval (CI) 1.1 to 4.0) and non-significant effect (OR, 1.6 with 95% CI 0.9 to 2.8), respectively, the latter with adjustment for multiple exposures and using prior probability of 0.3 for glyphosate as being carcinogenic. Contrary to common standards, the authors consider the result from the hierarchical model as significant. The description of the study design, analysis and results do not allow assessing methodological quality.	The internal validity cannot be assessed fully due to limitations in the reporting of the study. The past exposure status for a wide range of pesticides has been assessed in interviews, which is inherently prone to recall and interviewer bias. The study showed four out of 47 pesticides with lower limits of 95% confidence intervals greater than 1.0, indicative for a significant effect. The 47 pesticides may constitute multiple testing so that 5% of effects may show up by chance alone. The approximation of the relative risk using the OR is justified for NHL being a rare disease.	The relevance of the study for the current risk question is high. It is not known whether exclusion of females from the study population compromises the applicability of the findings to the general, European population.
McDuffie et al. (2001, ASB2011-364) mentioned a non-significant positive association between self-reported glyphosate exposure and NHL in a Canadian study.	OR_{adj} = 1.20 (0.83/1.74): low effect size, not significant; no unequivocal evidence for causation of NHL by glyphosate. Well performed case control study on the male Canadian population from 6 provinces with one	Low/medium Multiplicity of pesticide exposure reported, but not the correlations. Tiered approach starting with pesticide classes, but no adjustment	Low/medium Should be considered for assessment as it is a well performed study exploring the endpoint NHL, which however is a collection of diseases.

Short evaluation of the crucial studies in the draft of the Renewal Assessment Report (RAR) of the RMS	**Main RMS comment after IARC publication on strength of evidence (none, low, medium, high) based on study type, internal and external validity and estimated effect size**	**Internal validity, such as quality aspects of the study, sample size, measurement biases, statistical uncertainty.**	**External validity & relevance for the RMS assessment: how close is the measured endpoint to the health endpoint of concern**
	of four rare tumours (517 cases, 1506 controls). The study has some limitations typical of a case-control study (recall bias, misclassification of pesticide exposure) and without appropriate adjustment for multiple testing (multiple exposures and multiple endpoints).	for multiple testing (many pesticides, four tumours). While in this publication only NHL is considered, the study was planned and evaluated for four tumours.	The problem of multiple exposures is not easily overcome in reality; therefore it should not be over-stressed.
Eriksson et al. (2008, ASB2012-11614) reported a case-control study which included 910 cases of NHL and 1016 controls living in Sweden. The highest risk was calculated for MCPA. Glyphosate exposure was reported by 29 cases and 18 controls, and the corresponding OR was 2.02.	OR = 2.02 (1.10-3.71) medium effect size, significant; a multivariate analysis gave no significant results. Case control study in 4/7 Swedish regions; all new cases during 29 months. 910 cases, 1016 controls from population registry. The study has some limitations typical of a case-control study (recall bias, misclassification of pesticide exposure) and without appropriate adjustment for multiple testing (multiple exposures).	Low/medium OR values and confidence intervals cannot be reproduced. The reported dependency from use intensity sounds logical but might as well be attributable to reporting bias.	Medium Study reported NHL diagnosis and subtypes according to WHO classification
De Roos et al. (2005, ASB2012-11605) make use of the AHS cohort.	OR = 1.1 [0.7, 1.9] for NHL, adjusted for age, demographic and lifestyle factors, and other pesticides.	High/medium In contrast to case-control-studies, a prospective cohort study does not suffer recall-bias. However, the problems of multiple exposures and multiple testing remain.	High/medium This study is the best we can hope for: A prospective cohort study with sensible stratification is optimal for establishing a causal relation. However, the problems of multiple exposures and of the possible effect of frequently used co-formulants remain.
Orsi et al. (2009, ASB2012-11985) did not find an association between NHL and glyphosate handling in a French case control study (OR = 1.0).	OR = 1.0 [0.5, 2.2] for any exposure (12 cases, 24 controls), OR = 1.0 [0.3, 2.7] for professional exposure (5 cases, 24 controls). The study has some limitations typical of a case-control study (recall bias, misclassification of pesticide exposure) and without appropriate adjustment for multiple testing (multiple exposures).	Medium Sensible stratification.	Medium Study reported NHL diagnosis and subtypes according to ICD-O-3 classification

Short evaluation of the crucial studies in the draft of the Renewal Assessment Report (RAR) of the RMS	**Main RMS comment after IARC publication on strength of evidence (none, low, medium, high) based on study type, internal and external validity and estimated effect size**	**Internal validity, such as quality aspects of the study, sample size, measurement biases, statistical uncertainty.**	**External validity & relevance for the RMS assessment: how close is the measured endpoint to the health endpoint of concern**
Hardell et al. (2002, ASB2012-11839) This study pools data from Hardell and Eriksson (1999, ASB2012-11838) with data from Nordström et al. (1998, TOX1999-687). Case-control study which included 515 male cases of NHL/ HCL and 1141 controls living in North and Middle Sweden. NHL and HCL diagnosed between 1987-1992), each case matched with two male controls, for age and country.	Univariate: OR = 3.04 (1.08 - 8.52) –medium effect size, only 8 exposed case and 8 exposed controls Multivariate: OR = 1.85 (0.55 - 6.2) with adjustment for study, study area, vital status, other pesticides Low effect size, Logistic regression model provide no significant effect.	Not reliable as the study combines two studies with different endpoints in order to increase the power. Note that it might have been justified to combine the endpoints in the first place (if it is true that HCL can be considered a subtype of NHL) but combining two weak studies in order to strengthen the result is technically invalid. The results in the multivariate analysis must be interpreted with caution since exposure to different types of pesticides correlate.	Not relevant for the link between glyphosate and NHL as the study reported NHL and HCL diagnosis. Limited power for glyphosate exposure.

HCL, Hairy cell leukaemia; NHL, non-Hodgkin lymphoma; OR, odds ratio

The crucial studies used by IARC in the discussion on a relation between glyphosate exposure and risk of NHL were re-evaluated regarding strength of evidence and validity and there was no unequivocal evidence for a clear and strong association of NHL with glyphosate because of the limitations of these epidemiological studies such as being based on interviews with farmers or family members, the number of cases involved, and no knowledge of the actual amount of glyphosate or the type of glyphosate formula used. Even though the OR for an association between the exposure to glyphosate and NHL was slightly increased in all studies, it was not significant in the McDuffie study (ASB2011-364), significant in the Eriksson study (based on 29 cases) (ASB2012-11614) and not unequivocal in De Roos (2003, ASB2012-11606) (a further study with data from the AHS in 2005 by De Roos (ASB2012-11605) found no clear association between glyphosate and NHL, based on a large number of participating farmers), allowing no solid epidemiological statement on the basis of these three epidemiological studies. The studies need to be put in the context of the other epidemiological and experimental studies undertaken. Probably, further research needs to be carried out to study the usage and the impact of the formulation used in the field situation.

2.3 Case–control studies on other cancer sites

6 case control studies on other cancer sites were reported by IARC. The studies are summarized in Table 2.3-1.

One of these studies (Monge et al., 2007, ASB2012-11909) did not separately assess glyphosate. The other 5 studies reported no increased risk or even a reduced risk of the investigated cancers (adenocarcinoma of stomach and oesophagus, gliomas and soft-tissue sarcoma).

Table 2.3-1: Discussion of studies in section 2.3 'Case-control studies on other cancer sites' and section 2.4 'Meta-analyses' of the IARC monograph

Study (Author/year)	Subject	Evaluation IARC	Comment RMS on IARC evaluation	Study reported in RAR Draft April 2015	Final conclusion of RMS, considering IARC evaluation
Lee et al., 2004b, ASB2012-11883	Pesticide use and risk of adenocarcinomas of stomach and oesophagus	For ever use of glyphosate, the odds ratio was 0.8 (0.4 - 1.4) for cancer of the stomach, and 0.7 (0.3 - 1.4) for oesophageal cancer; the power of the study was limited.	Agreement	Yes, page 531	No increased risk of adenocarcinomas of stomach and oesophagus
Ruder et al., 2004, ASB2015-8078	Pesticide exposure and risk of gliomas	No association was found with any of the pesticides assessed, including glyphosate. Glyphosate use was assessed, but specific results were not presented.	Agreement	No	No increased risk of gliomas
Carreon et al., 2005, ASB2012-11585	Pesticide exposure and risk of gliomas	There was a reduced risk for glyphosate (OR 0.7 (0.4 - 1.3).	Agreement	Yes, page 531	Reduced risk of gliomas
Lee et al., 2005, ASB2012-11882	Pesticide use and risk of gliomas	There was a non-significant excess risk with glyphosate use for the overall group, but there was inconsistency between observations for self-responds and observations for proxy respondents. The study had limited power to detect an effect of glyphosate use and was difficult to interpret.	Agreement	Yes, page 530	Limited power of the study, difficult to interpret
Pahwa et al., 2011, ASB2014-9625	Pesticide exposure and risk of soft-tissue sarcoma	The fully adjusted odds ratio for glyphosate was 0.90 (0.58 - 1.40).	Agreement	Yes, page 532	No increased risk of soft-tissue sarcoma
Monge et al., 2007, ASB2012-11909	Pesticide exposure and risk of childhood leukaemia	Association of childhood cancer with glyphosate were reported only for an "other pesticides" category that also included other chemicals, glyphosate was not specifically assessed.	Agreement	Yes, page 530	No specific assessment of glyphosate

Study (Author/year)	Subject	Evaluation IARC	Comment RMS on IARC evaluation	Study reported in RAR Draft April 2015	Final conclusion of RMS, considering IARC evaluation
Schinasi and Leon, 2014, ASB2014-4819	Meta-analysis, exposure to pesticides and non-Hodgkin lymphoma	The meta-analysis for glyphosate included six studies and yielded a meta-risk ratio of 1.5 (1.1 - 2.0). The working group noted that the most fully adjusted risk estimates from the articles by Hardell et al. (2002, ASB2012-11839) and Eriksson et al. (2008, ASB2012-11614) were not used in this analysis. After considering the adjusted estimates of the two Swedish studies in the meta-analysis, the Working Group estimated a meta-risk-ratio of 1.3 (1.03 - 1.65).	Agreement, see separate assessment in this addendum (section 2.4).	Yes, page 531 and addendum	See separate assessment in this addendum (section 2.4).

OR, odds ratio

2.4 Meta-analyses

Meta-analysis is an accepted investigation tool to provide a statistical summary across a number of studies with the same research question and similar setting. RMS has reviewed the study of Schinasi and Leon (2014, ASB2014-4819) as it is described in the IARC monograph and a meta-risk ratio of 1.3 (95% CI 1.03 - 1.65) I2=0%, P for heterogeneity 0.589) for NHL and glyphosate (glyphosate-based formulations, see discussion in section 2.5), as elicited by the IARC Working Group for glyphosate, could be reproduced by the RMS. The type of selection of the studies by IARC can be followed. This is a matter of definition and weighting the OR/RR from the case-control and cohort studies. The meta-risk ratio - the result of the meta-analysis - appears to show a moderate effect. The result is based on only 6 studies (De Roos et al., 2003, ASB2012-11606; De Roos et al., 2005, ASB2012-11605; Eriksson et al., 2008, ASB2012-11614; Hardell et al., 2002, ASB2012-11839; McDuffie et al., 2001, ASB2011-364; Orsi et al., 2009, ASB2012-11985), which qualified according to the set criteria. Although one of these (De Roos et al., 2005, ASB2012-11605) is a prospective cohort study, it was not ranked higher. And one study (Hardell et al., 2002, ASB2012-11839) was included in the meta-analysis even though its definition of NHL differs from the other studies. Even in the article, it is pointed out that further studies are needed.

The review of epidemiological studies on glyphosate and cancer by Mink et al., (2012, ASB2014-9617) which was sponsored by Monsanto has not been discussed here as it is not mentioned in the IARC monograph. The authors conducted no meta-analysis, but list 7 cohort studies and 11 case-control studies; they found no evidence of consistent positive associations that would be indicative of a causal relationship between any site-specific cancer and exposure to glyphosate. Almost all of these studies were also reviewed by IARC and the RMS.

The conduct of systematic reviews and meta-analysis is considered primary research work and is typically not conducted by public agencies entrusted with assessing market authorisation studies.

2.5 Categorization of evidence from studies in humans

2.5.1 Contribution of co-formulants to the toxicity of glyphosate-based formulations

IARC concluded that the evidence relevant to carcinogenicity of glyphosate from studies in humans is classified into the category "*Limited evidence of carcinogenicity*".

IARC did not consider the differences of toxicity between the active substance glyphosate and of glyphosate-based formulations caused by the higher toxicity of co-formulants. The exposed cases in human studies are always exposed to glyphosate-based formulations and practically never to the active substance only.

All glyphosate-containing plant protection products contain surfactants or - if not present as an integral component - are to be mixed with surfactants as a compulsory additive to produce the ready-to-use dilution. As has already been discussed during the first Annex I inclusion procedure for glyphosate it became apparent that glyphosate-containing products were more toxic than glyphosate alone. This phenomenon was attributed to the presence of particular surfactants predominantly, namely the POE-tallowamines.

Already in the DAR on glyphosate (Germany, DAR, 1998, ASB2010-10302) that was prepared to support the first Annex I listing of the active substance, it was mentioned that surfactants could significantly contribute to the toxicity of glyphosate products.

Furthermore, a toxicological evaluation of tallowamine was prepared in 2010 and was included into the RAR (see pages 871-886 of the RAR (Volume 3 B.6), revised April 2015, ASB2015-1194).

With regard to nearly all toxicological endpoints under investigation, the POE-tallowamine was clearly

more toxic than glyphosate.

The higher toxicity of the surfactant might explain that also Roundup formulations when tested for different endpoints were more toxic than glyphosate ([redacted] 1982, TOX2002-693, and [redacted] 1983, TOX2002-694; Dallegrave et al., 2003, ASB2012-11600, and Dallegrave et al., 2007, ASB2012-2721).

Toxicological end points for which a higher toxicity of POE tallowamine in comparison to glyphosate was evidenced are summarized in Table 2.5-1.

Table 2.5-1: Comparison of toxicity data for glyphosate and the POE-tallowamine surfactant with CAS no. 61791-26-2 (from RAR, revised April 2015, ASB2015-1194).

End point	**Glyphosate**		**POE-tallowamine surfactant**	
Acute oral (rat)	LD_{50} > 5000 mg/kg bw		LD_{50}: 864 mg/kg bw	
Acute dermal (rabbit)	LD_{50} > 2000 mg/kg bw		LD_{50} >907 mg/kg bw	
Skin irritation	Not irritant		Irritant	
Eye irritation	Moderately to severely irritant		Severely irritant	
Skin sensitization	Negative		Sensitising	
DNA damage	Negative		Equivocal (some evidence at high and clearly toxic doses)	
	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)
Short-term toxicity (rat, oral, 90-day)	150	300	20	60
Short-term toxicity (dog, oral, approx. 3 month)	300	1000	21	42
Reproduction toxicity (rat)	700 (parental) 2000 (repro) 700 (offspring)	2000 (parental) >2000 (repro) 2000 (offspring)	38 (parental) 12 (repro) 12 (offspring)	74 (parental) 38 (repro) 38 (offspring)
Developmental studies (rat), maternal toxicity	300	1000	10.8	72
Developmental studies (rat), foetal effects	300	1000	72	216

Additionally to the above cited toxicological evaluation of tallowamine a large number of further, new studies demonstrated a higher toxicity of glyphosate-based formulations in comparison to the lower toxicity of the active substance glyphosate. Some of these studies are reported in the RAR (revised April 2015, ASB2015-1194) in chapter B.6.6.12 (in a comparison of the active substance glyphosate and glyphosate containing formulations concerning developmental and reproductive toxicity and endocrine disruption) and in chapter B.6.8.4 'Further published data released since 2000'.

Even in the new IARC monograph on glyphosate some studies have been reported which clearly demonstrate a higher toxicity of glyphosate-based formulations than of the active substance. (Gasnier et al., 2009, ASB2009-7384; Richard et al., 2005, ASB2009-9024; Benachour et al., 2007, ASB2009-9018, and Walsh et al., 2000, ASB2012-12046).

However, the evidence of a higher toxicity of glyphosate-based formulations caused by co-formulants was not noticed and not considered in the discussion by IARC.

Even though in some of the cited studies the authors clearly reported that a formulation was used, IARC discussed the effects only as glyphosate effects (e.g. IARC concluded in the study of Kreutz et al., 2001, ASB2015-8279, "*A positive association between exposure to glyphosate and immunotoxicity in fish has been reported.*"). However, no active substance glyphosate was used in this study but a formulation

including co-formulants.

2.5.2 Conclusions on the classification of the evidence relevant to carcinogenicity from studies in humans into the IARC-categories

The categories of IARC as explained in the document IARC Monographs on the Evaluation of Carcinogenic Risks to Humans Preamble, Lyon, 2006 explain the evaluation of epidemiological studies into certain categories (IARC, 2006, ASB2015-8291). On page 19 "Evaluation and rationale" it is stated: "*[…] It is recognized that the criteria for these evaluations, described below, cannot encompass all of the factors that may be relevant to an evaluation of carcinogenicity. In considering all of the relevant scientific data, the Working Group may assign the agent to a higher or lower category than a strict interpretation of these criteria would indicate.*"

These categories refer only to the strength of the evidence that an exposure is carcinogenic and not to the extent of its carcinogenic activity (potency). In other words, the categories describe whether there may be a possible carcinogenic effect of the substance, but not the severity of this effect.

IARC notes for categories:

"*1. Evidence suggesting the lack of carcinogenicity: there are several adequate studies covering the full range of levels of exposure that humans are known to encounter, which are mutually consistent in not showing a positive association between exposure to the agent and any studied cancer at any observed level of exposure […]*"

This is clearly not the case since the studies are not mutually consistent in not showing a positive association, instead results are inconsistent: a considerable number show no positive correlation, others may indicate a positive association. IARC states further "*Bias and confounding should be ruled out with reasonable confidence […]*". This is not the case for the epidemiological studies with glyphosate, since in most studies several chemicals are studied (and used) and the substance under consideration has been used in various mixtures with different co-formulants. Furthermore, a problem with estimating the exposure based on several studies using questionnaires and interviews should be considered since these instruments are prone to recall biases. The studies are not showing consistently a positive association. Most studies do not show an association, but some do. However, it is difficult to demonstrate or prove the lack of carcinogenicity using an epidemiological study. Therefore, RMS agrees that glyphosate cannot be classified in category 1.

"*2. Inadequate evidence of carcinogenicity: the available studies are of insufficient quality, consistency or statistical power to permit a conclusion regarding the presence or absences of a causal association between exposure and cancer, or no data on cancer in humans are available*" (as defined by IARC). IARC does not classify glyphosate in this category, since there were limited data available, even though a lot of the studies have low statistical power, when assessing them individually, due to the number of individuals involved. The AHS cohort-study does list a considerable number of participants. Furthermore, the epidemiological studies show serious limitations because of recall bias, mixture of several chemicals, and missing knowledge about the exact products used (formulations) and low sample sizes, etc. The adherence of each primary study to pertinent guidelines for epidemiological studies was not re-assessed by RMS.

Despite limitations of all involved individual primary studies, it would seem inadequate to neglect the body of evidence they can provide in combination. RMS agrees with IARC that glyphosate should not be classified in this category as the description does not fit the available data even though some of them are weak.

In the 3rd category: "*Limited evidence of carcinogenicity: a positive association has been observed between exposure to the agent and cancer for which a causal interpretation is considered by the working group to be credible, but chance, bias or confounding could not be ruled out with reasonable confidence*". This in other words means a trend in some studies is observed, however, no clear causal relationship can be established and no consistent positive association and the result can be an artefact due to chance or confounding. The IARC classifies the epidemiological evidence of glyphosate in this category. However, the authors of the meta-analysis (Schinasi and Leon, 2014, ASB2014-4819)

recommend for all pesticides further studies. The result could also be described as: most studies show no association, but a few studies do and in the most recent meta-analysis a weak trend between glyphosate NHL and a subgroup B cell lymphoma was observed. Therefore, an effect cannot be ruled out. Following the logic of the classification system of IARC, the RMS can accept this interpretation since the categories 1 and 2 do not appear to be correct, neither is the last category 4 with "sufficient evidence of carcinogenicity". It is a matter of expressing the remaining uncertainty in classifying glyphosate, since a lot of studies show no effect of glyphosate but some do with a weak carcinogenic potency as expressed through the odds ratios. It should be noted that the estimated OR of 1.3 by the IARC based on the meta-analysis of Schinasi and Leon, 2014, ASB2014-4819, indicates a rather weak association and that epidemiological associations cannot be interpreted as proof of causality. It is noteworthy that the most powerful study, the AHS, the prospective cohort-study, which in epidemiological terms is best suited to study the relationship, showed no association with cancer incidence overall or with most of the cancer subtypes, only a suggested association with multiple myeloma incidence was found, which needs to be followed up (De Roos et al., 2005, ASB2012-11605). Therefore, the evaluation of the RMS has a slightly different nuance than the evaluation of IARC, as the RMS is more cautious in describing the evidence for a positive relationship, even though the evaluation of the individual studies is similar.

The RMS sees a particular problem with the co-formulants of glyphosate-based formulations. As described in chapter 2.5.1 for the surfactants and thus for the glyphosate-based formulation a higher toxicity may be observed than for the glyphosate on its own. In the epidemiological studies it is not possible to differentiate between glyphosate itself and the other co-formulants, as well as different formulations used.

3 Cancer in Experimental Animals

In its Monograph Volume 112 IARC came to the conclusion, that there is "sufficient evidence" in experimental animals for the carcinogenicity of glyphosate (IARC, 2015, ASB2015-8421). In contrast and based on animal studies evaluated by the RMS Germany, the RMS had come to the conclusion that classification and labelling for carcinogenicity is not considered appropriate (RAR, April 2015, ASB2015-1194).

Potential explanations for the differences in the outcome of the evaluation may be that:

i) *a different database was used by both agencies and/or*

ii) *the data provided by the study reports was evaluated differently, and/or*

iii) *the overall database was interpreted differently, e.g. as the result of different decision criteria.*

Subsequently, all of these potential explanations are discussed.

i) Differences in the data basis

The database used by IARC and/or RMS for evaluation of neoplastic effects of glyphosate in laboratory animals is presented in the Table 3-1 (mice) and Table 3-2 (rats) below.

Overall, IARC evaluated three mouse and seven rat studies. Additionally IARC reported three further mouse studies and three more rat studies, which were however, not evaluated because these studies were not available in sufficient detail to the IARC Working Group.

Overall, RMS evaluated six mouse and ten rat studies. In addition to all studies assessed by IARC, RMS also evaluated the studies mentioned by IARC that were not fully assessed by the IARC Working Group. Hence, the data-basis considered by both agencies is essentially similar with three more mouse and three more rat studies fully evaluated by the RMS.

Table 3-1 and Table 3-2 summarize the studies reported by IARC and/or RMS, providing references and study owners, study type, duration, routes of exposure, dose levels, results (with respect to carcinogenicity) and the respective evaluations by both agencies.

Table 3-1: Animal studies in mice reported by IARC and/or RMS.

Reference, study ID, Lot, purity, owner	Study type duration route dose levels	Results (with respect to carcinogenicity)	Evaluation by IARC	Evaluation by RMS	Comments
[redacted] 1983, TOX9552381, Lots NB 1782608/3 and 1782610/7, 99.7%, Monsanto	Carcinogenicity, 2 year, CD-1, feeding 0, 1000, 5000, 30000 ppm (equal to 157/190; 814/955; 4841/5874 mg/kg bw/d in m/f)	*Males:* Renal tubule adenoma: 0/49, 0/49, 1/50 (2%), 3/50 (6%) [P for trend = 0.016] *Females:* No data provided on the kidney Report from the PWG of the EPA (1986): *Males:* Renal tubule adenoma: 1/49 (2%), 0/49, 0/50, 1/50 (2%) [NS] Renal tubule carcinoma: 0/49, 0/49, 1/50 (2%), 2/50 (4%) [P = 0.037; Cochran–Armitage trend test] Renal tubule adenoma or carcinoma (combined): 1/49 (2%), 0/49, 1/50 (2%), 3/50 (6%) [P = 0.034; Cochran–Armitage trend test]	Positive trend for renal tubule adenoma and carcinoma in male mice	No significant increase in tumour incidence observed in any groups of treated animals	Different statistical approaches reported by RMS and IARC. Due to differences in statistical evaluation RMS did not consider the renal tubule tumours as significant
[redacted] 1993, TOX9552382, Lot 206-JaK-25-1, 98.6%, Cheminova	Carcinogenicity, 2 year, CD-1, feeding 0, 100, 300, 1000 mg/kg bw/d (dietary levels regularly adjusted)	*Males*: Haemangiosarcoma: 0/50, 0/50, 0/50, 4/50 (8%) [P < 0.001, Cochran-Armitage] Histiocytic sarcoma in the lymphoreticular/ haemopoietic tissue: 0/50, 2/50 (4%), 0/50, 2/50 (4%) [NS] *Females:* Haemangiosarcoma: 0/50, 2/50 (4%), 0/50, 1/50 (2%) [NS] Histiocytic sarcoma in the lymphoreticular/ haemopoietic tissue: 0/50, 3/50 (6%), 3/50 (6%), 1/50 (2%) [NS]	Positive trend for haem-angiosarcoma in males	No significant increase in tumour incidence observed in any groups of treated animals	Different statistical approaches reported by RMS and IARC. Due to differences in statistical evaluation RMS did not consider the haemangiosarcomas as significant

Reference, study ID, Lot, purity, owner	Study type duration route dose levels	Results (with respect to carcinogenicity)	Evaluation by IARC	Evaluation by RMS	Comments
[redacted] 2009, ASB2012-11492, Lot H05H016A, 95.7%, Nufarm	Carcinogenicity, 18 month, CD-1 (ICR), feeding 0, 500, 1500, 5000 ppm (equal to 71/98; 234/299; 810/1081 mg/kg bw/d in m/f)	No relevant carcinogenic response reported	Study reported but not evaluated	No significant increase in tumour incidence observed in any groups of treated animals	Study not considered by IARC
[redacted] 2001, ASB2012-11491, Lot 01/06/97, >95.14%, ADAMA	Carcinogenicity, 18 month, Swiss albino, feeding 0, 100, 1000, 10000 ppm (15; 151; 1460 mg/kg bw/d, sexes combined since values were similar)	Higher incidence of malignant lymphoma at top dose level in males and females (significant according to Cochran-Armitage and Peto test)	Study reported but not evaluated	Considering historical control range and consistency, some evidence for carcinogenicity but not sufficient for classification	Study not considered by IARC
[redacted], 1997, ASB2012-11493, T-941209, 97.56% and T-950308, 94.61%, Arysta	Carcinogenicity, 18 month, CD-1 (ICR), feeding 0, 1600, 8000, 40000 ppm (165/153; 838/787; 4348/4116 mg/kg bw/d in m/f)	No relevant carcinogenic response reported	Study reported but not evaluated	No significant increase in tumour incidence observed in any groups of treated animals	Study not considered by IARC
George et al., 2010, ASB2012-11829, glyphosate based formulation (glyphosate, 41%; POEA, ~15%) (referred to as "glyphosate") dissolved in 50% ethanol; DMBA dissolved in 50% ethanol, and TPA dissolved in 50% acetone, Published study	Initiation–promotion study; Skin only 20 M/group Group I: untreated control Group II: glyphosate only: 25 mg/kg bw topically, 3 × /week, for 32 weeks Group III: single topical application of DMBA, 52 µg/mouse, followed 1 week later by TPA, 5 µg/mouse, 3 × /week, for	Skin tumours Group I: 0/20 Group II: 0/20 Group III: 20/20*, 7.8 ± 1.1 *P < 0.05 vs groups VI and VII Group V: 0/20 Group VI: 0/20 Group VII: 0/20 Group VIII: 8/20*, 2.8 ± 0.9 *P < 0.05 vs group VI	Inadequate study for the evaluation of glyphosate carcinogenicity	Inadequate study for the evaluation of glyphosate carcinogenicity	Both evaluations agree

Reference, study ID, Lot, purity, owner	Study type duration route dose levels	Results (with respect to carcinogenicity)	Evaluation by IARC	Evaluation by RMS	Comments
	32 weeks Group IV: single topical application of glyphosate, 25 mg/kg bw, followed 1 week later by TPA, 5 µg/mouse, 3 × /week, for 32 weeks Group V: 3 × /week topical application of glyphosate, 25 mg/kg bw, for 3 weeks, followed 1 week later by TPA, 5 µg/mouse, 3 × /week, for 32 weeks Group VI: single topical application of DMBA, 52 µg/mouse Group VII: topical application of TPA, 5 µg/mouse, 3 × /week, for 32 weeks Group VIII: single topical application of DMBA, 52 µg/mouse, followed 1 week later by topical treatment with glyphosate, 25 mg/kg bw, 3 × /week, for 32 weeks				

Table 3-2: Animal studies in rats reported by IARC and RMS.

Reference, study id, Lot, purity, owner	Study type duration route dose levels	Results	Evaluation by IARC	Evaluation by RMS	Comments
[redacted] 1993, TOX9750499,	Combined chronic toxicity/carcinogenicity; 2 year;	No relevant carcinogenic response reported	No significant increase in tumour incidence observed	No significant increase in tumour	Both evaluations agree

Reference, study id, Lot, purity, owner	Study type duration route dose levels	Results	Evaluation by IARC	Evaluation by RMS	Comments
229-JaK-5-1, 98.9% and 229-JaK-142-6, 98.7%, Cheminova	Sprague-Dawley; feeding		in any groups of treated animals	incidence observed in any groups of treated animals	
[redacted], 1996, TOX9651587, 2 batches used, 96.8/96.0%, ADAMA	Combined chronic toxicity/carcinogenicity; 2 year; Wistar; feeding 0, 100, 1000, 10000 ppm (6.3/8.6, 59.4/88.5, 595.2/886 mg/kg bw/d in m/f)	No relevant carcinogenic response reported	Study reported but not evaluated	No significant increase in tumour incidence observed in any groups of treated animals	No evaluation by IARC
[redacted] 1990, TOX9300244; XLH-264, 96.5%, Monsanto	Combined chronic toxicity/carcinogenicity; 2 year; Sprague-Dawley; feeding 0, 2000, 8000, 20000 ppm (89/113, 362/457, 940/1183 mg/kg bw/d in m/f)	*Males:* Pancreas (islet cell): Adenoma: 1/43 (2%), 8/45 (18%; P = 0.018), 5/49 (10%), 7/48 (15%; P = 0.042) Carcinoma: 1/43 (2%), 0/45 (0%), 0/49 (0%), 0/48 (0%) Adenoma or carcinoma (combined): 2/43 (5%), 8/45 (18%), 5/49 (10%), 7/48 (15%) Liver: Hepatocellular adenoma: 2/44 (5%; P for trend = 0.016), 2/45 (4%), 3/49 (6%), 7/48 (15%) Hepatocellular carcinoma: 3/44 (7%); 2/45 (4%), 1/49 (2%), 2/48 (4%) Hepatocellular adenoma or carcinoma (combined): 5/44 (11%), 4/45 (9%), 4/49 (8%), 9/48 (19%) *Females:* Pancreas (islet cell): Adenoma: 5/60 (8%), 1/60 (2%), 4/60 (7%), 0/59 Carcinoma: 0/60, 0/60, 0/60, 0/59 Adenoma or carcino-ma (combined): 5/60 (8%), 1/60 (2%), 4/60 (7%), 0/59 Thyroid: C-cell	Pancreas: There was no statistically significant positive trend in the incidence of pancreatic tumours, and no apparent progression to carcinoma but a significant increase in adenoma in males in two dose levels Liver: Significant positive trend for hepatocellular adenoma in males, no progression to malignancy Thyroid: Significant positive trend for C-cell adenoma in females	No significant increase in tumour incidence observed in any groups of treated animals	Due to differences in statistical evaluation RMS did neither consider the pancreatic islet cell tumours nor the hepatocellular adenomas nor the thyroid c-cell adenomas for classification

Reference, study id, Lot, purity, owner	Study type duration route dose levels	Results	Evaluation by IARC	Evaluation by RMS	Comments
		adenoma: 2/60 (3%), 2/60 (3%), 6/60 (10%), 6/60 (10%)			
[redacted] 1981, TOX2000-595 and TOX2000-1997, XHJ-64, 98.7%, Monsanto	Combined chronic toxicity/carcinogenicity; 26 months; Sprague-Dawley; feeding 0, 3/3.4, 10.3/11.2, 31.5/34 mg/kg bw/d in m/f (dietary levels adjusted according to values as measured in the 1st week)	*Males:* Pancreas (islet cell): Adenoma: 0/50 (0%), 5/49* (10%), 2/50 (4%), 2/50 (4%) Carcinoma: 0/50 (0%), 0/49 (0%), 0/50 (0%), 1/50 (2%) Adenoma or carcinoma (combined): 0/50 (0%), 5/49 (10%), 2/50 (4%), 3/50 (6%) *Females:* Pancreas (islet cell): Adenoma: 2/50 (4%), 1/50 (2%), 1/50 (2%), 0/50 (0%) Carcinoma: 0/50 (0%), 1/50 (2%), 1/50 (2%), 1/50 (2%) Adenoma or carcinoma (combined): 2/50 (10%), 2/50 (2%), 2/50 (74%), 1/50 (2%)	There was no statistically significant positive trend in the incidence of pancreatic tumours, and no apparent progression to carcinoma, but a significant increase in one of the treated groups of males	No significant dose dependent increase in tumour incidence observed in any groups of treated animals	Both evaluations basically agree; they disagree in the interpretation of the significant increase of pancreatic islet cell adenoma at the lowest dose group in males
[redacted] 2009, ASB2012-11490, H05H016A, 95,7%, Nufarm	Combined chronic toxicity/carcinogenicity; 2 year; Wistar; feeding Combined chronic toxicity/carcinogenicity; 2 year; Wistar; feeding	No relevant carcinogenic response reported	Study reported but not evaluated	No significant dose dependent increase in tumour incidence observed in any groups of treated animals	No evaluation by IARC
[redacted] 2001*, ASB2012-11488, P30, 97.6%, Syngenta	0, 2000, 6000, 20000 ppm (121/145, 361/437, 1214/1498 mg/kg bw/d in m/f)	No relevant carcinogenic response reported	No significant increase in tumour incidence observed in any groups of treated animals	No significant increase in tumour incidence observed in any groups of treated animals	Both evaluations agree
[redacted] 1997, ASB2012-11484, ASB2012-11485, ASB2012-11486, ASB2012-11487,	Combined chronic toxicity/carcinogenicity; 2 year; Sprague-Dawley; feeding 0, 3000, 10000, 30000 ppm (104/115, 354/393, 1127/1247 mg/kg bw/d	No relevant carcinogenic response reported	Study reported but not evaluated	No significant increase in tumour incidence observed in any groups of treated animals	No evaluation by IARC

Reference, study id, Lot, purity, owner	Study type duration route dose levels	Results	Evaluation by IARC	Evaluation by RMS	Comments
T-941209, 97.56% and T-950308, 94.61%, Arysta Life Sciences	in m/f)				
[redacted] 1996*#, TOX2000-1998, P24, 95.6%, Syngenta	Chronic toxicity; Wistar-derived; 12 months; feeding 0, 2000, 8000, 20000 ppm (141/167, 560/671, 1409/1664 mg/kg bw/d in m/f)	No relevant carcinogenic response reported	No significant increase in tumour incidence observed in any groups of treated animals	No significant increase in tumour incidence observed in any groups of treated animals	Both evaluations agree
Seralini et al., 2012, (re-published 2014) ASB2012-15514, Published study	24-month study (10 males and 10 females per group) Sprague Dawley Drinking water at 0, 5 x 10^{-5} mg/L, 400 mg/L and 2.25 g/L of total glyphosate from a glyphosate based formulation	*Males:* No significant increase in tumour incidence observed in any of the treated groups *Females:* Mammary tumours (mainly fibroadenomas and adenocarcinomas): 5/10 (50%), 9/10 (90%), 10/10 (100%)*, 9/10 (90%) Pituitary lesions (hypertrophy, hyperplasia, and adenoma): 6/10 (60%), 8/10 (80%), 7/10 (70%), 7/10 (70%)	Inadequate study for the evaluation of glyphosate carcinogenicity	Inadequate study for the evaluation of glyphosate carcinogenicity	Both evaluations agree
Chruzielska et al., 2000, ASB2013-9829, Published study	24 month-study Wistar drinking water containing 0, 300, 900 or 2700 mg/L, 55 m/f per group	No relevant carcinogenic response reported	No significant increase in tumour incidence observed in any groups of treated animals	No significant increase in tumour incidence observed in any groups of treated animals	Both evaluations agree

Summary of results by IARC:

Critical results with respect to carcinogenicity identified by IARC included the occurrence of renal tubular adenoma and carcinoma in CD-1 mice in one study ([redacted], 1983, TOX9552381), the occurrence of haemangiosarcoma in male mice in one other study ([redacted], 1993, TOX9552382) and the occurrence of pancreatic islet cell tumours and hepatocellular adenomas in rats ([redacted] 1990, TOX9300244).

IARC summarized: "*[…] there was a positive trend in the incidence of renal tubule carcinoma and of renal tubule adenoma or carcinoma (combined) in males in one feeding study in CD-1 mice. Renal tubule carcinoma is a rare tumour in this strain of mice. No significant increase in tumour incidence was seen in female mice in this study. In the second feeding study, there was a significant positive trend in the incidence of haemangiosarcoma in male CD-1 mice. No significant increase in tumour incidence was seen in female mice in this study. For the five feeding studies in rats, two studies in the Sprague-Dawley strain showed a significant increase in the incidence of pancreatic islet cell adenoma in males - one of these two studies also showed a significant positive trend in the incidences of hepatocellular adenoma in males and of thyroid C-cell adenoma in females. Two studies (one in Sprague-Dawley rats, one in Wistar rats) found no significant increase in tumour incidence at any site. One study in Wistar rats was inadequate for the evaluation because of the short duration of exposure. In the study in Wistar rats given drinking-water containing glyphosate, there was no significant increase in tumour incidence. A glyphosate-based formulation was found to be a skin-tumour promoter in the initiation-promotion study in male Swiss mice. The study of a glyphosate-based formulation in drinking-water in Sprague-Dawley rats was inadequate for the evaluation because of the small number of animals per group, and the limited information provided on tumour histopathology and incidence in individual animals. These studies of a chemical mixture containing glyphosate were considered inadequate to evaluate the carcinogenicity of glyphosate alone.*" (IARC, 2015, ASB2015-8421)

In addition, IARC reported but did not evaluate the studies by [redacted] (1997, ASB2012-11493) in CD-1 mice, [redacted] (2001, ASB2012-11491) in Swiss albino mice and [redacted] (2009, ASB2012-11490) in CD-1 mice.

Summary results by RMS:

As apparent from the Tables above, RMS had not considered any of the tumours listed by IARC as potentially relevant for classification due to a lack of statistical significance and limited consistency between the studies. Critical results in terms of carcinogenicity identified by the RMS included the occurrence of malignant lymphoma in Swiss mice. RMS argued, however, that the murine tumours are not to be considered for classification because of the high background level of these tumours in Swiss mice.

In summary, RMS stated: "*Taking all this information together, a treatment-related effect in the study by [redacted] (2001, ASB2012-11491) in Swiss albino mice cannot be completely excluded. However, the weak increase in malignant lymphoma even over the historical control of the performing laboratory was clearly confined to this single study and strain since it was not reproducible in four other valid long-term studies. Thus, there is only very limited evidence of a carcinogenic potential of glyphosate as a high-dose phenomenon in mice of a susceptible strain. Most likely, perhaps, age-related neoplastic changes might be exacerbated by long-lasting administration of high doses. Swiss albino mice with high background prevalence of malignant lymphoma could be more vulnerable than other strains.*

Since the more frequent occurrence of malignant lymphoma was confined to a very high dose level that was administered over a long period, glyphosate was considered unlikely to pose a carcinogenic risk in humans. Classification and labelling for carcinogenicity is not considered appropriate by the RMS because of the following considerations:

(1) The presumed effect was observed statistically significant in only one of five long-term studies in mice in a strain with a rather high background incidence of malignant lymphoma. Evidence coming from two other studies one more study is even more equivocal because a certain increase there did not gain statistical significance. In a third study, a (non-significant) increase in top

dose incidence was explained and contravened by historical control data. Taking into account the huge amount of information on historical control incidences, there was no evidence of a similar effect in any other study.

(2) *Although the increase in lymphoma incidence in the study by* [redacted] *(2001, ASB2012-11491) was statistically significant in both sexes, it was still within the (small) historical control range of the performing laboratory for females. No evidence of a similar effect in female mice was obtained in any other study.*

(3) *No evidence of carcinogenicity was obtained in a total of six valid 2-year studies in rats (see above) in which sufficiently high dose levels were employed.*

(4) *The dose with a significantly higher lymphoma incidence (1460 mg/kg bw/day) is more than 2900 times higher than the proposed ADI and the margin to the expected consumer exposure is even wider.*" (RAR, April 2015, ASB2015-1194)

ii) Differences in evaluation of individual study reports

Due to the application of different statistical approaches selected for evaluation, IARC and RMS came to diverging conclusions when evaluating cancer incidences in animal studies. IARC included a trend test (generally according to Cochran-Armitage) for statistical evaluation of the data (IARC, 2015, ASB2015-8421). In contrast, initially, the RMS relied on the statistical evaluation provided with the study reports, which was performed and documented as foreseen in the individual study plans (RAR, April 2015, ASB2015-1194). The later were mostly based on pairwise comparison of treatment groups using tests including Fishers exact test, Chi-Square test, or Z-test. As a consequence, IARC reported a positive carcinogenic response in some of these studies, while RMS did not. According to guidance documents for the evaluation of carcinogenicity studies published in support of respective OECD test guidelines (OECD 2012, ENV/JM/MONO(2011)47, ASB2015-8445 and OECD 2002, ENV/JM/MONO(2002)19, ASB2013-3754), both statistical approaches are appropriate.

In order to systematically assess the impact of choice of statistical method, a number of neoplastic endpoints in key-studies were re-evaluated by the RMS for this Addendum using the Fishers exact test and the Cochran-Armitage test, as both are explicitly recommended in the OECD guidance documents cited above. The Cochran-Armitage Test was performed using BMDS version 2.4.0.70. The Fisher-Yates test (Fisher´s exact test) was done using SigmaPlot version 11.2.0.5. The Fisher exact test was replaced by the Chi-square test if N was >50 for all groups.

(a) Renal adenoma and carcinoma in male mice:

The positive trend for renal adenoma and carcinoma in the study by [redacted] (1983, TOX9552381) as reported in the IARC evaluation could be confirmed (Table 3-3). When the trend test was also applied to the incidences of renal tubular tumours in male CD-1 mice as reported by [redacted] (1997, ASB2012-11493), and male Swiss albino mice as reported by [redacted] (2001, ASB2012-11491) further positive results were obtained (Table 3-4). The IARC Working Group did report but not evaluate these studies. In both cases, the pairwise comparison of treatment groups using the Fishers exact test did not show statistically significant differences (Table 3-3 and Table 3-4). No statistical association between renal neoplasia and glyphosate exposure was found in females of these three studies (incidences as follows: [redacted]: 0,0,0,0; [redacted] 0,0,0,0; [redacted] 0,0,0,0). No renal neoplasia were reported in mice of either sex by [redacted] (2009, ASB2012-11492). In the study of [redacted] (1993, TOX9552382), the incidences of renal tubular adenoma + carcinoma were 1+1, 1+1, 0, 0 in groups of males and 0 in all groups of females (control, low, medium, high dose). Incidences of renal tumours in rat studies were not statistically (re-)evaluated.

Table 3-3: **Renal adenoma and carcinoma in male CD-1 mice ([redacted] 1983, TOX9552381), originally reported data and re-evaluation by pathology working group (PWG). Fishers exact test was used to compare each treatment group to the respective control group, with p-values reported in brackets. For each endpoint a Cochran-Armitage trend test was performed, with p-values reported in a separate row.**

Dose		**report**	**Re-evaluation by PWG**		
(mg/kg bw)	N	adenoma	adenoma	carcinoma	combined
0	49	0	1	0	1
157	49	0 (1.000)	0 (1.000)	0 (1.000)	0 (1.000)
814	50	1 (1.000)	0 (0.495)	1 (1.000)	1 (1.000)
4841	50	3 (0.242)	1 (1.000)	2 (0.495)	3 (0.617)
Trend test (p-value)		0.0080	0.2473	0.0370	0.0339

Table 3-4: **Renal tubular tumours adenoma in two additional studies performed in CD-1 mice ([redacted] 1997, ASB2012-11493) and in Swiss albino mice ([redacted] 2001, ASB2012-11491). Fishers exact test was used to compare each treatment group to the respective control group, with p-values reported in brackets. A Cochran-Armitage trend test was performed, with p-values reported in a separate row.**

Animals	[redacted] **(1997, ASB2012-11493)**		[redacted] **(2001, ASB2012-11491)**	
per group	Dose (mg/kg bw)	adenoma	Dose (mg/kg bw)	adenoma
50	0	0	0	0
50	165	0 (1.000)	15	0 (1.000)
50	838	0 (1.000)	151	1 (1.000)
50	4348	2 (0.495)	1460	2 (0.495)
	Trend test (p-value)	0.0078	Trend test (p-value)	0.0390

b) Haemangiosarcoma in male mice:

The statistically positive trend test for haemangiosarcoma in the study by [redacted] (1993, TOX9552382) as reported by IARC could be confirmed. Direct comparison of the incidences in males of the high dose and the control group using the Fishers exact test resulted in a p-value of 0.059 just above the significance level of 0.05 (**Fehler! Ungültiger Eigenverweis auf Textmarke.**). In addition, there was a positive trend for haemangiosarcoma when the data from [redacted] (1997, ASB2012-11493) was included in the re-evaluation.

Table 3-5: Haemangiosarcoma in male CD-1 mice ([redacted] 1993, TOX9552382; [redacted] 1997, ASB2012-11493). Fishers exact test was used to compare each treatment group to the respective control group, with p-values reported in brackets. A Cochran-Armitage trend test was performed, with p-values reported in a separate row.

Dose		Haemangiosarcoma	Dose	Haemangiosarcoma
(mg/kg bw)	N	[redacted] (1993, TOX9552382)	(mg/kg bw)	[redacted] (1997, ASB2012-11493)
0	50	0	0	0
100	50	0 (1.000)	165	0 (1.000)
300	50	0 (1.000)	838	0 (1.000)
1000	50	4 (0.059)	4348	2 (0.495)
Trend test (p-value)		0.0004		0.0078

c) Malignant lymphoma in mice:

IARC and RMS reported a significantly increased incidence of malignant lymphoma in males of the high dose group in the study of [redacted] (2001, ASB2012-11491) compared to the concurrent control. Interestingly, when the analysis was performed using the Fischers exact test rather than the Z-test as done by the authors of the study report, a p-value of 0.077 > 0.05 instead of 0.002 < 0.01 was obtained. The trend test (not reported by IARC) also provided a p-value above the significance level of 0.05 (Table 3-6).

However, re-evaluation of the incidences if malignant lymphoma reported by [redacted] (2009, ASB2012-11490) and [redacted] (1997, ASB2012-11493) showed statistically significant increases with dose for male CD-1 mice (Table 3-7 and Table 3-8). Re-analysis of malignant lymphoma data reported by of [redacted] (1993, TOX9552382) confirmed the earlier evaluation, showing no treatment-related increases in incidence (Table 3-9).

Table 3-6: Malignant Lymphoma in Swiss albino mice ([redacted] 2001, ASB2012-11491). Fishers exact test was used to compare each treatment group to the respective control group, with p-values reported in brackets. For each sex, a Cochran-Armitage trend test was performed, with p-values reported in a separate row.

Dose	male		female	
(mg/kg bw)	N	Malignant Lymphoma	N	Malignant lymphoma
0	50	10	50	18
15	50	15 (0.356)	50	20 (0.837)
151	50	16 (0.254)	50	19 (1.000)
1460	50	19 (0.077)*	50	25 (0.225)*
Trend test (p-value)		0.0655		0.068

* The original study report indicated a statistically significant increase (p<0.05).

Table 3-7: Malignant Lymphoma in CD-1 mice ([redacted] 2009, ASB2012-11490). Chi square test was used to compare each treatment group to the respective control group, with p-values reported in brackets. For each sex, a Cochran-Armitage trend test was performed, with p-values reported in a separate row.

Dose	male		female	
(mg/kg bw)	N	Malignant Lymphoma	N	Malignant lymphoma
0	51	0	51	11
71	51	1 (1.000)	51	8 (0.611)
234	51	2 (0.475)	51	10 (1.000)
810	51	5 (0.067)*	51	11 (1.000)
Trend test (p-value)		0.0037		0.3590

* Chi –square test was chosen in accordance to the recommendations of the statistics package used. Using the Fishers exact test, a p-value of 0.056 (two-sided) is calculated. Depending on the tool used for calculation, the two-tailed Z-test produced p-values of 0.0220, 0.0219 and 0.067.

Table 3-8: Malignant Lymphoma in CD-1 mice ([redacted] 1997, ASB2012-11493). Fishers exact test was used to compare each treatment group to the respective control group, with p-values reported in brackets. For each sex, a Cochran-Armitage trend test was performed, with p-values reported in a separate row.

Dose	male		female	
(mg/kg bw)	N	Malignant Lymphoma	N	Malignant lymphoma
0	50	2	50	6
165	50	2 (1.000)	50	4 (0.741)
838	50	0 (0.495)	50	8 (0.774)
4348	50	6 (0.269)	50	7 (1.000)
Trend test (p-value)		0.0085		0.2971

Table 3-9: Malignant Lymphoma in CD-1 mice ([redacted] 1993, TOX9552382). Fishers exact test was used to compare each treatment group to the respective control group, with p-values reported in brackets. For each sex, a Cochran-Armitage trend test was performed, with p-values reported in a separate row.

Dose	male		female	
(mg/kg bw/d)	N	Malignant Lymphoma	N	Malignant lymphoma
0	50	4	50	14
100	50	2 (0.678)	50	12 (0.657)
300	50	1 (0.362)	50	9 (0.342)
1000	50	6 (0.741)	50	13 (1.000)
Trend test (p-value)		0.0760		0.4831

d) Pancreatic islet cell adenoma in rats:

IARC noted that based to the tumour incidences reported by [redacted] (1990, TOX9300244), there was a significant increase in pancreatic adenoma in males in two dose levels but no statistically significant positive trend nor a progression to carcinoma. In contrast, RMS did not report any statistically significant effect for pancreatic tumours in this study. When re-evaluating the reported incidences using Cochran-Armitage trend testing and Fishers exact test, absence of a statistically positive trend was confirmed and a significant difference to the incidence in the control group was found for the low dose group only (Table 3-10). The latter result is in agreement with the study summary provided in the revised RAR Volume 3 (April 2015, ASB2015-1194).

Table 3-10: Pancreatic islet cell tumors in SD rats ([redacted], 1990, TOX9300244). Fishers exact test was used to compare each treatment group to the respective control group, with p-values reported in brackets. A Cochran-Armitage trend test was performed, with p-values reported in a separate row.

Dose	male	
(mg/kg bw)	N	adenoma
0	43	1
89	45	8 (0.030)
362	49	5 (0.209)
940	48	7 (0.062)
Trend test (p-value)		0.1687

In addition, IARC reported for the study of [redacted] (1981, TOX2000-595, TOX2000-1997) in SD rats a significant increase in the incidence of pancreatic tumours in one of the treated groups of males in the absence of statistically significant positive trends over all dose groups and no indication for progression to carcinoma. The RMS did not report significant pancreatic tumour findings for this study. Re-evaluation confirmed a significantly increase number of adenomas and combined adenomas + carcinomas for the male low dose group when compared to the concurrent controls. In addition, a significantly positive trend for carcinomas in male animals was found that has not been previously reported. There were no significant findings for pancreatic tumours in the females (Table 3-11 and Table 3-12).

Table 3-11: Pancreatic tumors in male SD rats ([redacted] 1981, TOX2000-595, TOX2000-1997). Fishers exact test was used to compare each treatment group to the respective control group, with p-values reported in brackets. For each endpoint a Cochran-Armitage trend test was performed, with p-values reported in a separate row.

Dose	male			
(mg/kg bw)	N	adenoma	carcinoma	adenoma + carcinoma
0	50	0	0	0
3	49	5 (0.027)	0 (1.000)	5 (0.027)
10.3	50	2 (0.495)	0 (1.000)	2 (0.495)
31.5	50	2 (0.495)	1 (1.000)	3 (0.242)
Trend test (p-value)		0.5284	0.0496	0.3207

Table 3-12: Pancreatic tumors in female SD rats ([redacted] 1981, TOX2000-595, TOX2000-1997). Fishers exact test was used to compare each treatment group to the respective control group, with p-values reported in brackets. For each endpoint a Cochran-Armitage trend test was performed, with p-values reported in a separate row.

Dose	**female**			
(mg/kg bw)	N	adenoma	carcinoma	adenoma + carcinoma
0	50	2	0	2
3.4	50	1 (1.000)	1 (1.000)	2 (1.000)
11.2	50	1 (1.000)	1 (1.000)	2 (1.000)
34	50	0 (0.495)	1 (1.000)	1 (1.000)
Trend test (p-value)		0.9025	0.2969	0.7371

e) Hepatocellular adenoma and carcinoma in rats:

IARC reported a significantly positive trend for hepatocellular adenoma in males in the study of [redacted] (1990, TOX9300244) without indications for progression to malignancy. In contrast, RMS did not report any statistically significant effect for liver tumours in this study. When re-evaluating the reported incidences using Cochran-Armitage trend testing and Fishers exact test, the statistically positive trend was confirmed for adenomas and no positive trend was observed for adenoma and carcinoma combined. In accordance with evaluations by IARC and RMS, a significant difference to the incidence in the control group was not found for the respective treatment groups (Table 3-13).

Table 3-13: Liver cell tumors in SD rats ([redacted] 1990, TOX9300244). Fishers exact test was used to compare each treatment group to the respective control group, with p-values reported in brackets. For each endpoint a Cochran-Armitage trend test was performed, with p-values reported in a separate row.

Dose	**male**	**liver**	
(mg/kg bw)	N	adenoma	adenoma + carcinoma
0	44	2	5
89	45	2 (1.000)	4 (0.739)
362	49	3 (1.000)	4 (0.732)
940	48	7 (0.162)	9 (0.392)
Trend test (p-value)		0.0171	0.0752

f) Thyroid C-cell adenoma in rats:

The IARC Working Group reported a significant positive trend for C-cell adenoma in females of the study of [redacted] (1990, TOX9300244). The RMS did not report any statistically significant effect with respect to thyroid tumours for this study. The statistically significant positive trend could be confirmed using the Cochran-Armitage test (Table 3-14).

Table 3-14: Thyroid C-cell adenoma tumors in female SD rats ([redacted], 1990, TOX9300244). Fishers exact test was used to compare each treatment group to the respective control group, with p-values reported in brackets. A Cochran-Armitage trend test was performed, with p-values reported in a separate row.

Dose	female	Thyroid
(mg/kg bw)	N	C-cell adenoma
0	60	2
113	60	2 (1.000)
457	60	6 (0.167)
1183	60	6 (0.167)
Trend test (p-value)		0.0435

iii) Differences in decision criteria

In addition to the statistical significance, the RMS had taken into account consistency of results as a criterion for evaluation. Since no consistent significant increase in any of the tumour types was originally reported in the available studies the apparent effects were not considered sufficient for classification in the RAR (April 2015, ASB2015-1194).

As for the database, a part of the criteria used by both agencies is essentially similar while some deviations exist in terms of classification.

The IARC has used their own published criteria for evaluation of carcinogenic effects (IARC, 2006, ASB2015-8291) while RMS is generally bound to the classification criteria laid down in EU Regulation (EC) No 1272/2008 on Classification, Labelling and Packaging of Substances and Mixtures (in brief referred to as CLP-criteria) (2008, ASB2015-8591).

Criteria IARC:

When considering the level of evidence for a carcinogenic effect, both sets of criteria are similar.

The IARC and CLP criteria state, that:

"*Sufficient evidence of carcinogenicity*: [The Working Group considers that] a causal relationship has been established between the agent and an increased incidence of malignant neoplasms or of an appropriate combination of benign and malignant neoplasms in (a) two or more species of animals or (b) two or more independent studies in one species carried out at different times or in different laboratories or under different protocols. An increased incidence of tumours in both sexes of a single species in a well-conducted study, ideally conducted under Good Laboratory Practices, can also provide *sufficient evidence*.

A single study in one species and sex might be considered to provide *sufficient evidence of carcinogenicity* when malignant neoplasms occur to an unusual degree with regard to incidence, site, type of tumour or age at onset, or when there are strong findings of tumours at multiple sites.

"Limited evidence of carcinogenicity": The data suggest a carcinogenic effect but are limited for making a definitive evaluation because, e.g. (a) the evidence of carcinogenicity is restricted to a single experiment; (b) there are unresolved questions regarding the adequacy of the design, conduct or interpretation of the studies; (c) the agent increases the incidence only of benign neoplasms or lesions of uncertain neoplastic potential; or (d) the evidence of carcinogenicity is restricted to studies that demonstrate only promoting activity in a narrow range of tissues or organs." (IARC 2006, ASB2015-8291; Reg (EC) No 1272/2008, Annex 1, 3.6.2, ASB2015-8591).

Conclusion by IARC:

Based on these criteria it is obvious that IARC concludes on "sufficient evidence of carcinogenicity" in experimental animals, because the above criteria for this conclusion are fully met.

Additional Criteria CLP:

The CLP criteria are taking into account the IARC criteria. However, the CLP regulation also states that when evaluating carcinogenic effects, additional criteria have to be taken into account. In Annex I to Reg (EC) 1272/2008 it is summarized:

"*Annex I: 3.6.2.2.4.* Additional considerations (as part of the weight of evidence approach Beyond the determination of the strength of evidence for carcinogenicity, a number of other factors need to be considered that influence the overall likelihood that a substance poses a carcinogenic hazard in humans. The full list of factors that influence this determination would be very lengthy, but some of the more important ones are considered here.

Annex I: 3.6.2.2.5. The factors can be viewed as either increasing or decreasing the level of concern for human carcinogenicity. The relative emphasis accorded to each factor depends upon the amount and coherence of evidence bearing on each. Generally there is a requirement for more complete information to decrease than to increase the level of concern. Additional considerations should be used in evaluating the tumour findings and the other factors in a case-by-case manner.

Annex I: 3.6.2.2.6. Some important factors which may be taken into consideration, when assessing the overall level of concern are:

(a) tumour type and background incidence;

(b) multi-site responses;

(c) progression of lesions to malignancy;

(d) reduced tumour latency;

(e) whether responses are in single or both sexes;

(f) whether responses are in a single species or several species;

(g) structural similarity to a substance(s) for which there is good evidence of carcinogenicity;

(h) routes of exposure;

(i) comparison of absorption, distribution, metabolism and excretion between test animals and humans;

(j) the possibility of a confounding effect of excessive toxicity at test doses;

(k) mode of action and its relevance for humans, such as cytotoxicity with growth stimulation, mitogenesis, immunosuppression, mutagenicity." (Reg (EC) No 1272/2008, Annex 1, ASB2015-8591)

Conclusion RMS:

Considering these additional criteria when taking into account the rat studies RMS argued that:

"No evidence of carcinogenicity was obtained in any of these studies." and when considering the majority of mouse studies RMS argues (possibly referring to point (a) and (j)) that: "*Again, there was no evidence of carcinogenicity of glyphosate in any of the studies."*

Accordingly for the malignant lymphoma previously observed in one mouse study only, RMS argues, referring to point (a) of the aforementioned list: *"Taking all this information together, a treatment-related effect in the study by* [redacted] *(2001, ASB2012-11491) in Swiss albino mice cannot be completely excluded. However, the weak increase in malignant lymphoma even over the historical control of the performing laboratory was clearly confined to this single study and strain since it was not reproducible in four other valid long-term studies. Thus, there is only very limited evidence of a carcinogenic potential of glyphosate as a high-dose phenomenon in mice of a susceptible strain. Most likely, perhaps, age-related neoplastic changes might be exacerbated by long-lasting administration of high doses. Swiss albino mice with high background prevalence of malignant lymphoma could be more vulnerable than other strains.*

Since the more frequent occurrence of malignant lymphoma was confined to a very high dose level that was administered over a long period, glyphosate was considered unlikely to pose a carcinogenic risk in humans […]" (RAR , April 2015, ASB2015-1194).

Overall, based on the study results and the CLP criteria RMS concluded that the evidence of carcinogenicity is conclusive but not sufficient for classification.

Summary and conclusion:

The statistical analysis by IARC was confirmed and extended. Based on the data evaluated by the respective agencies and the different criteria used for concluding on a potential carcinogenic effect, it is evident that both agencies have come to reasoned conclusions. The OECD test guideline on the evaluation of carcinogenicity studies states: "*Significance in either kind of test is sufficient to reject the hypothesis that chance accounts for the result.*" (OECD 2002, 2012, ASB2013-3754, ASB2015-8445). Accordingly, renal tumours in male CD-1 mice would be considered as treatment-related based on positive trend tests in two studies ([redacted], 1983, TOX9552381, [redacted] 1997, ASB2012-11493). Malignant lymphoma in males could be considered treatment related in the study by [redacted] (2001, ASB2012-11491) using Swiss albino mice based on the original positive Z-test for the high dose males and the studies of [redacted] (2009, ASB2012-11490) and [redacted] (1997, ASB2012-11493) in CD-1 mice based on positive trend tests for males.

4 Mechanistic and Other Relevant Data

4.1 Toxicokinetic data

4.1.1 Introduction

The introduction in the IARC monograph is in line with the conclusions from the RAR (April 2015, ASB2015-1194). However, in the RAR a broader database was used to assess the microbial metabolism in the gut, suggesting a lower relevance as concluded by IARC.

4.1.2 Absorption

The data presented in the IARC monograph is also nearly completely reported in the RAR (April 2015, ASB2015-1194). The only additional study in the IARC monograph is an *in vitro* model by Vasiluk et al. (2005, ASB2012-12043), describing an increased paracellular permeability due to glyphosate at >10 mg/mL.

4.1.3 Distribution

In general the conclusion for the distribution of glyphosate is comparable between the IARC monograph and the RAR (April 2015, ASB2015-1194), suggesting short half-live times between 10 to 33 h. Also, tissue levels were identified to be highest in kidney.

Two studies presented in the IARC monograph were not reported in the RAR (Yue et al., 2008, ASB2012-12059 and Bernal et al., 2010, ASB2015-7858), however their results do not lead to different conclusions for the distribution of glyphosate.

4.1.4 Metabolism and modulation of metabolic enzymes

Both the IARC monograph and the RAR (April 2015, ASB2015-1194) concluded that glyphosate metabolized to a very small amount into AMPA in mammals. The IARC monograph relied on two studies not included in the RAR (Motojyuku et al., 2008, ASB2015-8160 and Bernal et al., 2010, ASB2015-7858). However in total the RAR provided a broader database for this endpoint. Concerning the modulation of metabolic enzymes all studies used by IARC were also presented in the RAR. No deviating conclusions were drawn in both documents.

4.1.5 Excretion

Except for one study on glyphosate and AMPA levels in urine of a rural population in Colombia (Varona, 2009, ASB2015-8039), which is in line with results from other studies, all references presented by IARC were also cited in the RAR. Also the conclusion that systemically absorbed glyphosate is not metabolized efficiently and is mainly excreted unchanged into the urine is identical. No discrepancies between the RAR (April 2015, ASB2015-1194) and the IARC monograph were identified.

4.2 Mechanisms of carcinogenesis

4.2.1 Genetic and related effects

Glyphosate has been studied for genotoxic potential in a wide variety of assays. The studies which were evaluated by IARC were carried out in exposed humans, in human cells *in vitro*, in other mammals *in vivo* and *in vitro*, and in non-mammalian systems *in vivo* and *in vitro*, respectively, are summarized in Tables 4.1-4.5 of the IARC monograph.

The IARC Working Group has reviewed only reports that have been published or accepted for publication in the openly available scientific literature as well as data from government agency reports that are publicly available.

In contrast, the RMS which undertakes the task of evaluating an active substance according to Regulation (EC) No 1107/2009 (2009, ASB2015-8589) shall review the complete dossier (that contains the full text of the individual test and study reports) and the scientific peer-reviewed open literature on the active substance and its relevant metabolites.

Thus, the RMS has assessed the relevant published data on genotoxicity of glyphosate which has also been reviewed by IARC, and additionally a number of regulatory studies which were not available to IARC, but a great many of them were evaluated in the review article of Kier and Kirkland (2013, ASB2014-9587). The regulatory studies were mostly generated in compliance with internationally agreed test guidelines, which include principles for conducting studies, reporting results, and analysing and interpreting data.

For regulatory purposes, test methods preferred for use are (ECHA, 2015: Guidance on information requirements and chemical safety assessment; Chapter R.7a: Endpoint specific guidance; Version 4.0, ASB2015-8657):

In vitro test methods: OECD 471, OECD 476, OECD 476, OECD 473, OECD 487.

In vivo test methods, somatic cells: OECD 475, OECD 474, OECD 488, OECD 486, OECD 489.

In vivo test methods, germ cells: OECD 483, OECD 478, OECD 488.

To be able to evaluate the mutagenic potential of a substance in a comprehensive way, information is required on its capability to induce gene mutations, structural chromosome aberrations (clastogenicity) and numerical chromosome aberrations (aneugenicity).

Classification of substances for (germ cell) mutagenicity according to CLP criteria:

Hazard classification for germ cell mutagenicity primarily aims to identify substances causing heritable mutations or being suspected of causing heritable mutations. A secondary aim is that the hazard class germ cell mutagenicity offers supporting information with respect to the classification of carcinogenic substances. This is expressed by the broad meaning of the hazard statements ‘H340: May cause genetic defects’ and ‘H341: Suspected of causing genetic defects’ which comprises heritable genetic damage as well as somatic cell mutagenicity. Thus, classification as a germ cell mutagen (Category 1A, 1B, and 2) classifies for the hazard heritable genetic damage as well as providing an indication that the substance could be carcinogenic.

Classification as a Category 1A mutagen:

Epidemiological studies have been to date unable to provide evidence to classify a substance as a Category 1A mutagen. Hereditary diseases in humans for the most part have an unknown origin and show a varying distribution in different populations. Due to the random distribution of mutations in the genome it is not expected that one particular substance would induce one specific genetic disorder. Therefore, it is unlikely that such evidence may be obtained by epidemiological studies to enable for classification of a substance as a Category 1A mutagen.

Classification as a Category 1B mutagen:

Classification in Category 1B may be based on positive results of at least one valid *in vivo* mammalian germ cell mutagenicity test. In case there are also negative or equivocal data, a weight of evidence approach using expert judgement has to be applied.

If there are only positive results of at least one valid *in vivo* mammalian somatic mutagenicity test but no respective data on mammalian germ cells are available, additional evidence is required to be able to classify as mutagen in Category 1B. Such additional data must prove that the substance or its metabolite(s) interacts *in vivo* with the genetic material of germ cells. It is also possible to obtain supporting evidence in an *in vivo* genotoxicity test with mammalian germ cells. In addition, genetic damage to germ cells in exposed humans proven to be caused by substance exposure may offer respective information. In case of other supporting evidence or where there are also negative or equivocal data, a weight of evidence approach using expert judgement has to be applied.

Classification as a Category 2 mutagen:

Classification in Category 2 may be based on positive results of a least one *in vivo* valid mammalian somatic cell mutagenicity test, indicating mutagenic effects in somatic cells. A Category 2 mutagen classification may also be based on positive results of a least one *in vivo* valid mammalian somatic cell genotoxicity test, supported by positive *in vitro* mutagenicity results. Genetic damage to somatic cells in exposed humans shown to be caused by substance exposure supported by positive *in vitro* mutagenicity results may also offer respective information warranting classification as a Category 2 mutagen. *In vitro* results can only lead to a Category 2 mutagen classification in a case where there is support by chemical structure activity relationship to known germ cell mutagens. In the case where there are also negative or equivocal data, a weight of evidence approach using expert judgement has to be applied.

Principles for the evaluation of published studies used by the RMS

For the analysis of published studies, the RMS made generally a comparison to the criteria in guidelines used for regulatory purposes. However, these criteria do not represent an absolute judgment standard but can provide a way for evaluating the quality of the protocols used in various published studies. Kier & Kirkland (2013, ASB2014-9587) have summarized a number of relevant issues to be considered: "*Some of the criteria are rarely met in scientific publications and should be given little or no weight in evaluating the studies. For example, data for individual cultures and individual animals are not commonly included in publications in scientific journals. These data are presumably collected but are usually summarized as group means with a measure of variance for the treatment and control groups. This is not considered to be a significant omission in a scientific publication. However, other guideline features are more essential as scientific quality standards and should be considered as having greater weight in evaluating a study. For example, there are consistent recommendations that assays involving visual scoring (e.g. chromosomal aberration, micronucleus and sister chromatid exchange (SCE) endpoints) should use slides that are independently coded so that scoring is performed without any knowledge of the treatment or practice and studies that do not explicitly include a description of coding or ''blind'' scoring in the methodology would appear to have a deficiency either in the methodology, or perhaps a limitation in the description of the methodology used if coding was actually used and either not indicated or was assumed to be indicated by a reference citation. Other examples of guideline features that have clear experimental scientific value are the use of concurrent negative and positive controls and concurrent measurement and reporting of toxicity endpoints in main experiments, especially in in vitro mammalian cell assays.*"

Glyphosate:

Assessment and conclusion of IARC:

According to the conclusion of IARC, there is strong evidence that glyphosate causes genotoxicity. The evidence base includes studies that gave largely positive results in human cells *in vitro* (IARC monograph, Table 4.2), in mammalian model systems *in vivo* (IARC monograph, Table 4.3) and *in vitro*

(IARC monograph, Table 4.4), and studies in other non-mammalian systems *in vivo* (IARC monograph, Table 4.5) and *in vitro* (IARC monograph, Table 4.6). *In vivo* studies in mammals gave generally positive results in the liver, with mixed results for the kidney and bone marrow. The end-points that have been evaluated in these studies comprise biomarkers of DNA adducts and various types of chromosomal damage. Tests in bacterial assays gave consistently negative results (IARC monograph, Table 4.6).

Assessment and conclusion of the RMS:

In vitro studies:

1. Bacterial assays gave consistently negative results.
2. *In vitro* mammalian cell gene mutation tests gave consistently negative results.
3. *In vitro* mammalian chromosome aberration tests and *in vitro* micronucleus tests: several regulatory studies conducted according to internationally agreed test guidelines which gave negative results at concentrations up to 1250 µg/ml (Table 4.2-1). In contrast, induction of chromosomal aberrations in bovine lymphocytes was reported in one non-guideline study without metabolic activation at concentrations of 3-30 µg/mL (Lioi et al., 1998, ASB2013-9836), and induction of micronucleus formation in CHO cells was reported in one non-guideline study with metabolic activation at concentrations of 5-100 µg/mL (Roustan et al., 2014, ASB2014-8086).
4. Further *in vitro* tests (indicator tests): Positive results for induction of sister chromatid exchange (SCE) were reported in cultured human and bovine lymphocytes without metabolic activation in two published non-guideline studies (Table 4.2-2).

   Positive results were also reported for induction of DNA strand breaks in *in vitro* mammalian cell assays in five published non-guideline studies (Table 4.2-2).

   There was no evidence of an increase in unscheduled DNA synthesis (UDS) in rat primary hepatocyte cultures *in vitro* in a published study and a regulatory study (Table 4.2-2).

In vivo studies (in mammals) in somatic cells:

1. Mutagenicity tests: Both the rodent bone marrow micronucleus test and the rodent bone marrow chromosome aberration test were used in a total of 16 studies to examine mutagenic effects of glyphosate.

   In 8 regulatory studies in rats and mice conducted according to internationally agreed test guidelines, glyphosate was administered by oral gavage at dose levels up to 5000 mg/kg bw, which is well above the limit dose of 2000 mg/kg bw according to OECD test guidelines 474 or 475. The tests gave consistently negative results (Table 4.2-3).
   In another 8 studies in rats and mice (4 publications and 4 regulatory studies), glyphosate was administered by intraperitoneal application at dose levels up to 600 mg/kg bw in mice and up to 1000 mg/kg bw in rats. These dose levels may have exceeded the maximum tolerated dose, since the intraperitoneal LD_{50} of glyphosate has been reported to be 134 mg/kg bw in mice (Bababunmi et al., 1978, ASB2015-8535). For rats, the intraperitoneal LD_{50} of glyphosate ranged from 238 mg/kg bw to 1383 mg/kg bw (Bababunmi et al., 1978, ASB2015-8535, [redacted] 1991, TOX9300330). Irrespective of the high dose levels tested, negative results were obtained in 6 studies (one chromosome aberration test in rats, 5 micronucleus tests in mice; Table 4.2-3).
   In one published study in mice (Bolognesi et al., 1997, Z59299), two i.p. doses of 150 mg/kg bw, administered 24 h apart, produced a statistically significant increase in micronuclei when bone marrow was examined 24 h after the second dose. However, the dose tested was in the range of the intraperitoneal LD_{50} of glyphosate reported for mice, and no information on signs of toxicity was provided in the publication.
   In second published study in mice (Mañas et al., 2009a, ASB2012-11892), two i.p. doses of 200 mg/kg bw, administered 24 h apart, produced a statistically significant increase in micronuclei

when bone marrow was examined 24 h after the second dose. However, the result of this study is flawed by a major deviation from internationally agreed test guidelines: "erythrocytes" instead of immature or "polychromatic erythrocytes" (PCE) were scored for micronuclei. In an assay with the reported treatment and sampling times, scoring of all erythrocytes instead of polychromatic erythrocytes would be inappropriate (test guideline OECD 474).

2. Further *in vivo* studies: Evidence for DNA adduct formation and for induction of DNA strand breaks following i.p. administration of glyphosate to mice at a single dose of 300 mg/kg bw has been reported in one publication (Bolognesi et al., 1997, Z59299). Induction of DNA strand breaks was also reported in a published study in mice after oral doses of 40 and 400 mg/kg bw per day over a period of 14 days (Mañas et al., 2013). In contrast, no evidence for DNA adduct formation was reported following intraperitoneal administration of glyphosate isopropylammonium salt to mice at a single dose of 270 mg/kg bw (Peluso et al., 1998, TOX1999-318).
   Since the induction of DNA strand breaks was observed at a dose close to or in excess of the i.p. LD_{50} of glyphosate in mice, the positive result of this assay may be caused by secondary effects of cytotoxicity.

In vivo studies (in mammals) in germ cells:

Glyphosate has been shown to be devoid of mutagenic activity in a dominant lethal assay in mice at oral doses up to 2000 mg/kg bw (EPA, 1980, ASB2015-8547; [redacted] 1980, TOX9552377) and in a dominant lethal assay in rats at oral doses up to 2000 mg/kg bw ([redacted] 1992, TOX9551102).

Overall conclusion:

Glyphosate has been tested in a broad spectrum of mutagenicity and genotoxicity tests *in vitro* and *in vivo*.

In vitro, bacterial assays and mammalian cell gene mutation assays gave consistently negative results. Also, the majority of *in vitro* chromosomal aberration tests and micronucleus tests were negative, in particular, all of the studies performed under GLP conditions resulted in negative findings. *In vitro* tests for induction of indicator endpoints gave positive results for induction of SCE and DNA strand breaks (comet assay) and a negative result for induction of DNA repair (UDS).

In vivo, 14 somatic cell tests for induction of chromosomal aberrations or micronuclei gave negative results, including all the 12 regulatory studies conducted under GLP conditions. Therefore, it is concluded that glyphosate does not induce chromosomal damage *in vivo*, although positive results are reported in two publications. Furthermore, there was no evidence for mutagenic activity in germ cells. Inductions of DNA strand breaks were reported in 2 publications following a high i.p. dose or repeated oral doses.

Taking into account all available data and using a weight of evidence approach, it is concluded that glyphosate does not induce mutations *in vivo* and no hazard classification for mutagenicity is warranted according to the CLP criteria.

AMPA:

AMPA has been tested for mutagenicity and genotoxicity *in vitro* and *in vivo* in an adequate range of assays.

In vitro, two bacterial assays and a mammalian cell gene mutation assay performed under GLP conditions gave negative results, while two micronucleus tests were positive. Two *in vitro* tests for induction of DNA repair (UDS) performed under GLP conditions gave negative results; while a test for induction of DNA strand breaks (comet assay) was positive.

In vivo, two bone marrow micronucleus tests conducted under GLP conditions gave negative results, while a positive result was reported in a published study flawed by methodological limitations. Induction of DNA strand breaks was reported in a publication following repeated oral doses.

Taking into account all available data and using a weight of evidence approach, it is concluded that AMPA does not induce mutations *in vivo* and no hazard classification for mutagenicity is warranted according to the CLP criteria.

Glyphosate-based formulations:

Glyphosate-based formulations have been extensively tested for mutagenicity and genotoxicity *in vitro* and *in vivo* in a wide range of assays. However, since formulation compositions are considered proprietary, the specific composition of the formulations tested was not available for the published studies.

In vitro, bacterial assays gave generally negative results. No regulatory studies of glyphosate-based formulations in *in vitro* mammalian cell chromosomal aberration or micronucleus assays were provided. However, published studies suggested the possibility of activity of glyphosate-based formulations in *in vitro* chromosomal damage assays. No regulatory studies of glyphosate-based formulations in *in vitro* mammalian cell assays for DNA damage were provided. In some published studies, however, positive results for DNA strand breakage and SCE induction were reported.

In vivo mammalian chromosomal aberration or micronucleus assays gave positive results in some published studies for specific glyphosate-based formulations. However, no regulatory studies for these endpoints were provided. Also, no regulatory studies for these endpoints were provided for *in vivo* mammalian assays for DNA damage. However, in some published studies positive results for DNA adducts, DNA strand breakage and SCE induction were reported for specific glyphosate-based formulations. The positive results may be associated with high organ toxicity (liver, kidney) that was primarily due to the non-glyphosate components of the formulation when administered at very high doses via the i.p. route of exposure.

In non-mammalian systems, positive results were reported in *in vivo* studies on chromosomal damage or DNA damage of fish, amphibians and reptiles with different formulations (IARC monograph, Table 4.5). For the representative formulation for the EU renewal procedure 'Roundup Ultra' two studies (Guilherme et al., 2012, ASB2014-7619, Guilherme et al., 2014, ASB2015-8631) reported positive results in comet assays using the European eel as test species.

However, in addition to some technical limitations, there is considerably less experience with these assay systems, and their relevance fur human health assessment is undecided.

Table 4.2-1: Glyphosate; mutagenicity tests in mammalian cells or bacteria *in vitro*

Reference	Evalu-ated by IARC	Test system (endpoint)	Test	Results: Without metabolic activation by authors	Results: With metabolic activation by authors	Concentration range	GLP, Test guideline	RAR 04/2015	Comments BfR
Mañas et al., 2009a, ASB2012-11892	Yes	Human Lymphocytes (Chromosomal damage)	Chromosomal aberrations	–	NT	0.2-6.0 mM (34 - 1015 µg/mL) Purity: 96%	NR, TG 473	p. 401, 436	Only 100 cells scored per treatment. Results not reported separately for replicate cultures.
Fox, 1998, TOX2000-1995	No	Human lymphocytes (Chromosomal damage)	Chromosomal aberration	–	–	-S9/+S9: 100 - 1250 µg/ml Purity: 95.6%	GLP, TG 473	p. 345, 353-357	
Mladinic et al., 2009a, ASB2012-11907	Yes	Human lymphocytes (Chromosomal damage)	Micronucleus formation	–	(+)	-S9/+S9: 0.5 - 580 µg/mL Purity: 98%	Non-GLP, NR	p. 401, 437	$P < 0.01$ (580 µg/mL) Independent coding of slides for scoring not indicated for visually scored slides. Results not reported separately for replicate cultures.
Van de Waart, 1995, TOX9651525	No	Human lymphocytes (Chromosomal damage)	Chromosomal aberration	–	–	-S9: 33 - 333 µg/mL +S9: 237 - 562 µg/mL Purity: 96%	GLP, TG 473	p. 345	
Wright, 1996, ASB2012-11476	No	Chinese hamster lung cells (Chromosomal damage)	Chromosomal aberrations	–	–	-S9/+S9: 312.5 - 1250 µg/mL Purity: 95.3%	GLP, NR	p. 345, 351-353	
Kyomu, 1995, ASB2012-11475	No	Chinese hamster lung cells	Chromosomal aberrations	–	–	-S9: 62.5 - 500 µg/mL +S9:	GLP, TG 473	p. 345-351	

Reference	Evalu-ated by IARC	Test system (endpoint)	Test	Results: Without metabolic activation by authors	Results: With metabolic activation by authors	Concentration range	GLP, Test guideline	RAR 04/2015	Comments BfR
		(Chromosomal damage)				250 - 1000 µg/mL Purity: 95.7%			
Lioi et al., 1998, ASB2013-9836	Yes	Bovine Lymphocytes (Chromosomal damage)	Chromosomal aberrations	+	NT	17 - 170 µM (3 - 30 µg/mL) Purity: ≥ 98%	NR	p. 387	$P < 0.05$ (17 µM) 150 metaphases per concentration were scored for CAs (200 or 300 needed acc. TG 1997 or 2014).
Roustan et al., 2014, ASB2014-8086	Yes	Hamster, Chinese CHO-K1 ovary cell line (Chromosomal damage)	Micronucleus formation	–	+	5 - 100 µg/mL Purity: not given	NR	p. 423-424	$P \leq 0.001$ (10 µg/mL) No continuous treatment (TG 2014).
Clay, 1996, TOX2000-1994	No	Mouse lymphoma cells /L5178Y TK$^{+/-}$ (Mutation)	Mouse lymphoma test	–	–	+/-S9: 296 - 1000 µg/mL Purity: 95.6%	GLP, TG 476	p. 338-341	
Jensen, 1991, TOX9552372	No	Mouse lymphoma cells/L5178Y	Mouse lymphoma test	–	–	-S9: 0.61 - 5.0 mg/mL +S9: 0.52 - 4.2 mg/mL Purity: 98.6%	GLP, TG 476	p. 338	
Li and Long, 1988, TOX9500253 also reported in RAR, TOX9552369, Z35243	Yes	Hamster, Chinese CHO-K$_1$BH$_4$ ovary, cell line (Mutation)	*Hprt* mutation	–	–	-S9: 2 - 22.5 mg/mL +S9: 5 - 22.5 or 25 mg/mL Purity: 98.7%	NR	p. 338	Not entirely clear from the original study report which dose level was actually the highest under activation conditions.

Reference	Evalu-ated by IARC	Test system (endpoint)	Test	Results: Without metabolic activation by authors	Results: With metabolic activation by authors	Concentration range	GLP, Test guideline	RAR 04/2015	Comments BfR
Li and Long, 1988, TOX9500253	Yes	*Salmonella typhimurium* TA1535, TA1537, TA1538, TA98, TA100 (Mutation)	Reverse mutation	–	–	10 - 5000 µg/plate Purity: 98.4%	NR	p. 305	2-aminoanthracen only used as positive control + S9. Only duplicate plating.
Li & Long, 1988, TOX9500253	Yes	Escherichia coli WP2 (Mutation)	Reverse mutation	–	–	10 - 5000 µg/plate Purity: 98.4%	NR	p. 305	2-aminoanthracen only used as positive control + S9. Only duplicate plating.

Results: +, positive; -, negative
NT, not tested; NR, not reported ; S9, 9000 × g supernatant; Hprt, hypoxanthine guanine phosphoribosyl transferase gene;

Table 4.2-2: Glyphosate; genotoxicity tests in mammalian cells or bacteria *in vitro*

Reference	Evaluat ed by IARC	Test system (endpoint)	Test	Results: Without metabolic activation by authors	Results: With metabolic activation by authors	Concentration range, purity of test substance	GLP, Test guideline	RAR 04/2015	Comments BfR
Mañas et al., 2009a, ASB2012-11892	Yes	Liver Hep-2 (DNA damage)	DNA strand breaks, comet assay	+	NT	3 - 7.5 mM (507.2 - 1268 µg/mL) Purity: 96%	NR	p. 404, 436	$P < 0.01$ (507.2 µg/mL), dose–response relationship No indication of pH or osmolality control. Results not reported separately for replicate cultures.
Mladinic et al., 2009b, ASB2012-11906	Yes	Human lymphocytes (DNA damage)	DNA strand breaks, standard and hOGG1	+	+	0.5-580 µg/mL Purity: 98%	NR	p. 437	$P < 0.05$ (3.5 µg/mL) With the hOGG1 modified comet assay, + S9, the increase was significant ($P < 0.01$) only at the highest dose tested (580 µg/mL).

Reference	Evaluated by IARC	Test system (endpoint)	Test	Results: Without metabolic activation by authors	Results: With metabolic activation by authors	Concentration range, purity of test substance	GLP, Test guideline	RAR 04/2015	Comments BfR
			modified comet assay						No indication of pH or osmolality control. Results not reported separately for replicate cultures. Authors state that no clear dose-dependent effect was observed.
Alvarez-Moya et al., 2014, ASB2014-6902	Yes	Human lymphocytes (DNA damage)	DNA strand breaks, comet assay	+	NT	0.0007-0.7 mM (0.118- 118 µg/mL) Purity: 96%	NR	p. 404	$P \leq 0.01$ (0.0007 mM) No indication of pH or osmolality control. Results not reported separately for replicate cultures. Inconsistent and not clear dose dependent. Test was conducted with glyphosate isopropylamine .
Monroy et al., 2005, ASB2012-11910	Yes	Fibroblast GM 39 and Fibrosarcoma HT1080 (DNA damage)	DNA strand breaks, comet assay	+	NT	4.0-6.5 nM ($6.76 \cdot 10^{-4}$ – $1.1 \cdot 10^{-3}$ µg/mL, GM39 cells), 4.5-6.5 nM ($7.6 \cdot 10^{-4}$-$1.1 \cdot 10^{-3}$ µg/mL HT1080) cells) Purity: not given	NR	p. 403	Fibroblast: $P < 0.001$ (4 nM) Fibrosarcoma: $P < 0.001$ (4.75 nM) No indication of pH or osmolality control. No concurrent measurement of toxicity reported. Independent coding of slides for scoring not indicated for visually scored slides. Results not reported separately for replicate cultures. Concentrations seem very low.
Lueken et al., 2004, ASB2012-11886	Yes	Fibroblast GM 5757 (DNA damage)	DNA strand breaks, comet assay	(+)	NT	75 mM (12.7 mg/ml) Purity: 98.4%	NR	–	Not regarded as glyphosate was only tested together with H_2O_2.
Koller et al., 2012, ASB2014-7618	Yes	Buccal carcinoma TR146 (DNA damage)	DNA strand breaks, comet assay	+	NT	10-2000 µg/mL Purity: 95%	NR	p. 404	$P \leq 0.05$ (20 µg/mL) No indication of pH or osmolality control. Results not reported separately for replicate cultures. No clear dose-response effect.

Reference	Evaluated by IARC	Test system (endpoint)	Test	Results: Without metabolic activation by authors	Results: With metabolic activation by authors	Concentration range, purity of test substance	GLP, Test guideline	RAR 04/2015	Comments BfR
									Higher activity of formulation than pure a. s.
Bolognesi et al., 1997, Z59299	Yes	Human lymphocytes (Chromosomal damage)	Sister-chromatid exchange	+	NT	0.33 and 6 mg/mL Purity: 99.9%	NR	p. 385, 390, 429	$P < 0.05$ (1 mg/ml) The number of only two subjects to be included in the study appears too low for meaningful evaluation. Furthermore, the data from two experiments were pooled for the two donors and individual values were not given. The study is performed with methodological and reporting deficiencies (no positive controls included in *in vitro* SCE). Test guideline deleted by now.
Li and Long, 1988, TOX9500253	Yes	Rat, Fisher F334 Hepatocytes (DNA damage)	Unscheduled DNA synthesis	–	NT	$1.25 \cdot 10^{-5}$-$1.25 \cdot 10^{-1}$ mg/ml Purity: 98%	NR	–	Only between 5 and 20 cells counted. Test guideline deleted by now.
Rossberger, 1994, TOX9400697	No	UDS assay/ Primary rat hepatocytes/Sprague Dawley	Unscheduled DNA synthesis	–	NT	0.2-111.7 mM (33.8 µg/ml-18.9 mg/ml) Purity: > 98%	GLP, TG 482	p. 342	Instead of autoradiography or LSC procedures, incorporation of radioactivity into DNA was determined on basis of UV absorbance measurement.
Lioi et al., 1998, ASB2013-9836	Yes	Bovine Lymphocytes (Chromosomal damage)	Sister-chromatid exchange	+	NT	17-170 µM (2.9-29 µg/ml Purity: ≥ 98%	NR	p. 387	$P < 0.05$ (17 µM) Data is pooled for the three donors and individual values were not given. Increase of SCE not dose related in highest dose group. Test guideline deleted by now.
Akanuma, 1995, ASB2012-11477	No	B. subtilis H17, M45 (DNA	Rec assay	–	–	7.5-240 µg/disk Purity: 95.7%	GLP, U.S. EPA FIFRA	p. 342-344	Rec assay is not a standard method for this endpoint (DNA damage and repair).

Reference	Evaluated by IARC	Test system (endpoint)	Test	Results: Without metabolic activation by authors	Results: With metabolic activation by authors	Concentration range, purity of test substance	GLP, Test guideline	RAR 04/2015	Comments BfR
		damage/repair)							

Results: +, positive; -, negative; (+) or (-) positive/negative in a study with limited quality
hOGG1, human 8-hydroxyguanosine DNA-glycosylase; NR, not reported; NT, not tested; S9, 9000 × g supernatant; vs, versus

Table 4.2-3: Glyphosate; somatic cell mutagenicity tests in mammals, *in vivo*

Reference	In IARC mono-graph	Species, test, tissue	Test substance, purity, application route, dose levels, sampling time	Results by authors	GLP, Test guideline	Result details	Comments BfR	In RAR 04/2015
Oral application								
[redacted] 1991, TOX9552374	No	Mouse, Micronucleus test, bone marrow	Glyphosate, 98.6 % oral, 1x 0 or 5000 mg/kg bw, sampled after 24, 48 and 72 h	Negative	GLP, OECD 474 (1983)	*MN/2000 PCE [mean (range)]:* Control: 2.7 (1-4) 24h, 5000 mg/kg: 3.2 (1-5) 48h, 5000 mg/kg: 2.8 (1-6) 72h, 5000 mg/kg: 1.7 (0-4) PosControl: 48.2 (32-58)	5 animals per sex and sampling time. 2000 PCE scored/animal. PCE/NCE: no effect.	p. 358, 364
[redacted] 1993, TOX9551100	No	Mouse, Micronucleus test, bone marrow	Glyphosate, 96.8 % oral, 2x 0, 50, 500 or 5000 mg/kg bw (24 h interval), sampled 24 h after second dose	Negative	GLP, OECD 474 (1984)	*% MNPCE [mean (range)], male/female:* Control: 0.69 (0.1-1.6)/0.51 (0.2-1.0) 50 mg/kg: 0.84 (0.2-1.4)/0.28 (0.0-0.5) 500 mg/kg: 0.73 (0.4-1.6)/0.52 (0.2-1.3) 5000 mg/kg: 0.89 (0.7-1.1)/1.05*(0.4-1.6) PosControl: 2.33* (1.5-3.2)/2.39* (1.4-3.4) *p<0.05	5 animals per sex and dose (Control: 10/sex). 2000 PCE scored/animal. PCE/NCE: no effect (but PosControl). *The MN incidence in females at 5000 mg/kg is within the range of controls considering both sexes.*	p. 357 ff.
[redacted] 1994, TOX940032	No	Mouse, Chromosome aberration test,	Glyphosate, 96.8 % oral, 2 x 0-5000 mg/kg bw	Negative	GLP, OECD 475	*No. of aberrations per 250-250-500 metaphases (male/female/total)* Control: 12/10/22	5 animals per sex. 50 metaphases/animal examined. *Mitotic index (%)*	p. 358

Reference	In IARC mono-graph	Species, test, tissue	Test substance, purity, application route, dose levels, sampling time	Results by authors	GLP, Test guideline	Result details	Comments BfR	In RAR 04/2015
3		bone marrow	(24 h interval), sampled 24 h after second dose		(1984)	5000 mg/kg: 10/11/21 PosControl: 139*/155*/294* *p<0.05	*(male/female/total)* Control: 13.3/17.4/15.3 5000 mg/kg: 8.9*/9.5*/9.2* PosControl: 14.7/5.5*/10.1*	
[redacted] 1996, TOX2000-1996	No	Mouse, Micronucleus test, bone marrow	Glyphosate, 95.6 % oral, 1x 0 or 5000 mg/kg bw, sampled after 24 and 48 h	Negative	GLP, OECD 474 (1997)	*MN/1000 PCE (mean±SD), male/female:* 24h, Control: 1.6±0.8/1.4±0.7 24h, 5000 mg/kg: 2.1±1.6/2.1±2.5 24h, PosControl: 22.2±6.1*/23.3±4.9* 48h, Control: 1.7 ±1.3/0.7±0.6 48h, 5000 mg/kg: 2.1±1.9/0.8±0.8 *p<0.01	5 animals per sex and sampling time. 2000 PCE scored/animal. PCE/NCE: no effect.	p. 359, 370 ff.
[redacted], 2008, ASB2012-11483	No	Mouse, Micronucleus test, bone marrow	Glyphosate, 99.1 % oral, 1x 0, 500, 1000 or 2000 mg/kg bw, sampled after 24 h 1x 0 or 2000 mg/kg bw, sampled after 48 h	Negative	GLP, OECD 474 (1997)	*MN/2000 PCE [mean (range)]:* 24h, Control: 1.4 (0-3) 24h, 500 mg/kg: 1.6 (1-2) 24h, 1000 mg/kg: 1.6 (1-2) 24h, 2000 mg/kg: 1.4 (0-2) 24h, PosControl: 63.0 (44-92)* 48h, Control: 1.4 (0-3) 48h, 2000 mg/kg: 1.6 (0-3) *p<0.01	5 males per group and sampling time. 2000 PCE scored/animal. PCE/NCE: no effect. Historical control data (293 studies): *% MNPCE [mean±SD, (range)]: 0.084±0.031 (0.01 – 0.18)*	p. 359, 372 ff.
[redacted] 2012, ASB2014-9277	No	Mouse, Micronucleus test, bone marrow	Glyphosate, 98.9 % oral, 2x 0 or 2000 mg/kg bw (24 h interval), sampled 24 h after second dose	Negative	GLP, OECD 474 (1997)	*% MNPCE [mean (range)]:* Control: 0.033 (0-0.05) 2000 mg/kg: 0.0 (0-0) PosControl: 2.49* (1.1-3.7) *p<0.01	6 males per group. 2000 PCE scored/animal. PCE/NCE: no effect at 2000 mg/kg, increased in PosControl. Historical control data (of 73 studies) *% MNPCE [mean±SD (range)]: 0.02±0.02 (0.0-0.07)*	p. 359, 374 ff.
[redacted] 2012, ASB2014-	No	Mouse, Micronucleus	Glyphosate, 96.3 % oral,	Negative	GLP, OECD	*MN/2000 PCE [mean±SD, (range)]:* 24h, Control: 3.2±3.6 (0-8)	7 males per group (Control and PosControl: 5 males each).	p. 359. 375 ff.

Reference	In IARC mono-graph	Species, test, tissue	Test substance, purity, application route, dose levels, sampling time	Results by authors	GLP, Test guideline	Result details	Comments BfR	In RAR 04/2015
9333		test, bone marrow	1x 0 or 2000 mg/kg bw, sampled after 24 and 48 h		474 (1997)	24h, 2000 mg/kg: 2.3±0.5 (2-3) 24h, PosControl: 40.2±18.2* (16-67) 48h, Control: 1.4±1.1 (0-3) 48h, 2000 mg/kg: 1.1±1.3 (0-3) *p<0.01	2000 PCE scored/animal. PCE/NCE: no effect. Historical control data (of 219 studies) *% MNPCE [mean±SD (range of mean group value)]:* 0.108±0.039 (0.01-0.25)	
[redacted] 2009, ASB2012-11479	No	Rat, Micronucleus test, bone marrow	Glyphosate, 98.8 % oral, 1x 0, 500, 1000 or 2000 mg/kg bw, sampled after 24 and 48 h	Negative	GLP, OECD 474 (1997)	*MN/2000 PCE (mean±SD), male/female:* 24h, Control: 1.6±1.1/1.8±0.4 24h, 500 mg/kg: 1.0±1.2/1.2±1.3 24h, 1000 mg/kg: 0.8±0.4/1.6±0.9 24h, 2000 mg/kg: 1.2±0.8/0.8±0.8 24h, PosControl: 30.2±10.5*/24.0±4.9* 48h, Control: 2.0 ±1.9/2.2 ±1.3 48h, 2000 mg/kg: 1.6±0.9/0.8±0.8 *p<0.05	5 animals per sex and dose and sampling time. 2000 PCE scored/animal. PCE/NCE: no effect. Historical control data (24, 48 and 72 h samplings combined): *MN/1000 PCE [mean and (range):* Males: 1.97 (0.4 – 5.7) Females: 1.86 (0.4 – 4.7)	p. 359. 376 ff.
i.p. application								
Li and Long, 1988, TOX9500253 [redacted] 1983, TOX9552369	Yes	Rat, Chromosome aberration test, bone marrow	Glyphosate, 98 % i.p., 1x 0 or 1000 mg/kg bw, sampled after 6, 12 and 24 h	Negative	No GLP, no reference to TG	*% aberrant cells (mean), male/female/total:* 6h, Control: 1.3/2.7/2.0 6h, 1000 mg/kg: 2.3/3.0/2.7 12h, Control: 1.0/1.5/1.2 12h, 1000 mg/kg: 2.0/2.5/2.3 24h, Control: 1.3/2.3/1.8 24h, 1000 mg/kg: 1.0/3.7/2.6 PosControl: 42.2*/23.8*/40.8* * p < 0.05	Consistent with OECD 475 (1984): 6 animals per sex and sampling time. Ca 50 metaphases/animal examined. Slides were coded and scored "blind". Original study reported in RAR as Li, 1983 (TOX9552375).	p. 358, 383
Rank et al., 1993,	Yes	Mouse, Micronucleus	Glyphosate isopropylamine salt,	Negative	No GLP, no	*% MNPCE (mean±SD):* 24h, Control: 0.27±0.11	Consistent with OECD 474 (1983):	p. 385, 388f.

Reference	In IARC mono-graph	Species, test, tissue	Test substance, purity, application route, dose levels, sampling time	Results by authors	GLP, Test guideline	Result details	Comments BfR	In RAR 04/2015
Z82234		test, bone marrow	purity not stated i.p., 1x 0, 100, 150 or 200 mg/kg bw sampled after 24 and 48 h		reference to TG	24h, 100 mg/kg: 0.20±0.13 24h, 150 mg/kg: 0.2±0.13 24h, 200 mg/kg: 0.25±0.10 24h, PosControl: 2.53±0.59 48h, 150 mg/kg: 0.13±0.09 48h, 200 mg/kg: 0.12±0.09	Mostly 5 animals per sex and dose and sampling time. 1000 PCE scored/animal. Slides were scored randomly. PCE/NCE: no effect.	
Bolognesi et al., 1997, Z59299	Yes	Mouse, Micronucleus test, bone marrow	Glyphosate, 99.9 % i.p., 2x 150 mg/kg bw (24 h interval), sampled 6 or 24 h after second dose	Positive	No GLP, no reference to TG	*MN/1000 PCE (mean±SD):* Control: 0.75±0.46 6h, 2x 150 mg/kg: 1.4±0.9 24h, 2x 150 mg/kg: 2.4±1.5* 24h, PosControl: 80.0±8.5* * $p < 0.05$	6 males in Control and PosControl group. 3000 PCE scored/animal. PCE/NCE: 0.73±0.06 in Control, 0.6±0.05 at 6h, 0.5±0.2 at 24h. *Deviations from OECD 474 (1997):* *Only 3(4) males examined per sampling time.* *Sampling time of Control not stated.* *Independent coding of slides not stated.*	p. 385, 389
Mañas et al., 2009a, ASB2012-11892	Yes	Mouse, Micronucleus test, bone marrow	Glyphosate, 96 % i.p., 2x 50, 100 or 200 mg/kg bw (24 h interval), sampled 24 h after second dose	Positive	No GLP, OECD 474 (1997)	*MN/1000* ***Erythrocytes*** *(mean±SD):* Control: 3.8 ±0.8 2x 50 mg/kg: 3.7±0.5 2x 100 mg/kg: 4.2±0.5 2x 200 mg/kg: 13.0±3.5* PosControl: 19.2±3.9* * $P < 0.01$	5 animals per dose. PCE/NCE no effect. *Deviations from OECD 474 (1997):* *Sex of animals not reported.* *1000* ***erythrocytes*** *(not* ***PCE****) scored/animal.* *Independent coding of slides not stated.*	p. 402, 410
[redacted] 1999,	No	Mouse, Micronucleus test, bone marrow	Glyphosate, 95 % i.p., 2x 0, 187.5, 375 or 562.5 mg/kg bw (24 h	Negative	GLP, internal SOP	*MN/1000 PCE [mean (range)], male/female:* Control: 0.4 (0-1)/0.8 (0-2) 188 mg/kg: 0.0 (0)/0.6 (0-3)	5 animals per sex and dose. 1000 PCE and 1000 NCE scored per animal. PCE/NCE: no effect (but	p. 358, 367 ff.

Reference	In IARC mono-graph	Species, test, tissue	Test substance, purity, application route, dose levels, sampling time	Results by authors	GLP, Test guideline	Result details	Comments BfR	In RAR 04/2015
ASB2012-11482			interval), sampled 24 h after second dose			375 mg/kg: 0.6 (0-3)/0.6 (0-2) 563 mg/kg: 0.4 (0-2)/0.6 (0-1) PosControl: 4.8* (4-7)/4.8* (2-12) *p<0.05	PosControl). MN/1000 NCE: no effect (but PosControl). *$LD50_{i.p.}$=750 mg/kg*	
[redacted] 2006, ASB2012-11478	No	Mouse, Micronucleus test, bone marrow	Glyphosate, 95.7 % i.p., 1x 0, 150, 300 or 600 mg/kg bw, sampled after 24 and 48 h	Negative	GLP, OECD 474 (1997)	*% MNPCE [mean±SD, (range)]:* 24h, Control: 0.06±0.06 (0.0-0.15) 24h, 150 mg/kg: 0.07±0.04 (0.0-0.10) 24h, 300 mg/kg: 0.06±0.05 (0.0-0.15) 24h, 600 mg/kg: 0.19±0.07* (0.05-0.25) 24h, PosControl: 3.03±0.49*** (2.20-3.35) 48h, Control: 0.1±0.12 (0.0-0.35) 48h, 600 mg/kg: 0.09±0.11 (0.0-0.30) *p<0.05, ***p<0.001	7 males per group and sampling time. 2000 PCE scored/animal. *Pre-test: Mortality at 800-1000 mg/kg, clinical signs at 150 mg/kg and above.* PCE/NCE: reduced at 600 mg/kg (not in PosControl). Stat. sign. increase in MNPCE at 600 mg/kg (24 h), within historical control. *Control data from 60 groups (24h):* *0.0-0.9 MN/1000 PCE: 40x (67%)* *1.0-1.4 MN/1000 PCE: 14x (23%)* *1.5-2.0 MN/1000 PCE: 3x (5%)* *2.1-2.5 MN/1000 PCE: 3x (5%)*	p. 358, 359 ff.
[redacted] 2008, ASB2012-11481	No	Mouse, Micronucleus test, bone marrow	Glyphosate, 98 % i.p., 2x 0, 15.6, 31.3 or 62.5 mg/kg bw (24 h interval), sampled 24 h after second dose	Negative	GLP, OECD 474 (1997)	*MN/2000 PCE [mean (range)], male/female:* Control: 0.0 (0)/0.0 (0) 15.6 mg/kg: 0.0 (0)/0.0 (0) 31.3 mg/kg: 0.0 (0-1)/0.0 (0) 62.5 mg/kg: 0.6 (0-3)/0.0 (0) PosControl: 23.0* (8-30)/12.2* (7-26) *p<0.01	5 animals per sex and dose. 2000 PCE scored/animal. *Pre-test: Mortality at 500-1000 mg/kg, decreased PCE/NCE at 250 mg/kg and above.* PCE/NCE no effect. Historical control: ca. 3 MN/1000 PCE	p. 358. 364 ff.
[redacted] 2010,	No	Mouse,	Glyphosate, 98 %	Negative	GLP,	*MN/2000 PCE [mean (range)],*	5 animals per sex and dose.	p. 358.

Reference	In IARC mono-graph	Species, test, tissue	Test substance, purity, application route, dose levels, sampling time	Results by authors	GLP, Test guideline	Result details	Comments BfR	In RAR 04/2015
ASB2014-9284		Micronucleus test, bone marrow	i.p., 2x 0, 125, 250 or 375 mg/kg bw (24 h interval), sampled 24 h after second dose		OECD 474 (1997)	*male/female:* Control: 0.4 (0-2)/0.4 (0-1) 125 mg/kg: 0.2 (0-1)/0.0 (0-1) 250 mg/kg: 0.0 (0)/0.0 (0) 375 mg/kg: 0.2 (0-1)/0.0 (0-1) PosControl: 8.0* (5-11)/6.4* (5-9) *p<0.01	2000 PCE scored/animal. *Clinical signs at 125 mg/kg and above.* PCE/NCE: slight increase at 250 and 375 mg/kg and in PosControl. Historical control: ca. 3 MN/1000 PCE	364 ff.

NCE, normochromatic erythrocytes; MN, micronucleus; MNPCE%, percent of micronucleated polychromatic erythrocytes; PCE, polychromatic erythrocytes; SD, standard deviation

Table 4.2-4: Glyphosate; further tests on DNA adducts and DNA strand breaks in mammals, *in vivo*

Reference	In IARC monograph	Species, test, tissue	Test substance, purity, route, dose levels, sampling time	Results by authors	GLP, Test guideline	Result details	Comments BfR	In RAR 04/2015
Bolognesi et al., 1997, Z59299	Yes	Mouse DNA adduct (8-OHdG by LC/UV), liver	Analytical grade glyphosate (purity 99.9 %) i.p.; 1 × 300 mg/kg bw; sampled after 8 and 24 h	- (4 h) + (24 h)	No GLP, no reference to TG	(Estimated from figure in report) Control: approx. 0.6 moles 8-OHdG/10^5 moles dG 4 h: approx. 0.9 moles 8-OHdG/10^5 moles dG 24 h: approx. 3.6 moles 8-OHdG/10^5 moles dG*	3 male animals per group, at least 3 independent repeat experiments	p. 386
Bolognesi et al., 1997, Z59299	Yes	Mouse DNA adduct (8-OHdG by LC/UV), kidney	Analytical grade glyphosate (purity 99.9 %) i.p.; 1 × 300 mg/kg bw; sampled after 8 and 24 h	– (4 & 24 h)	No GLP, no reference to TG	(Estimated from figure in report) Control: approx. 0.6 moles 8-OHdG/10^5 moles dG 4 h: approx. 0.5 moles 8-OHdG/10^5 moles dG 24 h: approx. 0.4 moles 8-OHdG/10^5 moles dG*	3 male animals per group, at least 3 independent repeat experiments	p. 386

Reference	In IARC monograph	Species, test, tissue	Test substance, purity, route, dose levels, sampling time	Results by authors	GLP, Test guideline	Result details	Comments BfR	In RAR 04/2015
Peluso et al., 1998, TOX1999-318	Yes	Mouse DNA adduct (^{32}P-DNA post labelling), kidney	Glyphosate isopropylammonium salt i.p.; 1 × 0, 130 or 270 mg/kg bw; sampled after 24 h	–	No GLP, no reference to TG	Not reported	6 animals in control group, 6 in low dose group and 3 in high dose group, sex of animals not clear	p. 386
Peluso et al., 1998, TOX1999-318	Yes	Mouse DNA adduct (^{32}P-DNA post labelling), liver	Glyphosate isopropylammonium salt i.p.; 1 × 0, 130 or 270 mg/kg bw; sampled after 24 h	–	No GLP, no reference to TG	Not reported	6 animals in control group, 6 in low dose group and 3 in high dose group, sex of animals not clear	p. 386
Bolognesi et al., 1997, Z59299	Yes	Mouse DNA strand breaks (alkaline elution assay), liver	Analytical grade glyphosate (purity 99.9 %) i.p.; 1 × 300 mg/kg bw; sampled after 4 and 24 h	+ (4 h) - (24 h)	No GLP, no reference to TG	(Estimated from figure in report) Control: approx. 15 *10^3/mL 4 h: approx. 47 *10^3/mL* 24 h: approx. 20 *10^3/mL	3 male animals per group, at least 4 independent repeat experiments	p. 385
Bolognesi et al., 1997, Z59299	Yes	Mouse DNA strand breaks (alkaline elution assay), kidney	Analytical grade glyphosate (purity 99.9 %) i.p.; 1 × 300 mg/kg bw; sampled after 4 and 24 h	+ (4 h) - (24 h)	No GLP, no reference to TG	(Estimated from figure in report) Control: approx. 17 *10^3/mL 4 h: approx. 55 *10^3/mL* 24 h: approx. 25 *10^3/mL	3 male animals per group, at least 4 independent repeat experiments	p. 385
Manas et al., 2013, ASB2014-6909	No	Mouse comet assay, blood cells	Glyphosate (96%) Drinking water, 14 days, 0, 40 or 400 mg/kg bw per day; sampled after treatment period	+	No GLP, no reference to TG	Tail moment (mean ± SEM): Control: 2.98±1.08 40 mg/kg bw per day: 8.54***±7.82 400 mg/kg bw per day: 9.06***±5.15	6 animals per group sex of animals not clear	p. 404

Reference	In IARC monograph	Species, test, tissue	Test substance, purity, route, dose levels, sampling time	Results by authors	GLP, Test guideline	Result details	Comments BfR	In RAR 04/2015
Manas et al., 2013, ASB2014-6909	N	Mouse comet assay, liver cells	Glyphosate (96%) Drinking water, 14 days, 0, 40 or 400 mg/kg bw per day; sampled after treatment period	+	No GLP, no reference to TG	Tail moment (mean ± SEM): Control: 7.14±3.41 40 mg/kg bw per day: 7.92*±3.99 400 mg/kg bw per day: 20.59***±15.47	6 animals per group sex of animals not clear	p. 404

8-OHdG, 8-hydroxy-2' -deoxyguanosine; dG, deoxyguanosine; SEM, standard error of the mean; SCGE, single cell gel electrophoresis

Table 4.2-5: Glyphosate; germ cell mutagenicity tests in mammals, *in vivo*

Reference	In IARC mono-graph	Species, test, tissue	Test substance, purity, application route, dose levels, mating period	Results by authors	GLP, Test guideline	Result details	Comments BfR	In RAR 04/2015
EPA, 1980, ASB2015-8547 ([redacted] 1980, TOX9552377	Yes	Mouse, Dominant lethal test	Glyphosate, 98.7 % oral, 1x 0, 200, 800 or 2000 mg/kg bw 8 successive one-week mating periods (1 male/2 females)	Negative	GLP, no reference to TG	No increase in post-implantation loss in treated groups. PosControl: stat. significant increase in post-implantation loss.	Only 10 males per group. Post-implantation loss evaluated after mating of non-treated females with glyphosate-treated male mice. Original study reported in RAR as Wrenn et al., (1980, TOX9552377).	p. 378
[redacted] 1992, TOX9551102	No	Rat, Dominant lethal test	Glyphosate, 96.8 % oral, 1x 0, 200, 800 or 2000 mg/kg bw 10 successive one-week mating periods (1 male/1 female)	Negative	GLP, OECD 478 (1984)	No increase in post-implantation loss in treated groups. PosControl: stat. significant increase in post-implantation loss.	30 males per group (Control: 10 males, PosControl: 2 x 5 males). Post-implantation loss evaluated after mating of non-treated females with glyphosate-treated male mice.	p. 378

Table 4.2-6: AMPA; mutagenicity and genotoxicity tests, *in vitro*

Reference	Evaluated by IARC	Test system (endpoint)	Test substance	Results: Without metabolic activation by authors	Results: With metabolic activation by authors	Concentration range	GLP, Test guideline	RAR 04/2015	Comments BfR
Callander, 1988, TOX9500043	No	*Salmonella typhimurium* TA1535, TA1537, TA1538, TA98, TA100 (reverse mutation)	AMPA, >99%	Negative	Negative	1.6-5000µg/plate	GLP, OECD 471 (1983)	p. 735	No evidence of genotoxicity. The slight increase in revertant numbers in one strain in the first experiment was rather weak and was sufficiently contravened by subsequent trials in which the test material proved clearly negative.
[redacted] 1993, TOX9300378	No	Salmonella typhimurium TA1535, TA1537, TA98, TA100 (reverse mutation)	AMPA, 99.2%	Negative	Negative	310-5000µg/plate	GLP, OECD 471 (1983)	p. 95, 727	
[redacted] 1993; TOX9300380	No	L5178Y mouse lymphoma cells, gene mutation, TK locus	AMPA, 99.2%	Negative	Negative	310-5000µg/mL	GLP, OECD 476 (1983)	p. 727	
Mañas et al., 2009b, ASB2012-11891	Yes	Human lymphocytes, Chromosomal aberrations	Analytical grade AMPA (99%).	Positive	NT	1.8 mM [200 µg/mL] $P < 0.05$	No GLP, no reference to TG		Methodological deficiencies (only 2 dose levels used).
Roustan et al., 2014, ASB2014-	Yes	CHO cells, Micronucleus formation	AMPA, purity not stated	Positive	Positive	-S9: 0.005-0.1 µg/ml +S9: 0.1-5 µg/ml	No GLP, no reference	p. 423	-S9: ≥0.01 µg/mL $P < 0.05$ +S9: ≥0.1 µg/mL $P < 0.01$

Reference	Evaluated by IARC	Test system (endpoint)	Test substance	Results: Without metabolic activation by authors	Results: With metabolic activation by authors	Concentration range	GLP, Test guideline	RAR 04/2015	Comments BfR
8086							to TG		
Bakke, 1991, TOX9552409	No	Primary rat hepatocytes (Fischer F334) (UDS test)	AMPA, 94.38%	Negative	Negative	5-5000µg/mL	GLP, no reference to TG	p. 728, 962 steht nur in der Über-sichts-tabelle	Negative up to 2500 µg/mL, meaningful evaluation of higher concentrations not possible due to cytotoxicity.
Nesslany, 2002, ASB2012-11508	No	Primary rat hepatocytes (Fischer) (UDS test)	AMPA, 99.9%	Negative	Negative	0.625 – 10 mM	GLP, OECD 482 (1986)	p. 728, 743	Negative under the condition of the experiment
Mañas et al., 2009b, ASB2012-11891	Yes	Liver Hep-2, DNA strand breaks, comet assay	Analytical grade AMPA (99%).	Positive	NT	Range 2.5-7.5 µM $P < 0.05$ at 4.5 mM [500 µg/mL]; $P < 0.01$ at up to 7.5 mM Dose–response relationship ($r \geq 0.90$; $P < 0.05$)	No GLP, no reference to TG	p. 422, 434	

Results: +, positive; -, negative
NT, not tested; NR, not reported ; S9, 9000 × g supernatant; Hprt, hypoxanthine guanine phosphoribosyl transferase gene;

Table 4.2-7: AMPA; mutagenicity and genotoxicity tests in mammals, *in vivo*

Reference	In IARC monograph	Species, test, tissue	Test substance, purity, route, dose levels, sampling time	Results by authors	GLP, Test guideline	Result details	Comments BfR	In RAR 04/2015
Manas et al.,	Yes	Mouse	Analytical grade	Positive	No GLP,	MNE/1000 analysed cells:	5 animals per group	p. 422, 434

Reference	In IARC monograph	Species, test, tissue	Test substance, purity, route, dose levels, sampling time	Results by authors	GLP, Test guideline	Result details	Comments BfR	In RAR 04/2015
2009b, ASB2012-11891		micronucleus test, bone marrow	AMPA (purity 99 %) i.p.; 2 × 100 or 200 mg/kg bw per day; sampled 24 h after second injection		OECD 474 (1997)	Control: 3.8 ±1.8 100 mg/kg bw: 10.0**±1.9 200 mg/kg bw: 10.4**±3.3 PosControl: 19.2**±3.9 PCE/NCE: Control: 0.85±0.17 100 mg/kg bw: 1.14±0.22 200 mg/kg bw: 1.07±0.04 PosControl: 0.80.+-0.20	Sex of animals not reported. *1000 **erythrocytes** (not **PCE**) scored/animal.* *Independent coding of slides not stated.*	
[redacted] 1993, TOX9300379	No	Mouse micronucleus test, bone marrow	AMPA (99.2 %) oral; 1x 5000 mg/kg bw; sampled after 24, 48 and 72 h	Negative	GLP, OECD 474 (1983)	MN/1000 PCE [mean (range)] Control: 0.50 (0-1) 24 h, 5000 mg/kg: 0.20 (0-1) 48 h, 5000 mg/kg: 0.40 (0-1) 72 h, 5000 mg/kg:: 0.60 (0-1) PosControl: 13.1** (10-19)	5 males and 5 females per group. 1000 PCE scored/animal. 1000 NCE scored/animal	728 (mentioned but not reported in detail)
[redacted] 1993, TOX9552413 Study also mentioned by Williams et al., 2000, ASB2012-12053	No	Mouse micronucleus test, bone marrow	AMPA (94.38 %) i.p.; 1x 100, 500, 1000 mg/kg bw; sampled 24, 48 and 72 h	Negative	GLP, OECD 474 (1983)	Mean MN/1000 PCE 24 h, males/females: Control: 0.2±0.4/1.0±1.4 100 mg/kg bw: 0.2±0.4/0.8±0.8 500 mg/kg bw: 0.1±0.3/2.0±2.9 1000 mg/kg bw: 0.8±1.3/0.8±0.8 PosControl: 18.3**±10.9/12.0*±12.3 48 h, males/females: Control: 0.6±1.3/0.4±0.9 100 mg/kg bw: 0.0±0.0/0.2±0.4 500 mg/kg bw: 0.6±0.9/0.2±0.4 1000 mg/kg bw: 0.2±0.4/0.0±0.0 72 h, males/females: Control: 0.2±0.4/0.0±0.0 100 mg/kg bw: 0.0±0.0/1.6*±1.1	5 males and 5 females per group. 1000 PCE scored/animal. *Pre-test: Mortality at 606 mg/kg and above*	728 (mentioned but not reported in detail)

Reference	In IARC monograph	Species, test, tissue	Test substance, purity, route, dose levels, sampling time	Results by authors	GLP, Test guideline	Result details	Comments BfR	In RAR 04/2015
						500 mg/kg bw: 0.0±0.0/0.8±0.8 1000 mg/kg bw: 0.0±0.0/0.4±0.9		
Manas et al., 2013, ASB2014-6909	N	Mouse comet assay, blood cells	AMPA (99%) Drinking water, 14 days, 0 or 100 mg/kg bw per day; sampled after treatment period	Positive	No GLP, no reference to TG	Tail moment (mean ± SEM): *Blood cells* Control: 2.98 ± 1.08 100 mg/kg bw per day: 8.45*** ± 6.43 Liver cells Control: 7.14 ± 3.41 100 mg/kg bw per day: 14.99*** ± 9.09	6 animals per group sex of animals not clear	p. 404

MN, micronucleus; MNE, micronucleated erythrocytes; NCE, normochromatic erythrocytes; PCE, polychromatic erythrocytes; SEM, standard error of the mean

4.2.2 Receptor-mediated mechanisms

In section 4.4.2 of the IARC monograph 13 studies are reported. The studies including comments of RMS are summarized in Table 4.2-8.

4 studies compared endocrine disrupting activity of glyphosate and glyphosate-based formulations (Gasnier et al., 2009, ASB2009-7384; Richard et al., 2005, ASB2009-9024; Benachour et al., 2007, ASB2009-9018 and Walsh et al., 2000, ASB2012-12046). The results demonstrate that glyphosate-based formulations have a higher sex hormone disrupting activity than the active substance glyphosate.

Other studies used only a formulation. Based on the results no conclusion on the active substance is possible.

2 studies investigated endocrine disrupting potential of pesticides in general and did not report results on glyphosate.

Based on the study of Thongprakaisang et al. (2013, ASB2013-11991) it was concluded that proliferative effects of glyphosate on T4/D cells would be mediated by oestrogen receptors. However the results of all animal studies and of epidemiological studies demonstrated that glyphosate and glyphosate-based formulations did not cause breast cancer in animals and humans.

Glyphosate was included into the U.S. EPA Endocrine Disruptor Screening Program's (EDSP). Levine et al. (2012, ASB2014-9609) published a short summary of the results. They concluded that, based on the Tier 1 assays that had been performed at different independent laboratories and taking into account the 'higher tier' regulatory safety studies glyphosate might not be considered an endocrine disrupter. Later on, Bailey et al. (2013, ASB2013-3464) summarized results of the male and female pubertal assays in which glyphosate did not exhibit evidence of endocrine disruption.

Table 4.2-8: Discussion of studies in section 4.2.2 Receptor mediated mechanisms of the IARC monograph

Study (Author/year)	Subject	Evaluation by IARC	Comment RMS on IARC evaluation	Study reported in RAR Draft April 2015	Final conclusion of RMS, considering IARC evaluation
Thongprakaisang et al., 2013, ASB2013-11991	Glyphosate effects on human breast cell cancer growth	The findings suggested that the proliferative effects of glyphosate on T4/D cells are mediated by oestrogen receptors.	Agreement with the reported results.	Yes, page 672	It must be emphasised that no increase in mammary tumours was reported in any of the numerous long-term studies in rats or mice and no increased risk of mammary tumours was found in the epidemiological studies.
Gasnier et al., 2009, ASB2009-7384	Toxicity and endocrine disrupting activity of glyphosate in human cell lines	In human hepatocarcinoma HepG2 cells, four glyphosate-based formulations had a marked effect on the activity and transcription of aromatase, while glyphosate alone differed from controls, but not significantly so. Additionally, although four glyphosate-based formulations dramatically reduced the transcription of ERα and ERβ in ERE-transfected HepG2 cells, glyphosate alone had no significant effect. A stronger effect of the formulations was also reported for the effects on androgen-receptor transcription in a breast cell line.	Agreement with the reported results. The study confirms the clearly higher activity of formulations than of the active substance alone.	Yes, page 671, 686	The study confirms the higher activity of formulations (Roundup) than of the active substance alone. This important difference was already highlighted in the first DAR and also in the RAR.
Richard et al., 2005, ASB2009-9024	Effects of glyphosate and Roundup on human placental cells and aromatase	A glyphosate-based formulation caused decreased aromatase activity in human placental cells. Glyphosate alone was without effect.	Agreement with the reported results. The authors Richard et al., 2005 conclude that endocrine and toxic effects of Roundup, not just glyphosate, can be observed in	Yes, page 328, 671, 676 and 682	The study confirms the clearly higher activity of formulations (Roundup) than of

Study (Author/year)	Subject	Evaluation by IARC	Comment RMS on IARC evaluation	Study reported in RAR Draft April 2015	Final conclusion of RMS, considering IARC evaluation
			mammals.		the active substance alone. This important difference was already highlighted in the first DAR and also in the RAR.
Benachour et al., 2007, ASB2009-9018	Time- and dose-dependent effects of Roundup on human embryonic and placental cells	Glyphosate, at non-overtly toxic concentrations, decreased aromatase activity in fresh human placental microsomes and transformed human embryonic kidney cells transfected with human aromatase cDNA. A glyphosate-based formulation, at non-overtly toxic concentrations, had the same effect. The formulation was more active at equivalent doses than glyphosate alone.	The study confirms the higher activity of formulations (Roundup) than of the active substance alone.	Yes, pages 671, 678 and 683-684	The study confirms the higher activity of formulations (Roundup) than of the active substance alone. This important difference was already highlighted in the first DAR and also in the RAR.
Kojima et al., 2004, ASB2010-14389	Estrogen and androgen activities of pesticides	In human androgen receptor and ERα and ERβ reporter gene assays using the Chinese hamster ovary cell line (CHO-K1), glyphosate had neither agonist nor antagonist activity.	Agreement	No	Several of the 200 tested pesticides were found to have endocrine-disrupting potential; however, no activity of glyphosate was reported.
Kojima et al., 2010, ASB2015-7815	Endocrine disrupting potential of pesticides	In human androgen receptor and ERα and ERβ reporter gene assays using the Chinese hamster ovary cell line (CHO-K1), glyphosate had neither agonist nor antagonist activity.	Agreement	No	Several of the 200 tested pesticides were found to have endocrine-disrupting potential; however, no activity of glyphosate was

Study (Author/year)	Subject	Evaluation by IARC	Comment RMS on IARC evaluation	Study reported in RAR Draft April 2015	Final conclusion of RMS, considering IARC evaluation
					reported.
Walsh et al., 2000, ASB2012-12046	Inhibition of steroidogenesis by roundup	A glyphosate-based formulation markedly reduced progesterone production in mouse leydig cell tumour cells. The inhibition was dose-dependent. The formulation also disrupted steroidgenic acute regulatory protein expression. Glyphosate alone did not affect steroidogenesis.	Agreement	Yes, pages 327, 328, 332, 677, 678	The study confirms the clearly higher activity of formulations (Roundup) than of the active substance alone. No effects of glyphosate alone have been observed. This important difference was already highlighted in the first DAR and also in the RAR.
Forgacs et al., 2012, ASB2012-11621	Effects of glyphosate and further chemicals on steroidogenesis in a novel murine Leydig cell model	Glyphosate had no effect on testosterone production in a novel murine Leydig cell line. Glyphosate did not modulate the effect of recombinant human chorionic gonadotropin.	Agreement	Yes, page 677	No effects of glyphosate on steroidogenesis.
Omran and Salama, 2013, ASB2014-7614	Endocrine disrupting effects of glyphosate and atrazine in snails.	A glyphosate-based formulation reduced levels of testosterone in gonadal tissue of snails and induced degenerative changes in the ovotestis. CYP450 was increased.	Agreement with the reported results. Only a formulation was tested, therefore, no conclusion on the active substance glyphosate alone is possible.	Yes, page 673	Only a formulation was tested, therefore, no conclusion on the active substance glyphosate alone is possible.
Xie et al., 2005, ASB2012-12056	Estrogenic activities of herbicides and surfactants	Glyphosate did not increase plasma vittelogenin levels in juvenile rainbow trout.	Agreement	Yes, page 332,	No estrogenic activity of glyphosate
Vainio et al., 1983, Z31881	Hypolipidaemia and peroxisome proliferation induced by pesticides	Glyphosate had no effect on formation of peroxisomes or the activity of hepatic carnitine acetyltransferase and catalase, and did not	Agreement	No, study published before 2000	Glyphosate does not have peroxisome proliferator activated

Study (Author/year)	Subject	Evaluation by IARC	Comment RMS on IARC evaluation	Study reported in RAR Draft April 2015	Final conclusion of RMS, considering IARC evaluation
		cause hypolipidaemia, suggesting that glyphosate does not have peroxisome proliferator activated receptor activity.			receptor activity.
Takeuchi et al., 2008, ASB2013-6443	*In vitro* screening for aryl hydrocarbon receptor agonistic activity in 200 pesticides.	Glyphosate was not an agonist for the aryl hydrocarbon receptor in mouse hepatoma Hepa1c17 cells transfected with a reporter plasmid containing copies of dioxin-responsive element.	Agreement	No	No effect of glyphosate
Paganelli et al., 2010, ASB2010-11410	Teratogenic effects of glyphosate-based herbicides by impairing retinoic acid signalling.	Retinoic acid activity in tadpoles exposed to a glyphosate based formulation was measured. Retinoic activity was increased by the formulation, and a retinoic acid antagonist blocked the effect.	The formulation Roundup classic was used in this study. Therefore, no conclusion on the active substance glyphosate alone is possible.	Yes, page 671, 675, 676, 680	The formulation Roundup classic was used in this study. Therefore, no conclusion on the active substance glyphosate alone is possible.

4.2.3 Oxidative stress, inflammation, and immunosuppression

4.2.3.1 Oxidative stress

Human cells *in vitro*, data on glyphosate:

Gehin et al. (2005, ASB2012-11826) investigated effects of pre-incubation of HaCaT with 100 or 200 µM Vit C, Vit E or both for 0, 24 or 48 h on glyphosate cytotoxicity at doses of up to 25 mM for 24 h. IC_{50} for glyphosate alone, pre-incubated with Vit C, Vit E or both in ranges from 20.9 - 23.9 mM, 20.6 - 23.9 mM, 21.6 - 23.6 mM or 19 - 21.3 mM, respectively. No information is available on the purity of the tested substance.

Elie-Caille et al. (2010, ASB2012-11610) investigated the formation of reactive oxygen species (ROS) after treatment of HaCaT cells at the IC_{50} using 2′,7′-dichlorodihydrofluorescein diacetate. Treatment with 50 mM glyphosate (purity 95%) for 30 min resulted in "*overproduction of H_2O_2*" determined as "*a thicker and more intense fluorescent area*". No quantitative estimate is available.

Kwiatkowska et al. (2014, ASB2014-9603) examined the production of ROS in human erythrocytes (without metabolic activation) using dihydrorhodamine 123. Cells were exposed to glyphosate concentrations of 0.01 - 5.0 mM for 1 h. Positive results are observed from 0.25 mM up to the highest tested concentration that induces cytotoxic effects (increase in percent of haemolysis). No information is available on the purity of the tested substance.

Mladinic et al. (2009, ASB2012-11906) investigated possible effects of *in vitro* exposure of glyphosate on oxidative DNA damage and on oxidative stress parameters (total antioxidant capacity and lipid peroxidation) in human lymphocytes with and without metabolic activation. Cells were exposed to concentrations of 0.5 - 580 µg/mL (up to ca. 3.4 mM). Regarding the induction of cytotoxic effects significantly increased early apoptosis and necrosis at the highest tested concentration of 580 µg/mL were observed. In a modified comet assay oxidative DNA damage was observed without metabolic activation only at a concentration of 3.5 µg/mL whereas an obviously more relevant effect was observed with metabolic activation at the highest tested concentration of 580 µg/mL. Both, determinations of total antioxidant capacity (TAC) as well as the lipid oxidation (determination by level of thiobarbituric reactive substances) indicate an increase of oxidative stress with and without metabolic activation at the highest tested concentrations of 580 µg/mL.

Chaufan et al. (2014, ASB2014-7616, ASB2014-9314) evaluated the effect of glyphosate (purity: 95%) on oxidative stress in HepG2 cells with 2',7'dichlorohydrofluorescin diacetate. Treatment of the cells with 900 mg/mL glyphosate for 24 h does not lead to an increase in ROS. Concentrations up to 1000 mg/mL did not affect the cell viability (MTT test).

Human cells *in vitro*, data on AMPA:

Kwiatkowska et al. (2014, ASB2014-9603) examined the production of ROS in human erythrocytes (without metabolic activation) with dihydrorhodamine 123. Cells were exposed to AMPA concentrations of 0.01 - 5.0 mM for 1 h. Positive results are observed from 0.25 mM up to the highest tested concentration that induces cytotoxic effects (increase in percent of haemolysis). No information is available on the purity of the tested substance.

Chaufan et al. (2014, ASB2014-7616) evaluated the effect of AMPA on oxidative stress in HepG2 cells with 2',7'dichlorohydrofluorescin diacetate. AMPA exposure of the only tested concentration of 900 mg/mL for 24 h does not lead to an increase in ROS. Concentrations up to 1000 mg/mL did not affect the cell viability (MTT test). No information is available on the purity of the tested substance.

Human cells *in vitro*, data on formulations containing glyphosate:

Gehin et al. (2005, ASB2012-11826) investigated effects of pre-incubation of HaCaT with 100 or 200 µM Vit C, Vit E or both for 0, 24 or 48 h on cytotoxicity of a glyphosate-based formulation (containing 21% (p/p) isopropylamine glyphosate salt (170 g/L), 8% (p/p) POEA and 71% (p/p) water and others minor ingredients) at doses of up to 25 mM for 24 h. IC_{50} for Roundup 3 plus® alone, pre-incubated with Vit C, Vit E or both ranged from 17.1 - 18.2 mM, 16.9 - 18.1 mM, 16 - 17.6 mM or 16.7 - 21.8 mM, respectively. The authors inferred a protective effect of vitamin pretreatment indicating that ROS formation might be a mechanism for cytotoxicity of glyphosate-based formulations.

George and Shukla (2013, ASB2014-8034) investigated ROS formation after treatment of HaCaT cells with doses of 0.01, 0.025, 0.05 and 0.1 mM of a glyphosate-based formulation (containing glyphosate 41%, polyethoxethyleneamine (POEA) ≅15%) using 2′,7′-dichlorodihydrofluorescein diacetate. An up to 1.9-fold increase in ROS formation was detected when compared to control and antioxidant N-acetylcysteine (NAC) treated HaCaT cells. The effect was comparable with 10 nM 12-otetradecanoyl-phorbol-13-acetate. The positive control of 100 mM H_2O_2 is questionable as peroxide concentration is expected to decrease in cell cultures after 24 h at 37°C. Pretreatment with NAC statistically significantly decreased ROS formation below vehicle control (apparently not pre-treated with NAC). Some cell proliferation occurred upon treatment with Roundup. However, it was statistically significantly increased only at 0.1 mM glyphosate and after 72 h, but not at lower doses or shorter treatment. The proliferative effect at 0.1 mM after 72 h could be statistically significantly decreased by NAC. Cytotoxicity of the glyphosate formulation occurred from 0.5 mM glyphosate on upwards.

Coalova et al. (2014, ASB2014-7615) examined the impact of a glyphosate-based formulation (glyphosate as isopropylamine salt, 48%) on oxidative stress in HEp-2 cells with 2',7'-dichlorohydrofluorescin diacetate. The exposure of the only tested concentration of 376.4 mg/mL for 24 h leads to an increase in ROS. The tested concentration is equivalent to the determined LC_{50} value for a 24 h-exposure. The exposure of the formulation also increased glutathione and catalase activity whereas glutathione-S-transferase activity and superoxide dismutase activity (SOD) were not affected.

Chaufan et al. (2014, ASB2014-7616) evaluated the effect of a glyphosate-based formulation (74.4% monoammonium salt of N-phosphonomethylglycine) on oxidative stress in HepG2 cells with 2',7'-dichlorohydrofluorescin diacetate. An increase in ROS was observed at the only tested concentration of 40 mg/mL after an exposure of 24 h. The tested concentration is equivalent to the determined LC_{50} value of 41.22 mg/mL for a 24 h-exposure (MTT test).

Non-human mammalian experimental systems, data on glyphosate:

Astiz et al. (2009b, ASB2012-11550) investigated the effect of glyphosate, dimethoate and zineb administered alone or in combination on defence systems of the liver, kidney, brain and plasma antioxidant. Male Wistar rats, weighing 190 ± 20 g, were randomly divided into nine groups (4/group). Animals of one group were injected intraperitoneally (i.p.) with 10 mg glyphosate/kg bw (purity: commercial grade) in polyethylene-glycol 400 (PEG-400) three times a week for five weeks. Two groups served as controls (one group without treatment and one group receiving i.p. injections of PEG-400. Six further test groups were used to examine either zineb or dimethoate or a mixture of glyphosate, dimethoate and zineb (these groups are not further discussed here). At the end of the treatment the animals were killed, blood was collected and plasma was prepared. Homogenates from brains, livers, and kidneys were prepared. Various biomarkers of oxidative stress and cell damage were measured. Lipid peroxidation was assessed as thiobarbituric acid-reactive substances (TBARS); the sum of nitrates and nitrites ([NOx]) was measured as the main end-metabolite products of nitric oxide (NO) and peroxinitrite anion ($ONOO^-$), protein carbonyls as a biomarker of oxidative damage to proteins; enzymatic and non-enzymatic biomarkers of the antioxidant defence system: Ferric Reducing Ability of Plasma assay (FRAP, total antioxidant ability in plasma, Vitamin E (α-Tocopherol) levels in liver and brain), total glutathione (GSH) in plasma and brain; catalase activity (CAT), superoxide dismutase activity (SOD), glutathione peroxidase (GPx) activity, glutathione-S-transferase (GST), glutathione reductase (GR) activity in liver, brain, and kidney; lactate dehydrogenase (LDH) in plasma as a biomarker of cellular damage, and γ-glutamyl transpeptidase (γ–GT) activities as a biomarker of hepato-cellular damage. Results: At the end of treatment with glyphosate no effects were observed on animal

behaviour, body weight or body weight gain. Also no clinical signs of toxicity or observations of tremors or gait abnormalities (open field) were observed during the entire experimental period. The analytical examinations showed the following results: Increase of lipid peroxidation in liver, brain, kidney, plasma (significant, $p < 0.01$); slight increase (not significant) of oxidative damage to proteins seen as protein carbonyls in plasma; increase of [NOx] concentration (significant, $p < 0.01$) in brain and plasma; lower values (significant, $p < 0.01$) of FRAP in plasma, liver kidney and brain; progressive loss (significant, $p < 0.01$, approx. 30%) of α-tocopherol in liver and brain; increase (significant, $p < 0.01$) of GSH (GSH and GSSG, glutathione disulphide, oxidized Glutathione, hydrogen acceptor) in plasma; the following values were determined for the various antioxidant enzyme activities: increase (significant, $p < 0.01$) of SOD in liver and brain, decrease (significant, $p < 0.01$) of CAT in brain, slight increase (not significant) of SOD, CAT, GPx, GR, GST activity in kidney; no effect of LDH in plasma, increase (significant, $p < 0.01$) of γ–GT in plasma. Overall, repeated i.p. injection of glyphosate over a period of 5 weeks resulted in a lower antioxidant status in liver, brain, kidney and plasma, higher oxidized protein and glutathione levels in plasma with a decreased concentration of α-tocopherol in brain and liver. SOD was decreased in liver and brain. Glutathione reductase was inhibited in liver while glutathione peroxidase and transferase were unaffected. Plasma lactate dehydrogenase was not affected, but γ-glutamyl transpeptidase activity was increased. In conclusion the IARC statement can be supported that there are indications of oxidative stress in the blood plasma, liver, brain and kidney of rats upon exposure to glyphosate.

Bolognesi et al. (1997, Z59299) examined the genotoxic activity of glyphosate and its technical formulation 'Roundup'. Glyphosate (purity: 99.9%) was tested in a battery of genotoxicity tests *in vitro* and *in vivo*. These data were documented as part of the summarized data on *in vitro* and *in vivo* genotoxicity testing with glyphosate in section 4.2.1 of IARC Monographs Volume 112 (2015, ASB2015-8421). No information regarding 'increased biomarkers of oxidative stress in liver and kidney' is given.

Non-human mammalian experimental systems, data on AMPA:

No data available.

Non-human mammalian experimental systems, data on formulations containing glyphosate:

Bolognesi et al. (1997, Z59299) examined the genotoxic activity of glyphosate and its technical formulation 'Roundup'. Roundup formulate (30.4% glyphosate as active agent) was tested in a battery of genotoxicity tests *in vitro* and *in vivo*. No information regarding 'increased biomarkers of oxidative stress in liver and kidney' is given. As mentioned above this study was disregarded in the assessment.

Cavusoglu et al. (2011, ASB2012-11588) evaluated the protective effect of Ginkgo biloba L. leaf extract against Roundup® (Roundup Ultra-Max, containing 450 g/L glyphosate as active ingredient) in Swiss albino mice. Male Swiss albino mice (12 - 14 weeks old and weighing 25 - 30 g) were randomly divided into six groups, each consisting of six animals. The control animals received single intraperitoneal (i.p.) injection with dimethyl sulfoxide (0.2 mL). One group received single i.p. injection of 50 mg/kg bw Roundup. Two further groups were given orally G. biloba at doses of, respectively, 50 and 150 mg/kg bw for 8 consecutive days. The fifth group was given orally G. biloba at the dose of 50 mg/kg bw and i.p. injection of 50 mg/kg bw Roundup. The sixth group was given orally G. biloba at the dose of 150 mg/kg of body weight and i.p. injection with 50 mg/kg bw Roundup. For the fifth and sixth group, G. biloba application was started 5 days before exposure to Roundup and was continued alone for 3 consecutive days after single-dose applications of Roundup. Animals were sacrificed at the end of treatment (72 h). Blood, bone marrow, and liver and kidney tissues were investigated. Serum analysis involved the following parameters: aspartate aminotransferase (AST), alanine aminotransferase (ALT), blood urea nitrogen (BUN), and creatinine. For the determination of lipid peroxidation and glutathione activity the liver and kidney tissues of each animal were processed for biochemical measurements. Tissue glutathione (GSH) and malondialdehyde (MDA) levels were measured. For evaluation of genotoxic effects the mouse erythrocyte micronucleus (MN) assay, a modified mouse MN test that conventionally scores the MN frequencies in bone marrow polychromatic erythrocytes, was used. For

determination of chromosomal aberrations (CAs) animals were sacrificed 2 h after treatment under ether anesthesia and bone marrow from the femur was aspirated, washed, fixed in Carnoy's fixative, and stained with 5% Grünwald–Giemsa stain. Histopathological examination of the liver and kidneys was performed. Results of Roundup treatment without pre-treatment with the antioxidant: Serum AST, ALT, BUN, and creatinine levels were significantly increased ($p < 0.05$) in mice. The examination of the lipid peroxidation products showed significantly decreased ($p < 0.05$) levels of GSH and significantly increased ($p < 0.05$) levels of MDA in the liver and kidney tissues. The frequency of micronucleated cells was clearly increased (significant, $p < 0.05$) in mature normachromatic erythrocytes, and the mean number of micronucleated cells was significantly higher ($p < 0.05$) compared to controls. Roundup induced an increase in the frequency of CAs and the number of AMNs in bone marrow metaphases. It also significantly decreased the rate of MI. A significant stimulation in the frequency of CA types such as chromatid breaks, acentric fragments, and chromatid gaps in bone marrow cells was noted. Histopathology of the liver revealed severe degenerative and necrotic changes. There were hydropic degeneration, nuclear pyknosis, and loss of some nuclei of hepatocytes in periacinar and midsonal areas. Kupffer cell proliferation and fibrosis were seen in some portal areas. In the kidneys glomerular basement membranes were thickened, accumulation of hyaline droplets and cylinders was detected in some tubular lumina, and some tubular epithelial cells were degenerated.

Results of Roundup treatment with pre-treatment with the antioxidant: The treatment of Roundup together with G. biloba caused a significant reduction in the above described effects of Roundup, especially in indices of hepatotoxicity, nephrotoxicity, lipid peroxidation, and genotoxicity. The strongest effect was observed with G. biloba at 150 mg/kg bw.

Overall, results of serum analysis, evaluation of genotoxic effects and the histopathology indicate that Roundup induced (cyto-)toxicity in liver and kidney, higher frequencies of CAs, MNs, and abnormal metaphases compared with the controls, and oxidative stress in Swiss albino mice. The pre-treatment with G. biloba induced a weakening of oxidative stress by the glyphosate-based formulation. The IARC statement can be supported that there are indications of increases in biomarkers of oxidative stress in liver and kidney of mice upon exposure to the glyphosate-based formulation (Roundup). The supplementation with the antioxidant G. biloba extract can protect against glyphosate toxicity by reduction effects of free radicals.

Jasper et al. (2012, ASB2014-9583) investigated biochemical, hematological and oxidative parameters of glyphosate-Roundup® (= 41% Glyphosate as active ingredient and 16% polyoxyethylene amine (POEA) and apparently other surfactants (not further specified)). Male and female Swiss albino rats (10/sex/dose) received daily oral gavage doses of 50 or 500 mg/kg bw/d Roundup for 15 days (vehicle/control: distilled water). Liver toxicity was assessed by serum enzymes ALT, AST, and γ-GT, renal toxicity assessed by urea and creatinine. Haematology was assessed by RBC, WBC, hemoglobin, hematocrit, MCV, MCH, and MCHC. Oxidative damage assessed by TBARS (thiobarbituric acid reactive substances) and NPSH (non-protein thiols) in liver. There was a significant dose-dependent reduction in body weight gain in both sexes. Significant increases in ALT, AST, and γ-GT at both dose levels, no considerable differences by histology. No significant changes in renal parameters. Hematology: Significant anemic alterations at high dose in both sexes: Reduction of RBC, hematocrit, and hemoglobin, significant increase of MCV. Lipid peroxidation: Males: at both dose levels important increases in lipid peroxidation together with an NPSH reduction in the hepatic tissue. Females: Significant increase in TBARS at both doses, significant decreases in NPSH only at high dose. Results indicate that glyphosate-Roundup® causes anemic effects and increased activities of liver enzymes that indicate liver cell dysfunction (although no abnormal morphology was observed) at subacute exposure and which could be related to the induction of reactive oxygen species.

Cattani et al. (2014, ASB2014-3919) investigated rat hippocampus. The herbicide Roundup Original® (Homologation number 00898793) containing glyphosate 360 g/L (commercial formulation registered in the Brazilian Ministry of Agriculture) was used, no further information on components are given. Wistar rats were exposed to 1% Roundup in drinking water during pregnancy up to lactation day 15 and from their pups, slices of hippocampus were prepared. TBARS assay was used to assess oxidation products, reduced GSH was measured with DTNB (both photometric assays). The experimental procedure is in part unclear: "*After preincubation, hippocampal slices were incubated in the presence or absence of 0.01% Roundup for 30 min*", but values are reported as being from 8 animals from each

treated group. TBARS levels were statistically significantly increased, ($p < 0.05$), GSH levels were statistically significantly decreased ($p < 0.01$). Remarks: It appears that the results might be a combination of *ex vivo* and *in vivo* results. Positive control is lacking, experimental details are missing. Unusual test setting, the reliability of the test system seems to be questionable. Uncertainties on the test method remain as a preparation of tissue slices was reported, but on the other hand, a homogenate was described. It is unknown whether a homogenate from slices was prepared and tested. Conclusion: From the poor description/questions arising from experimental procedure and due to lack of positive control, this study should be disregarded.

George et al. (2010, ASB2012-11829) investigated both, carcinogenicity and the change of expression of proteins by proteomics in skin of mice dermally treated with a glyphosate formulation (Roundup original®). Only the proteomics part is assessed here as it relates to oxidative stress. Method: Four male Swiss albino mice were treated each with a single dose of 50 mg/kg bw of glyphosate in a glyphosate formulation (Roundup original®, glyphosate 41%, POEA = 15%-Monsanto Company, St. Louis, MO, USA, 360 g/L glyphosate) by topical application at the dorsal region (2 cm^2, hair clipped). Untreated controls were included. After 24 h animals were sacrificed and skin tissues from the treatment site were excised and homogenized. Protein spots with a >2 fold change (compared to controls) were considered as differentially expressed, excised and identified via MALDI-TOF/TOF. To confirm the observed changes in protein expression an immunoblot analysis for some of the differently expressed proteins was performed. Results: Changes in expression levels of proteins in skin tissues of treated mice compared to controls, which were confirmed by immunoblot analysis, were observed for the three proteins calcyclin (increased expression, about 2.5 fold change), calgranulin-B (increased expression, about 9.5 fold change) and superoxide dismutase (SOD) (decreased expression, about 5 fold change). SOD is a biomarker of oxidative stress and provides a protective response against ROS. The expression of SOD is supposed to be up-regulated if ROS occur. As a down-regulation of SOD was observed it can be concluded that no direct induction of ROS occurred upon treatment with the glyphosate formulation. Calcyclin and calgranulin-B are not directly linked to ROS or oxidative stress. Calgranulin-B is a protein supposed to be involved in chronic inflammation and calcyclin is a calcium-binding protein often detected up-regulated in expression in proliferating cells. Remarks: Only results of the proteomics experiment confirmed by immunoblot analysis were considered as true changes in protein expression levels as only a small number of animals (4), skin samples and one dose were tested. Moreover, the gels were stained with the semi-quantitative silver staining and the detailed procedure of data analysis was not shown (including the total number of gels performed, the expression data of each protein spot on each gel, the significance value for each observed fold change in expression level of a protein spot compared to controls and the group formation for statistical analysis).

Overall, the conclusions drawn by George et al. (2010, ASB2012-11829) do not support the statement in the IARC report. The study was performed with a glyphosate formulation and not with pure glyphosate as described in the IARC report. No production of free radicals or oxidative stress after dermal exposure to a glyphosate formulation has been observed. An alteration of the expression level of an antioxidant enzyme was found (expression of SOD was down-regulated) but the observed down-regulation of SOD is not indicative of increased ROS formation. Conclusion: The IARC statement that glyphosate increases biomarkers of oxidative stress in skin based on the study of George et al. (2010, ASB2012-11829) cannot be supported.

Non-human mammalian experimental systems, data on mixtures of active substances including glyphosate:

Astiz et al. (2013, ASB2014-7493) treated male Wistar rats with a mixture of Zineb (99% pure, 15 mg/kg/d), glyphosate (99% pure, 10 mg/kg/d) and dimethoate (98% pure, 15 mg/kg/d) i.p., 5 x per week for 5 weeks to investigate the association between oxidative stress and inflammation/steroidogenesis. After treatment period, plasma was sampled and testis homogenates were prepared. For determination of oxidative damage, TBARS and protein carbonyls were determined. Further, the sum of nitrates and nitrites was determined. Statistical analysis was performed. Compared to untreated controls, levels of all biomarkers of oxidative damage were significantly increased in

plasma and testis homogenate. No positive control for oxidative stress was included. As glyphosate was only tested in combination with two other pesticides, no conclusion on glyphosate is possible. The IARC text is in principle correct but a more careful wording on the relevance of the study appears appropriate.

Overall conclusion on Oxidative stress:

In general the documentation of the majority of studies on oxidative stress in section 4.2.3 of IARC Monographs Volume 112 (2015, ASB2015-8421) can be confirmed. It is noted that here is a lack of positive controls for oxidative stress in all *in vitro* and *in vivo* studies described in section 4.2.3 *(ii) Non-human mammalian experimental systems* of the IARC monograph. From the available data on glyphosate, there is some indication of induction of oxidative stress from testing in human cell cultures and in mammalian (*in vivo*) experimental systems. In particular, the IARC statement that there are indications of oxidative stress in the blood plasma, liver, brain and kidney of rats upon exposure to glyphosate can be supported. However, only one of the cited studies (Astiz et al., 2009b, ASB2012-11550) investigated oxidative stress in animals with pure glyphosate. This study was conducted in rats and no other species was tested and increased oxidative stress was observed in combination with cytotoxic/degenerative effects of the targeted organs.

Only *in vitro* data were available on induction of oxidative stress by AMPA. There was no indication for such activity.

A glyphosate-based formulation increased biomarkers of oxidative stress in livers and kidneys of mice treated orally for 1 day or 15 days.

Considering the low level of metabolism and the chemical structure of glyphosate, glyphosate radical formation initiating oxidative stress appears unlikely. However, uncoupling or inhibition of mitochondrial oxidative phosphorylation also represents an established mechanism for ROS generation. Notably, uncoupling of oxidative phosphorylation by glyphosate has been reported in rat liver microsomes (Bababunmi et al., 1979, ASB2015-8535) and a glyphosate formulation (but not glyphosate) (Peixoto, 2005, ASB2012-11994).

Induction of oxidative stress, in general, can provide a mechanistic explanation for any observed cytotoxic/degenerative and indirectly genotoxic effects of substances (Chapter 3.6.2.3.2 Additional considerations for classification of Guidance on the Application of the CLP criteria, ECHA-13-G-10-EN, ECHA 2013, ASB2015-8592). However, from the sole observation of oxidative stress and the existence of a plausible mechanism for induction of oxidative stress through uncoupling of mitochondrial oxidative phosphorylation alone, genotoxic or carcinogenic activity in humans cannot be deduced for glyphosate and glyphosate based formulations.

4.2.3.2 Inflammation and immunomodulation

Six studies were reported by IARC in section 4.2.3 (b). The studies including comments of the RMS are summarized in Table 4.2-9 and are described in detail below.

(i) Humans:

Human cells *in vitro*:

Data on glyphosate:

Nakashima et al. (2002, ASB2012-11919) tested the proliferative activity and the release of cytokines of 1-1000 µM glyphosate on PHA-stimulated human peripheral blood mononuclear cells (PBMC) of unknown origin.

After 24 h incubation, glyphosate had a slight (not significant) inhibitory effect on cell proliferation, INF-y was significantly reduced at 1000 µM glyphosate (-30%) and a minimal reduction of IL-2 was

recorded. No effects on TNF-alpha or IL-1 beta. The authors concluded glyphosate showed only a little damage to the immune system.

Remarks: The study of Nakashima et al. (2002, ASB2012-11919) is limited due to the Japanese language. Only a summary and some figures with labelling in English is available, lack of information on the test method, numerical results and the details on the cell donator. The *in vitro* finding (reduction in INF-Y) is opposite to the *in vivo* response in BAL (increase in INF-y) seen in Kumar et al (2014, ASB2015-8276). The relevance of this study seems to be questionable. The highest test concentration of 1 mM that inhibited cell proliferation may be close to a cytotoxic concentration (no data).

Most of the information was correctly cited by IARC. The reported finding 'modestly inhibited the production of IFN-gamma' can be accepted for IFN-gamma (-30%), but no clear effect was seen for IL-2 up to 1000 µM glyphosate.

(ii) Non-human mammalian experimental systems:

Data on glyphosate:

The study of Kumar et al. (2014, ASB2015-8276) used the 'murine intranasal challenge model' with daily intranasal applications for 7 days or 3x/week for 3 weeks of glyphosate-rich air samples (called as 'Real Env.') suspended in PBS (8.66 µg/mL) or reagent grade glyphosate (of unknown purity) at concentrations 100 ng, 1 µg or 100 µg in 30 µl in wild-type of TLR 4-/- mice. (Cell numbers by flow cytometric analysis on BAL and lung tissue, cytokine levels in BAL, serum, immunohistochemistry in lung tissue).

Increases in numbers of cells, eosinophils, neutrophils per lung or BAL fluid at 1 µg and 100 µg glyphosate, but no dose-response was observed. No effect occurred at 100 ng glyphosate. No increase in mast cell number/lung tissue, but higher serum MCPT-1 indicating increased mast cell degranulation was found.

1 or 100 µg glyphosate induced increased release of cytokines (IL-5, Il-10, IL-13 without dose-response for IL-5 and IL-13) to BAL fluid. Although no dose response was recognized, IFN-Y was increased nasal application of glyphosate at both dose levels. In contrast the increase was not confirmed for the 'Real Env.' exposure. IL-4 was increased for 'Real Env.' but not for glyphosate.

At 1 µg glyphosate, 3-4-fold higher levels of IL-33 and TSLP in BAL and (a qualitative) confirmation by positively immuno-stained (bronchiolar?) lung tissue was reported.

Remarks: The study aimed to identify the potential of glyphosate to induce asthma. To our knowledge there are no validated models to assess the potential for respiratory sensitization.

The validity of the administration route and frequency is limited to assess effects after repeated inhalation. Due to the single intranasal injection of the test fluid there is lack of homogenous concentration and lack of constant exposure conditions over 6 hour per day. This method did not produce a continuously homogeneous test atmosphere at the mucosal surface of the airways. As the test material concentrations will be highest in the nasal cavity, the nasal tissues are the preferred sites for cytokine and morphological examinations. In addition, it remains unclear how many animals/sex/dose were treated and how many samples of BAL and lung tissue per animals were examined.

More weight should be given on the testing of glyphosate. Testing of the glyphosate-rich air samples are considered as less informative as the analytical concentration, composition, homogeneity and stability of the air samples were not examined. In comparison with the sham-(PBS) exposed mice the study identified an increase of biomarkers of airway inflammation as shown by increased numbers of cells and increased numbers of inflammatory cells (eosinophils, neutrophils) and elevated cytokine concentrations in BAL. The positive response could be interpreted as qualitative information indicating a potential for airway inflammation since for the majority of cell parameters and cytokines no dose-response was identified. The absence of a dose-response relationship might have been related to the application mode. Increased levels of IL-33 and TSLP in BAL and abundant staining in lung tissue were interpreted as indicative of (asthma-like) type 2 pathology. These effects as well as increased

concentrations of released cytokines that are related to asthma (IL-5, IL-10, IL-13) and mast cell degranulation were also seen following ovalbumin administration with a similar dosing scheme. The authors interpreted the results as indicating that glyphosate triggers allergic inflammation. As there is no validated model on respiratory sensitization and due to the weaknesses of the study, this conclusion needs confirmation by other studies or human data.

The study results were (almost) correctly reported by IARC. In contrast to the IARC text, no effect was seen at 1 ng glyphosate.

In the study of Chan and Mahler (1992, TOX9551954) Groups of 10 male and 10 female F344N rats and B6C3F1 mice were given glyphosate in feed at dietary concentrations of 0, 3125, 6250, 12500, 25000 or 50000 ppm (corresponding to 0, 205, 410, 811, 1678 or 3393 mg/kg for males rats and 0, 213, 421, 844, 1690 or 2293 mg/kg bw/d for female rats). Ten additional rats/sex were included at each dietary level for evaluation of hematologic and clinical pathology parameters (on days 5, and 21, and at the end of treatment after 13 weeks).

In male rats, reduced body weight (bw) gains were observed in the 25000 and 50000 ppm groups. The final bw in these groups were significantly lower than that of the control group. At necropsy the bw of the 50000 ppm male group was 18% less than that of controls. In female rats of this dose there was only a marginal effect on bw gain with the high dose group 5% lighter than controls at the end of study. In male rats of this dose, small increases in relative organ weight were observed for the liver, kidney, and testicle; a decrease in absolute weight and relative weight was observed for the thymus. The relative weight was 0.80% for high dose males versus 0.92% in control males. No treatment-related effects in females and on food consumption were observed.

Mild increases in haematocrit and RBC were observed in male rats at 13 weeks at ≥12500 ppm and increased haemoglobin in male rats at ≥25000 ppm. In female rats, minimal but significant increases occurred in lymphocyte and platelet counts, WBC, MCH and MCV. Treatment-related alterations in clinical chemistry parameters included increases in alkaline phosphatases in males and females at all-time points, ALAT in males and females at all-time points except 90 days, total bile acid at days 23 and 90 in males and at day 23 in females, total protein in females at all-time points, and sporadic increases in urea nitrogen and albumin.

In the 13-week study in mice, significantly lower final bw, lower relative thymus weights and increased relative weights of liver, heart, testes, lungs and kidneys were seen in high dose male mice, significantly lower final bw and lower absolute thymus and liver weights were observed in high dose female mice. A dose-related cytoplasmatic alteration of the parotid salivary gland in male mice and female mice at all doses (except the low dose) were seen. No data on haematology and clinical chemistry were available.

Remarks: The 13-week studies were conducted in 1988; the used method is not comparable to the current OECD test guideline standard. Increased haematocrit and RBC may indicate a lower water consumption and dehydration status of the animals (no data on water consumption available). Elevated ALAT and total bile acids could be related to hepatobiliary dysfunctions (in the absence of histopathological findings reported). Lower absolute and relative thymus weight alone in high dose males without any corresponding (microscopic) effect on immune organs or immune compartments in other tissues is not sufficient to indicate an immunosuppressive effect of glyphosate. More likely it could be interpreted as a nonspecific (toxic) response together with a lower bw gain that resulted in 18% lower final bw at 50000 ppm. Based on the limited information available it can be concluded that the observations in rats are in agreement with the findings in mice.

To the IARC Documentation:

IARC summed up the main findings as ‘pathological effects of glyphosate on the immune system’ without giving an interpretation of the effects seen. Based on a weight of evidence analysis of the available data from the studies in rats and mice one should conclude that there is no clear indication of an immunosuppressive effect.

Glyphosate-based formulation:

In the study of Blakley (1997, ASB2015-7878) female CD-1 mice received drinking water for 26 days at concentrations from 0, 0.35%, 0.7% or 1.05% Roundup (corresponding to 0, 335, 670 or 1000 mg/kg glyphosate/ kg/day. On day 21 mice were i.p. injected with sheep red blood cells (SRBC) and the production of the T-lymphocyte, macrophage dependent antibody response was evaluated on day 26.

No treatment-related effect on bw gain or water consumption. Roundup did not affect the T-cell mediated antibody production.

Remarks: There is no indication that the humoral immune response is adversely affected in mice that received Roundup for 26 days of treatment.

IARC correctly summed up the study results. The lack of effects on the immune system has not been reflected in their overall conclusion.

Overall conclusion on section (b) inflammation and immunomodulation:

IARC documented the results of one *in vitro* and three *in vivo* studies that examined for glyphosate-related effects on the mammalian immune system in this section.

With regards to the underlying mode of action for the carcinogenic effects IARC concluded that there is 'weak evidence that glyphosate may affect the immune system, both the humoral and cellular response' (section 5.4).

RMS concludes that the evidence from available data do not allow to conclude that glyphosate caused immunosuppression. However it is to note that due to the small number of studies assessed and the fact than all studies show limitations, no robust information is available to conclude on the immunomodulatory action of glyphosate.

Conclusion on glyphosate:

The main study results of the above mentioned studies were correctly summed up by IARC. Some details of the reporting could be improved. In the study of Kumar et al. (2014, ASB2015-8276) no effect was seen at the low dose tested (100 ng glyphosate) in mice. A critical analysis of the limitations of the studies (e.g. on the exposure regimen) is lacking.

The effects of the 13-week study in rats (Chan and Mahler, 1992, TOX9551954) were described by IARC as 'pathological effects of glyphosate on the immune system'. The only finding was a reduced absolute/relative thymus weight in male rats at the highest dose. No other corroborating effect in the immune organs was seen. The lower weight of the thymus is likely to be linked to nonspecific toxic effects such as a lower bw gain and a 18% lower final bw in male rats. No such effect was seen in female rats of this study. No clear pathological (immune suppressive) effect on the immune system can be identified from this study.

The study of Kumar et al. (2014, ASB2015-8276) indicated that glyphosate may induce inflammatory effects in the respiratory tract that by the authors was supposed as being predictive to induce asthma-like effects. Additional and more robust data are needed to confirm this assumption. A potential for inflammatory responses of the respiratory tract is the only immunomodulatory effect identified so far.

Conclusion on glyphosate-containing formulation (Roundup):

The negative results for glyphosate of the Chan and Mahler study (1992, TOX9551954) are in agreement with the negative finding for effects on the immune system of the study of Blakley (1997, ASB2015-7878). Although both studies had limitations (in comparison to current test guideline standards or the test material), the negative outcome was not reflected by IARC. The glyphosate-containing formulation tested in the Blakley study (1997, ASB2015-7878) was negative for T-cell dependent antibody response up to 1000 mg/kg bw/d glyphosate and did not indicate that the humoral and cellular immune responses were affected.

Table 4.2-9: Discussion of studies in chapter 4.2.3 (b) Inflammation and immunomodulation of the IARC monograph

Study (Author/year)	Subject	Evaluation by IARC	Comment RMS on IARC evaluation	Study reported in RAR Draft April 2015	Final conclusion of RMS, considering IARC evaluation
Nakashima et al., 2002, ASB2012-11919	Effects of glyphosate on cytokines production by human peripheral blood mononuclear cells	Glyphosate had a slight inhibitory effect on cell proliferation, and modestly inhibited the production of IFN-gamma and IL_2. The production of TNF-alpha and IL-1 Beta was not affected by glyphosate.	Agreement. The authors conclude that glyphosate might be a pesticide with only a little damage to the immune system. The study of Nakashima et al. (2002) is limited due to the Japanese language. Only a summary and some figures with labelling in English is available, lack of information on the test method, numerical results and the details on the cell donator. The *in vitro* finding (reduction in INF-Y) is opposite to the *in vivo* response in BAL (increase in INF-y) seen in Kumar et al., 2014, ASB2015-8276. The relevance of this study seems to be questionable. The highest test concentration of 1 mM that inhibited cell proliferation may be close to a cytotoxic concentration (no data).	No	The relevance of this study seems to be questionable.
Kumar et al., 2014, ASB2015-8276	Pro-inflammatory effects of glyphosate and farm air samples in mice	Airway exposure to glyphosate significantly increased the total cell count, eosinophils, neutrophils, and IgG1 and IfG2a levels and produced pulmonary inflammation. Glyphosate-rich farm air increased circulating levels of IL-5, IL-10, IL-13 and IL-14 in wildtype and TLR4-/- mice. In wildtype mice glyphosate increased levels of IL-5, IL-10, IL-13 and IFN-Gamma (but not IL-4).	Agreement with reported results. The study aimed to identify the potential of glyphosate to induce asthma. The positive response could be interpreted as qualitative information indicating a potential for airway inflammation since for the majority of cell parameters and cytokines no dose-response was identified. Testing of the glyphosate-rich air samples are considered as less informative as the	No	Agreement with reported results; the positive response could be interpreted as qualitative information indicating a potential for airway inflammation.

Study (Author/year)	Subject	Evaluation by IARC	Comment RMS on IARC evaluation	Study reported in RAR Draft April 2015	Final conclusion of RMS, considering IARC evaluation
			analytical concentration, composition, homogeneity and stability of the air samples were not examined.		
Chan and Mahler, 1992, TOX9551954	NTP report on toxicity studies of glyphosate in mice	In subchronic studies in rats and mice effects on thymus weight and haematological parameters have been observed.	Further effects on clinical chemistry parameters, body weight and salivary gland have been reported. The 13-week studies were conducted in 1988; the used method is not comparable to the current OECD test guideline standard. The results are not sufficient to indicate an immunosuppressive effect of glyphosate. More likely they could be interpreted as a nonspecific (toxic) response together with a lower bw gain that resulted in 18% lower final bw at 50000 ppm.	Yes, page 259	Supplementary information on subchronic toxicity of glyphosate in rats and mice additionally to the large number of studies reported in the RAR; The results are not sufficient to indicate an immunosuppressive effect of glyphosate. More likely they could be interpreted as a nonspecific (toxic) response.
Blakley, 1997, ASB2015-7878	Effect of Roundup on antibody production in mice	The humoral immune response (antibody production against sheep erythrocytes) was not affected by glyphosate.	Agreement	No, reported before 2000	No effect of glyphosate on humoral immune response.
Kreutz et al., 2011, ASB2015-8279	Effects of glyphosate on haematological and immunological parameters in catfish	"A positive association between exposure to glyphosate and immunotoxicity in fish has been reported."	No agreement with conclusion of IARC. Obviously, no glyphosate but a glyphosate containing formulation was used in this study. Without further information it is a mixture of unknown substances. Therefore, no conclusion on glyphosate is possible.	Yes, page 147	No agreement with conclusion of IARC. Obviously, no glyphosate but a glyphosate containing formulation was used in this study. Without further

Study (Author/year)	Subject	Evaluation by IARC	Comment RMS on IARC evaluation	Study reported in RAR Draft April 2015	Final conclusion of RMS, considering IARC evaluation
					information it is a mixture of unknown substances. Therefore, no conclusion on glyphosate is possible.
El-Gendy et al., 1998, ASB2015-8422	Effects of glyphosate on the immune response and protein biosynthesis of fish	Effects of a glyphosate-based formulation on immune response in bolti fish are reported.	Some effects are described by IARC as glyphosate effects. However, a formulation was used in this study. Therefore, no conclusion on the active substance glyphosate is possible.	No, reported before 2000	Some effects are described by IARC as glyphosate effects. However, a formulation was used in this study. Therefore, no conclusion on the active substance glyphosate is possible.

4.2.4 Cell proliferation and death

Information on apoptosis and proliferation in neuroprogenitor cells from humans (ReN CX) and mice (mCNS) is available from a HTS assay reported (refer to section 4.3).

4.3 Data relevant to comparisons across agents and end-points

IARC stated that no HTS or other relevant data was available to its working group. This included any data from Tox21 or the ToxCast initiatives.

In the RAR (April 2015, ASB2015-1194) information on androgenic and estrogenic effects from the U.S. EPA Endocrine Disruptor Screening Programme are reported. Based on Tier 1 studies of this programme as well as results published as part of the OECD validation of the steroidogenesis assay, and taking into account higher tier regulatory safety studies, it was concluded that there is no evidence for effects on the androgenic or estrogenic pathways of the endocrine system (refer to section 4.2.2).

In addition, the RAR contained information from a HTS assay for apoptosis and proliferation in neuroprogenitor cells from humans (ReN CX) and mice (mCNS). Glyphosate did not activate proliferation (BrdU assay) or apoptosis (caspase 3, p53 pathways) in concentrations between 0.001 and 100 µM in these tests.

DNA microarray data is available for Japanese medaka treated with 16 mg/L glyphosate or its mixture with 0.5 mg/L surfactant for 48 h (Uchida et al., 2012, ASB2015-8590). None of 138 genes that were induced in the liver by the treatment with the combination was associated with mutagenesis or carcinogenesis. Glyphosate alone did not lead to significant hepatic gene expression changes in this fish.

4.4 Cancer susceptibility data

IARC stated that studies examining relevant susceptibility factors were not identified.

In contrast, the RMS considered Swiss albino mice as a potentially susceptible strain for certain tumours: "*Swiss albino mice with high background prevalence of malignant lymphoma could be more vulnerable than other strains.*" (RAR, April 2015, ASB2015-1194). It was discussed that although it could not be completely excluded that the increase in malignant lymphoma incidence over the historical control of the laboratory reported by Kumar ([redacted] ASB2012-11491) was treatment-related, this (potential) effect was "*confined to this single study and strain*".

In its communication entitled "Does glyphosate cause cancer? Preliminary assessment of the carcinogenic risk of glyphosate with regard to the recent IARC evaluation", it was later noted by the BfR: "*Apart from the statistically significant increase in Swiss mice, a higher number of affected top dose males was also seen in two other studies ([redacted] 1997 [22] and [redacted], 2009 [23]) but was contravened later by historical control data.*" (BfR, 2015, ASB2015-8593). The following comparative table was provided:

Table 4.4-1: Total incidence of malignant lymphoma in long-term studies with glyphosate in different mouse strains (Table reproduced from BfR-communication entitled: "Does glyphosate cause cancer? Preliminary assessment of the carcinogenic risk of glyphosate with regard to the recent IARC evaluation" (BfR, 2015, ASB2015-8593).

Study, Strain		Males				Females			
[redacted] 2009, ASB2012-11492 Crl:CD-1 (ICR) BR	Dose (ppm)	0	500	1500	5000	0	500	1500	5000
	Affected	0/51	1/51	2/51	5/51	11/51	8/51	10/51	11/51
[redacted] 2001, ASB2012-11491 HsdOLA:MF1 (Swiss albino)	Dose (ppm)	0	100	1000	10000	0	100	1000	10000
	Affected	10/50	15/50	16/50	19/50*	18/50	20/50	19/50	25/50*
[redacted] 1997, ASB2012-11493 Crj:CD-1 (ICR)	Dose (ppm)	0	1600	8000	40000	0	1600	8000	40000
	Affected	2/50	2/50	0/50	6/50	6/50	4/50	8/50	7/50
[redacted]., 1993, TOX9552382, CD-1 (not further specified)	Dose (mg/kg bw/d)	0	100	300	1000	0	100	300	1000
	Affected**	4/50	2/50	1/50	6/50	14/50	12/50	9/50	13/50

* increase statistically significant, for females based on percentage and not on total number of affected mice
** based on histological examination of lymph nodes with macroscopic changes

4.5 Other adverse effects

A number of further (adverse) effects observed in humans and laboratory animals were discussed by both IARC and BfR. Respective findings have been taken into account in the chapters above as far as these were considered relevant for the assessment of carcinogenic and/or mutagenic potential.

5 Summary of Data Reported

5.1 Exposure data

Results of four occupational and two para-occupational studies using various glyphosate-containing plant protection products have been cited in the IARC monograph. The studies were carried out between 1988 and 2007 in different countries of North America and Europe. Four of these studies (Centre de Toxicology du Québec, 1988 (ASB2015-7889), Lavy et al., 1992 (TOX9650912), Johnson et al., 2005 (ASB2012-11859) and Curwin et al., 2007 (ASB2012-11597)) have not yet been included in the RAR (April 2015, ASB2015-1194) because a refinement of operator exposure was not necessary.

Within the scope of the risk assessment for the representative formulation in the European procedure for renewal of approval of glyphosate the exposure calculations according to the common models demonstrate safe use of the product.

Nevertheless, all six exposure studies have been roughly evaluated now (see Table A-5.5-2).

In all cases but one, the recorded values in the studies were below or in the same order of magnitude as those predicted in the RAR (April 2015, ASB2015-1194). Thus, it can be stated that there is no glyphosate based health risk anticipated for operators for intended uses applied for in the European Union provided that the plant protection product is used correctly and as intended.

However, in one study (Johnson et al., 2005, ASB2012-11859) the reported glyphosate air concentrations for some operators (vehicle application) were strikingly high, i.e. higher than the air concentrations detected in all other studies by a factor of 1000. But it is assumed that the data in this study were obtained with invalid calibration. For more details see Table A-5.5-2.

In summary, for resources on dietary exposure and for results on biological markers IARC refers to several selected reports from national food- and bio-monitoring programmes as well as to some studies in the public literature. Most of the data on dietary consumer exposure are not included in the RAR (April 2015, ASB2015-1194) due to the GAP-based “safe-use” approach for the assessment of active substances under Regulation (EU) 1107/2009 (2009, ASB2015-8589). All studies on biomarkers were also included in the RAR. No deviating conclusions between RAR and IARC were identified.

5.2 Human carcinogenicity data

Based on the studies on cancer in humans IARC concluded: „*There is limited evidence in humans for the carcinogenicity of glyphosate.*” RMS agrees with IARC that the other IARC categories (Evidence suggesting lack of carcinogenicity, inadequate evidence of carcinogenicity and sufficient evidence of carcinogenicity) are not suitable for the classification of the evidence from studies in humans. The evaluation of the epidemiological studies by the RMS is similar to IARC. However, RMS adopts a more cautious view since no consistent positive association is observed, with the most powerful study showing no effect. The IARC interpretation is more precautionary based on the objectives and scope of the IARC Monographs which represent a first step in carcinogen risk assessment, which involves examination of all relevant information in order to assess the strength of the available evidence that an agent could alter the age-specific incidence of cancer in humans and that the Monographs may also indicate where additional research efforts are needed, specifically when data immediately relevant to an evaluation are not available. Therefore, no recommendation is given with regard to regulation or legislation, which is the responsibility of individual governments or other international organizations.

It was also noted that in the epidemiological studies a differentiation between the effects of glyphosate and the co-formulants is not possible. However, data on glyphosate containing formulations indicate a significantly higher toxicity compared to the pure active substance.

5.3 Animal carcinogenicity data

Based on carcinogenicity studies in experimental animals IARC concluded: „*There is sufficient evidence in animals for the carcinogenicity of glyphosate*" on a positive trend in the incidence of renal neoplasms in male CD-1 mice, a significant positive trend in the incidence of haemangiosarcoma in male CD-1 mice and a significant increase in the incidence of pancreatic islet cell adenoma in two studies in the Sprague-Dawley rats.

A much larger number of animal studies have been performed to evaluate the carcinogenic potential of glyphosate than necessary by the legal requirements. In mice, a total of five long-term carcinogenicity studies using dietary administration of glyphosate were considered. In rats, seven chronic toxicity and carcinogenicity studies using dietary administration of glyphosate and two studies with application via drinking-water were reviewed.

In order to support the interpretation and evaluation of the tumour incidences observed in the CD-1 mice studies Table 5.3-1 was prepared (see below).

Renal tumours

In four studies in CD-1 mice and one study in Swiss albino mice, the incidences of renal tumours in male mice were reconsidered for statistical evaluation. In the first study [redacted] 1983 TOX9552381), the combined incidences for renal adenoma and carcinoma in males were 1, 0, 1 or 3 for the control, low, mid or high dose group, respectively, based on the result of the histopathological re-examination and 0, 0, 1, 3 when based on the original study report. In the second study ([redacted] 1997, ASB2012-11493), the incidences for renal adenoma were 0, 0, 0 or 2 for the control, low, mid or high dose group males, respectively. In Swiss albino mice ([redacted] 2001, ASB2012-11491) reported incidences in males were 0, 0, 1, 2. For these three studies, the statistical analysis with the Cochran-Armitage test for linear trend yielded a significant result, whereas the analysis by pair-wise comparisons (Fisher's exact test) indicated no statistically significant differences between the groups. In the two other studies, as well as the females of all studies, there was no indication for induction of renal adenoma.

For both studies in CD-1 mice, the observed renal tumours were considered spontaneous and unrelated to treatment by the study pathologists. Furthermore, extensive pathological and biometrical re-evaluations of the data from the first study reached the conclusion that the absence of any pre-neoplastic kidney lesion in treated males provided sufficient evidence that the occurrence of these tumours was spontaneous rather than substance-induced [redacted], 1986, TOX9552381). This assessment is supported by the fact that, in both studies, the increased incidences of renal tumours at the high dose groups were not statistically significant when compared with the concurrent controls, and the incidences were within the historical control range for adenomas and carcinomas combined (up to 6%).

The EU CLP regulation provides further important factors which should be taken into consideration for the interpretation and assessment of animal carcinogenicity data. If increased tumour incidences are found only at the highest doses used in a lifetime study, the possibility of a confounding effect of excessive toxicity cannot be excluded. In both studies, the highest dose levels tested (4841 or 4348 mg/kg bw per day) were well in excess of the limit dose for carcinogenicity testing (1000 mg/kg bw per day) as recommended by OECD guidance document 116 (OECD 2012). Also, the OECD test guideline for carcinogenicity studies states that the highest dose level should elicit signs of minimal toxicity, with depression of body weight gain of less than 10%. In both studies, however, the body weight gain in high dose males was decreased by more than 15% compared to controls, and there was a significant increase in central lobular hepatocyte hypertrophy, central lobular hepatocyte necrosis, and chronic interstitial nephritis in high dose males in one study ([redacted] 1983 TOX9552381).

Table 5.3-1: Summary of selected tumour incidences in male CD-1 mice.

	Historical control incidences			**Tumour incidence/number of animals examined**															
Dose (mg/kg bw per day)	*Mean*	*Min*	*Max*	0	0	0	0	71	100	157	165	234	300	810	814	838	1000	4348	4841
Study ID				A	B	C	D	D	B	A	C	D	B	D	A	C	B	C	A
Study duration (months)	*NR*	*18*	*24*	24	24	18	18	18	24	24	18	18	24	18	24	18	24	18	24
Survival	*NR*	*18.3%*	*94%*	20/50	26/50	26/50	39/51	41/51	25/50	16/50	34/50	39/51	29/50	35/51	17/50	27/50	25/50	29/50	26/50
Renal tumours#	*0.43%*	*3.43%*	*6.0%*	1/49	2/50	0/50	0/51	0/51	2/50	0/49	0/50	0/51	0/50	0/51	1/50	0/50	0/50	2/50	3/50
Malignant lymphoma	*4.09%*	*1.45%*	*21.7%*	2/48	4/50	2/50	0/51	1/51	2/50	5/49	2/50	2/51	1/50	5/51	4/50	0/50	6/50	6/50	2/49
Haemangiosarcoma	*1.13%*	*1.67%*	*12.0%*	0/48	0/50	0/50	0/51	0/51	0/50	0/49	0/50	0/51	0/50	0/51	1/50	0/50	4/50	2/50	0/49

Study ID: A = [redacted] (1983, TOX9552381), re-evaluation; B = [redacted] (1993, TOX9552382); C = [redacted] (1997, ASB2012-11493); D = [redacted] (2009, ASB2012-11492).

Renal tumours: combined incidence of adenoma and carcinoma.

HC: Historical control data for Crl:CD-1 (ICR)BR mice ([redacted], 2000). The data was gathered from 51 studies of at least 78 weeks duration which were initiated between January 1987 and December 1996.

Mean: Mean (in percent of total); Min: Minimum (in percent found); Max: Maximum (in percent found).

NR: Not reported.

Haemangiosarcoma

In two studies in CD-1 mice, the incidences of haemangiosarcoma in male mice were reconsidered for statistical evaluation. In the first study ([redacted] (1993, TOX9552382), the combined incidences for haemangiosarcoma were 0, 0, 0 or 4 for the control, low, mid or high dose group. In the second study ([redacted] 1997, ASB2012-11493), the incidences for haemangiosarcoma were 0, 0, 0 or 2 for the control, low, mid or high dose group, respectively. For both studies, the statistical analysis with the Cochran-Armitage test for linear trend yielded a significant result, whereas the analysis by pair-wise comparisons (Fisher's exact test) indicated no statistically significant differences between the groups.

The background incidences for haemangiosarcoma in male CD-1 mice provided by [redacted] (2000; from 51 studies, initiated between 1987 and 1996) were up to 6/50 (12%) if multiple organs were considered, and were up to 5% or 8% in liver and spleen, respectively. Therefore, the conclusion of the study pathologists that the observed incidences for haemangiosarcoma were spontaneous and unrelated to treatment is supported by the RMS.

Pancreatic and other tumours

The statistically significant increase in pancreatic tumours incidences in the male rats of the low dose groups of [redacted] (1981, TOX2000-595, TOX2000-1997) and [redacted] (1990, TOX9300244) are considered incidental. With regard to the positive trend for liver cell adenoma in male rats and thyroid C-cell adenoma in females for the study of [redacted] (1990, TOX9300244), IARC noted lack of evidence for progression.

Malignant lymphoma

IARC did also consider a review article (Greim et al., 2015, ASB2015-2287) containing information on five long-term bioassay feeding studies in mice, in which a statistically significant increase in the incidence of malignant lymphoma was reported, but the IARC Working Group was unable to evaluate this study because of the limited experimental data provided in the review article and supplemental information.

In three studies in CD-1 mice, the incidences of malignant lymphoma in male mice were reconsidered for statistical evaluation. For the control, low, mid or high dose group, the respective incidences in the first study were 0, 2, 2 or 5 ([redacted]., 2009, ASB2012-11492), in the second study the incidences were 2, 2, 0, 6 ([redacted] 1997, ASB2012-11493), and in the third study the incidences were 4, 2, 1, 6 ([redacted]., 1993, TOX9552382). For the first and second study, the statistical analysis with the Cochran-Armitage trend test yielded a significant result, whereas the analysis by pair-wise comparisons (Fisher's exact test) indicated no statistically significant differences between the groups for all three studies.

A study in Swiss albino mice ([redacted] 2001, ASB2012-11491) was also reconsidered for statistical evaluation. The incidences in males were 10, 15, 16 or 19 for the control, low, mid or high dose group, respectively. Neither the Cochran-Armitage trend test nor the pair-wise comparisons using Fisher`s exact test yielded a significant result. However, using the Z-test, the pair-wise comparison between the control and high dose group gave a statistically significant result, as reported in the RAR.

For the assessment of the biological significance of these findings, it is important to consider that malignant lymphomas are among the most common spontaneously occurring neoplasms in the mouse. For the CD-1 mouse strain, incidences of up to 13/60 (21.7%) have been reported in male control groups. Thus, the incidences observed in the above studies, with a maximum of 6/50 (12%), were all within the historical control range. Also in the study with Swiss mice, which have considerably higher background incidences for malignant lymphomas, the observed incidences were within the historical control range. Therefore, the conclusion of the study pathologists that the observed malignant lymphomas were spontaneous and unrelated to treatment is supported by the RMS.

For an overall conclusion, the large volume of animal data for glyphosate should be evaluated using a weight of evidence approach. It should be avoided to base any conclusion only on the statistical

significance of an increased tumour incidence identified in a single study, without consideration of the biological significance of the finding.

In summary, based on the data from five carcinogenicity studies in mice and seven chronic toxicity and carcinogenicity studies in rats, the weight of evidence suggests that no hazard classification for carcinogenicity is warranted for glyphosate according to the CLP criteria.

5.4 Mechanistic and other relevant data

Glyphosate has been tested in a broad spectrum of mutagenicity and genotoxicity tests *in vitro* and *in vivo*. Taking into account all available data and using a weight of evidence approach, it is concluded that glyphosate does not induce mutations *in vivo* and no hazard classification for mutagenicity is warranted according to the CLP criteria.

AMPA has been tested for mutagenicity and genotoxicity *in vitro* and *in vivo* in an adequate range of assays. Taking into account all available data and using a weight of evidence approach, it is concluded that AMPA does not induce mutations *in vivo* and no hazard classification for mutagenicity is warranted according to the CLP criteria.

Glyphosate-based formulations have been extensively tested for mutagenicity and genotoxicity *in vitro* and *in vivo* in a wide range of assays. However, since formulation compositions are considered proprietary, the specific composition of the formulations tested was not available for the published studies. Positive results from *in vitro* chromosomal damage assays and tests for DNA strand breakage and SCE induction were reported in published studies. Also, for specific glyphosate-based formulations, *in vivo* mammalian chromosomal aberration or micronucleus assays as well as tests for DNA adducts, DNA strand breakage and SCE induction gave positive results in some published studies. However, no regulatory studies for these endpoints were provided. Thus, for the different glyphosate-based formulations, no firm conclusions can be drawn with regard to a need for classification according to the CLP criteria.

In general the documentation of the majority of studies on oxidative stress can be confirmed, but it is noted that there is a lack of positive controls for oxidative stress in all *in vitro* and *in vivo* studies described in the IARC monograph. From the available data on glyphosate, there is some indication of induction of oxidative stress from testing in human cell cultures and in mammalian (*in vivo*) experimental systems. In particular, the IARC statement that there are indications of oxidative stress in the blood plasma, liver, brain and kidney of rats upon exposure to glyphosate can be supported. However, only one of the cited studies investigated oxidative stress in animals with pure glyphosate. This study was conducted in rats and no other species was tested and increased oxidative stress was observed in combination with cytotoxic/degenerative effects of the targeted organs.

Considering the low level of metabolism and the chemical structure of glyphosate, glyphosate radical formation initiating oxidative stress appears unlikely. However, uncoupling or inhibition of mitochondrial oxidative phosphorylation also represents an established mechanism for ROS generation. Notably, uncoupling of oxidative phosphorylation by glyphosate has been reported in rat liver microsomes and a glyphosate formulation (but not glyphosate).

Induction of oxidative stress can provide a mechanistic explanation for any observed cytotoxic/ degenerative and indirectly genotoxic effects of substances. However, from the sole observation of oxidative stress and the existence of a plausible mechanism for induction of oxidative stress through uncoupling of mitochondrial oxidative phosphorylation alone, genotoxic or carcinogenic activity in humans cannot be deduced for glyphosate and glyphosate based formulations.

Furthermore, the RMS concludes that the evidence from available data do not allow to conclude that glyphosate caused immunosuppression.

Glyphosate was included into the U.S. EPA Endocrine Disruptor Screening Program's (EDSP). Which concluded that, based on the Tier 1 assays that had been performed at different independent laboratories and taking into account the 'higher tier' regulatory safety studies Glyphosate might not be considered an endocrine disrupter.

5.5 Further conclusions and recommendations

In result of the now available additional data and information on glyphosate formulations it is concluded and recommended:

- The data requirement for the evaluation and authorisation of plant protection products should be general verified and extended, in particular in consideration of possible genotoxic properties and effects caused by the mixture of different active substances or in combination with co-formulants. The described information on the genotoxicity of the different glyphosate formulations clearly shows that a prediction on the genotoxicity based on the single ingredients of a formulation according to the CLP-Regulation (ECHA, 2013, ASB2015-8592) is insufficient. Therefore, in general a specific data requirement for the evaluation and assessment of genotoxic properties of plant protection products is necessary.
- For the representative formulation for the EU renewal procedure 'Roundup Ultra' two studies (Guilherme et al., 2012, ASB2014-7619, Guilherme et al., 2014, ASB2015-8631) reported positive results in comet assays using the European eel as test species. According to Point 7.1.7 of Regulation (EU) No 284/2013 (EU, 2013, ASB2015-8658) the competent Authorities have to discuss case by case the need to perform supplementary studies. The RMS recommends further genotoxicity studies performed in compliance with OECD test guidelines for the representative formulation as confirmatory information for the authorisation of plant protection products.

6 Environmental risk assessment

In addition to the evaluation of the IARC monograph in terms of human health and classification aspects, the significance of the corresponding studies for the environmental risk assessment has also been assessed. Taking into account the results of existing reproduction studies in fish, the genotoxicological effects observed in biomarker studies with the active substance glyphosate listed in the IARC monograph are considered not to be manifest on the population level. However, the genotoxicological effects observed in some studies with POEA-containing glyphosate formulations are considered to be relevant for the authorisation of glyphosate products in the Member States.

7 References relied on

Number	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not
1	Acquavella, J.F., Alexander, B.H., Mandel, J.S., Gustin, C., Baker, B., Chapman, P., Bleeke, M.	2004	Glyphosate biomonitoring for farmers and their families: Results from the farm family exposure study Environmental Health Perspectives vol.112, 3 (2004) 321-326 GLP: No Published: Yes BVL-2309536, ASB2012-11528
2	Akanuma, M.	1995	HR-001: DNA Repair Test (Rec-Assay) IET 94-0141 GLP: Yes Published: No BVL-2309325, ASB2012-11477
3	Alavanja, M.C., Samanic, C., Dosemeci, M., Lubin, J., Tarone, R., Lynch, C.F., Knott, C., Thomas, K., Hoppin, J.A., Barker, J., Coble, J., Sandler, D.P., Blair, A.	2003	Use of agricultural pesticides and prostate cancer risk in the Agricultural Health Study cohort Am J Epidemiol vol.157, 9 (2003) 800-814 GLP: No Published: Yes BVL-2309554, ASB2012-11535
4	Alavanja, M.C.R.; Sandler, D.P.; McMaster, S.B.; Zahm, S.H.; McDonnell, C.J.; Lynch, C.F.; Pennybacker, M.; Rothman, N.; Dosemeci, M.; Bond, A.E.; Blair, A.	1996	The agricultural health study Environmental Health Perspectives, Volume 104, Number 4, April 1996 362-369 ASB2015-7849
5	Alvarez-Moya, C.; Reynoso Silva, M.; Valdez Ramírez, C.; et al.;	2014	Comparison of the in vivo and in vitro genotoxicity of Glyphosate Isopropylamine salt in three different organisms page 105-110 Genetics and Molecular Biology, 37, 1, 105-110 (2014) GLP: No Published: Yes BVL-2716311, ASB2014-6902
6	Andreotti, G., Freeman, L.E.B., Hou, L., Coble, J., Rusiecki, J., Hoppin, J.A., Silverman, D.T., Alavanja, M.C.R.	2009	Agricultural pesticide use and pancreatic cancer risk in the Agricultural Health Study Cohort International Journal of Cancer vol.124, 10 (2009) 2495-2500 GLP: No Published: Yes BVL-2309572, ASB2012-11544
7	Anon.	2005	Glyphosate Plate R121608 ! 3000081 ASB2015-7847
8	Astiz, M., de Alaniz, M.J.T., Marra, C.A.	2009	Antioxidant defense system in rats simultaneously intoxicated with agrochemicals Environmental Toxicology and Pharmacology vol.28, 3 (2009) 465-473 GLP: No Published: Yes BVL-2309584, ASB2012-11550
9	Astiz, M.; de Catalfo, G.; Garcia, M.	2013	Pesticide-induced decrease in rat testicular steroidogenesisis differentially prevented by lipoate and tocopherol page 129-138 Ecotoxicology and Environmental Safety 91 (2013) 129–138

Number	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not
			GLP: No Published: Yes BVL-2716336, ASB2014-7493
10	[redacted]	1993	Glyphosate: 104 week dietary carcinogenicity study in mice 7793 ! IRI 438618 GLP: Open (22) Yes (30) Published: No (29) Open (23) BVL-1345023, TOX9552382
11	[redacted]	1993	Glyphosate: 104 week combined chronic feeding/oncogenicity study in rats with 52 week interim kill (results after 104 weeks) IRI 438623 ! IRI 7867 ! Page: 1-1510 GLP: Open (20) Yes (12) Published: No (25) Open (7) BVL-1345018, TOX9750499
12	Bababunmi, E.A.; Olorunsogo, O.O.; Bassir, O.;	1979	The uncoupling effect of N-(phosphonomethyl)glycine on isolated rat liver mitochondria Biochem Pharmacol. 1979. 28(6):925-7 ASB2015-8535
13	Bailey, J.; Hauswirth, J.; Stump, D.;	2013	No evidence of endocrine disruption by Glyphosate in male and female pubertal assays. Abstract The Toxicologist. 52nd Annual Meeting and ToxExpo, March 10-14, 2013, Texas, USA. GLP: No Published: Yes BVL-2716229, ASB2013-3464
14	Bakke, J. P.	1991	Evaluation of the potential of AMPA to induce unscheduled DNA synthesis in the in vitro hepatocyte DNA repair assay using the male F-344 rats 2495-V01-91 ! SR-91-234 GLP: Open (8) Yes (32) Published: No (34) Open (6) BVL-1345048, TOX9552409
15	Benachour, N.; Sipahutar, H.; Moslemi, S.; Gasnier, C.; Travert, C.; Seralini, G. E.	2007	Time- and dose-dependent effects of Roundup on human embryonic and placental cells Page: 126-133 Arch. Environ. Contam. Toxicol. 53, 126–133 (2007) GLP: No Published: Yes BVL-1872545, ASB2009-9018
16	Bernal, J.; Bernal, J.L.; Martin, M.T.; Nozal, M.J.; Anadón, A.; Martínez-Larranaga, M.R.; Martínez, M.A.;	2010	Development and validation of a liquid chromatography–fluorescence–mass spectrometry method to measure glyphosate and aminomethylphosphonic acid in rat plasma doi:10.1016/j.jchromb.2010.10.013 Journal of Chromatography B, 878 (2010) 3290–3296 ASB2015-7858
17	BfR	2015	Does glyphosate cause cancer? Preliminary assessment of the carcinogenic risk of glyphosate with regard to the recent IARC evaluation ASB2015-8593
18	Blair, A.; Thomas, K.; Coble, J.; Sandler, D.P.; Hines, C.J.; Lynch, C.F.; Knott, C.; Purdue, M.P.; Zahm, S.H.; Alavanja, M.C.R.; Dosemeci, M.; Kamel, F.; Hoppin, J.A.; Freeman, L.B.; Lubin, J.H.;	2011	Impact of pesticide exposure misclassification on estimates of relative risks in the agricultural health study doi:10.1136/oem.2010.059469 Occup Environ Med. 2011 July ; 68(7): 537–541 ASB2015-7868
19	Blakley, B.R.	1996	Effect of Roundup and Tordon 202C herbicides on antibody production

Number	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not
			in mice PMID:9251167 Vet Hum Toxicol, 39(4):204–6 ASB2015-7878
20	Bolognesi, C.; Bonatti, S.; Degan, P. et al.	1997	Genotoxic activity of Glyphosate and its technical formulation Roundup page 1957-1962 J. Agric. Food Chem. 1997, 45, 1957-1962 GLP: No (2) Open (1) Published: Open (1) Yes (2) BVL-2309628, Z59299
21	Botero-Coy, A.M.; Ibánez, M.; Sancho, J.V.; Hernández, F.;	2012	Improvements in the analytical methodology for the residue determination of the herbicide glyphosate in soils by liquid chromatography coupled to mass spectrometry http://dx.doi.org/10.1016/j.chroma.2012.12.007 Journal of Chromatography A, 1292 (2013) 132– 141 ASB2015-7882
22	Botero-Coy, A.M.; Ibánez, M.; Sancho, J.V.; Hernández, F.;	2013	Direct liquid chromatography–tandem mass spectrometry determination of underivatized glyphosate in rice, maize and soybean http://dx.doi.org/10.1016/j.chroma.2013.07.037 Journal of Chromatography A, 1313 (2013) 157– 165 ASB2015-7883
23	[redacted]	2001	Glyphosate Acid: Two Year Dietary Toxicity and Oncogenicity Study in Rats CTL/PR1111 GLP: Yes Published: No BVL-2309368, ASB2012-11488
24	Brown, L. M.; Burmeister, L. F.; Everett, G. D.; Blair, A.	1993	Pesticide exposures and multiple myeloma in Iowa men GLP: No (4) Open (2) Published: Open (2) Yes (4) BVL-1884430, TOX2002-1000
25	Brown, L.M.; Blair, A.; Gibson, R.; Everett, G.D.; Cantor, K.P.; Schuman, L.M.; Burmeister, L.F.; Van Lier, S.F.; Dick, F.	1990	Pesticide exposures and other agricultural risk factors for leukemia among men in iowa and minnesota Cancer research (1990) 6586-6591 GLP: No Published: Yes TOX2003-999
26	Callander, R. D.	1988	Aminomethyl phosphonic acid: An evaluation of mutagenic potential using S. typhimurium and E. coli CTL/P/2206 ! 3C.1/3 GLP: Open (1) Yes (4) Published: No (4) Open (1) BVL-2154325, TOX9500043
27	Cantor, K.P.; Blair, A.; Everett, G.; Gibson, R.; Burmeister, L.F.; Brown, L.M., Schuman, L.; Dick, F.R.;	1992	Pesticides and Other Agricultural Risk Factors for Non-Hodgkin's Lymphoma among Men in Iowa and Minnesota Cancer Research 52. 2447-2455. May 1. 1992 ASB2015-7885
28	Carreon, T., Butler, M.A., Ruder, A.M., Waters, M.A., Davis-King, K.E., Calvert, G.M., Schulte, P.A., Connally, B., Ward, E.M., Sanderson, W.T., Heinemann, E.F., Mandel, J.S., Morten, R.F., Reding, D.J., Rosenmann, K.D., Talaska, G.	2005	Gliomas and farm pesticide exposure in women: The Upper Midwest Health Study Environmental Health Perspectives vol.113, 5 (2005) 546-551 GLP: No Published: Yes BVL-2309660, ASB2012-11585

Number	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not
29	[redacted]	1999	A micronucleus study in mice for glifosate técnico Nufarm RF-G12.79/99 GLP: Yes Published: No BVL-2309335, ASB2012-11482
30	Cattani, D.; de Liz Oliveira Cavalli, V. L.; Heinz Rieg, C. E. et al.	2014	Mechanisms underlying the neurotoxicity induced by Glyphosate-based herbicide in immature rat hippocampus: Involvement of glutamate excitotoxicity page 34-45 Toxicology 320 (2015) 34–45 GLP: No Published: Yes BVL-2716340, ASB2014-3919
31	Cavusoglu, K., Yapar, K., Oruç, E., Yalçin, E.	2011	Protective effect of Ginkgo biloba l. leaf extract against lyphosate toxicity in swiss albino mice page 1263-1272 J Med Food 14 (10) 2011, 1263–1272 GLP: No Published: Yes BVL-2309666, ASB2012-11588
32	Chan, P. C.; Mahler, J. F.	1992	NTP technical report on toxicity studies of Glyphosate administered in dosed feed to F344/N rats and B6C3F1 mice 92-3135 GLP: No (41) Open (16) Yes (1) Published: Open (16) Yes (42) BVL-1344981, TOX9551954
33	Chang, F.C.; Simcik, M.F.; Capel, P.D.;	2010	Occurrence and fate of the herbicide glyphosate and its degradate aminomethylphosphonic acid in the atmosphere doi:10.1002/etc.431 PMID:21128261 Environ Toxicol Chem, 30(3):548–55 ASB2015-7895
34	Chaufan, G.; Coalova, I.; Molina, M.	2014	Glyphosate commercial formulation causes cytotoxicity, oxidative effects, and apoptosis on human cells: Differences with its active ingredient page 29-38 International Journal of Toxicology 2014, Vol. 33(1) 29-38 GLP: No Published: Yes BVL-2716312, ASB2014-9314
35	Chaufan, G.; Coalova, I.; Ríos de Molina, M. C.;	2014	Glyphosate commercial formulation causes cytotoxicity, oxidative effects, and apoptosis on human cells: Differences with its active ingredient DOI: 10.1177/1091581813517906 International Journal of Toxicology, Vol. 33(1) 29-38 ASB2014-7616
36	Chen, M.-X.; Cao, Z.-Y.; Jiang, Y.; Zhu, Z.-W.;	2012	Direct determination of glyphosate and its major metabolite, aminomethylphosphonic acid, in fruits and vegetables by mixed-mode hydrophilic interaction/weak anion-exchange liquid chromatography coupled with electrospray tandem mass spectrometry Journal of Chromatography A, 1272 (2013) 90– 99 ASB2014-8087
37	Chruscielska, K.; Brzezinski, J.; Kita, K. et al.	2000	Glyphosate: Evaluation of chronic activity and possible far - reaching effects - Part 1. Studies on chronic toxicity Page: 11-19 Pestycydy, 2000, (3-4), 11-20. GLP: No Published: Yes BVL-2716174, ASB2013-9829

Number	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not
38	Clay, P.	1996	Glyphosate acid: L5178Y TK+/- mouse lymphoma mutation assay CTL/P/4991 ! VV 0123 GLP: Open (1) Yes (3) Published: No BVL-2154316, TOX2000-1994
39	Coalova, I.; Ríos de Molina, M. C.; Chaufan, G.;	2014	Influence of the spray adjuvant on the toxicity effects of a Glyphosate formulation page 1306-1311 Toxicology in Vitro 28 (2014) 1306–1311 GLP: No Published: Yes BVL-2716413, ASB2014-7615
40	Cocco, P.; Satta, G.; Dubois, S.; Pili, C.; Pilleri, M.; Zucca, M.; Martine 't Mannetje, A.; Becker, N.; Benavente, Y.; de Sanjosé, S.; Foretova, L.; Staines, A.; Maynadié, M.; Nieters, A.; Brennan, P.; Miligi, L.; Ennas, M. G.; Boffetta, P.;	2012	Lymphoma risk and occupational exposure to pesticides: results of the Epilymph study page 91-98 Occup Environ Med 2012;0:1–7 GLP: No Published: Yes BVL-2716321, ASB2014-7523
41	[redacted]	2010	Amendment No. 1 to report: Evaluation of the mutagenic potential of Glyphosate technical by micronucleus assay in mice 3996.402.395.07 BioagriI Laboratorios Ltda. GLP: Yes Published: No BVL-2715988, ASB2014-9284
42	[redacted]	2008	Evaluation of the mutagenic potential of Glyphosate technical by micronucleus assay in mice RF - 3996.402.395.07 GLP: Yes Published: No BVL-2309333, ASB2012-11481
43	Curwin, B.D., Hein, M.J., Sanderson, W.T., Nishioka, M.G., Reynolds, S.J., Ward, E.M., Alavanja, M.C.	2005	Pesticide contamination inside farm and nonfarm homes Journal of Occupational and Environmental Hygiene vol.2, 7 (2005) 357-367 GLP: No Published: Yes BVL-2309682, ASB2012-11595
44	Curwin, B.D., Hein, M.J., Sanderson, W.T., Striley, C., Heederik, D., Kromhout, H., Reynolds, S.J., Alavanja, M.C.	2007	Urinary pesticide concentrations among children, mothers and fathers living in farm and non-farm households in iowa doi:10.1093/annhyg/mel062 Ann Occup Hyg vol.51, 1 (2007) 53-65 GLP: No Published: Yes BVL-2309686, ASB2012-11597
45	Dallegrave, E., Mantese, F.D., Coelho, R.S., Pereira, J.D., Dalsenter, P.R., Langeloh, A.	2003	The teratogenic potential of the herbicide glyphosate-Roundup (R) in Wistar rats page 45-52 Toxicology Letters 142 (2003) 45-52 GLP: No Published: Yes BVL-2309692, ASB2012-11600
46	Dallegrave, E.; Mantese, F.D.; Oliveira, R.T.; Andrade A.J.; Dalsenter, P.R.; Langeloh, A.	2007	Glyphosat: Pre-and postnatal toxicity of the commercial glyphosate formulation in Wistar rats page 665-673 Arch Toxicol (2007) 81:665–673 GLP: No Published: Yes BVL-2309694, ASB2012-2721

Number	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not
47	De Roos, A.J., Blair, A., Rusiecki, J.A., et al.	2005	Cancer incidence among glyphosate-exposed pesticide applicators in the agricultural health study page 49-54 Environmental Health Perspectives, VOLUME 113, NUMBER 1 GLP: No Published: Yes BVL-2309704, ASB2012-11605
48	De Roos, A.J., Zahm, S.H., Cantor, K.P., Weisenburger, D.D., Holmes, F.F., Burmeister, L.F., Blair, A.	2003	Integrative assessment of multiple pesticides as risk factors for non-Hodgkin's lymphoma among men Occupational and Environmental Medicine vol.60, 9 (2003) GLP: No Published: Yes BVL-2309706, ASB2012-11606
49	De Roos, A.J.; Svec, M.A.; Blair, A.; Rusiecki, J.A.; Dosemeci, M.; Alavanja, M.C. et al.	2005	Glyphosate results revisited: De Roos et al. respond. Environ Health Perspect, 113(6):A366–7. doi:10.1289/ehp.113-a366 doi:10.1289/ehp.113-a366 Environmental Health Perspectives; vol. 113, number 6, June 2005, page a366 ASB2015-8437
50	Dennis, L.K.; Lynch, C.F.; Sandler, D.P.; Alavanja, M.C.	2010	Pesticide use and cutaneous melanoma in pesticide applicators in the Agricultural Health Study. doi:10.1289/ehp.0901518 ! PMID:20164001 Environ Health Perspect, 118(6):812–7. ASB2015-8439
51	[redacted]	2006	Glyphosate Technical: Micronucleus Test In The Mouse 2060/014 GLP: Yes Published: No BVL-2309327, ASB2012-11478
52	ECHA	2013	Guidance on the Application of the CLP Criteria. Guidance to Regulation (EC) No 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures ECHA-13-G-10-EN ASB2015-8592
53	ECHA	2015	Guidance on Information Requirements and Chemical Safety Assessment. Chapter R.7a: Endpoint specific guidance ASB2015-8657
54	el-Gendy, K.S.; Aly, N.M.; el-Sebae, A.H.	1998	Effects of edifenphos and glyphosate on the immune response and protein biosynthesis of bolti fish (Tilapia nilotica) doi:10.1080/03601239809373135 J Environ Sci Health B, 33(2):135–49 ASB2015-8422
55	Elie-Caille, C., Heu, C., Guyon, C., Nicod, L.	2010	Morphological damages of a glyphosate-treated human keratinocyte cell line revealed by a micro- to nanoscale microscopic investigation Cell Biol Toxicol vol.26, 4 (2010) 331-339 GLP: No Published: Yes BVL-2309714, ASB2012-11610
56	Engel, L.S., Hill, D.A., Hoppin, J.A., Lubin, J.H., Lynch, C.F., Pierce, J., Samanic, C., Sandler, D.P., Blair, A., Alavanja, M.C.	2005	Pesticide use and breast cancer risk among farmers' wives in the agricultural health study American Journal of Epidemiology vol.161, 2 (2005) 121-135 GLP: No Published: Yes BVL-2309720, ASB2012-11613

Number	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not
57	[redacted]	1997	HR-001: 24-Month Oral Chronic Toxicity and Oncogenicity Study in Rats, Vol. 1 (Seite 1-500) IET 94-0150 Vol.1 GLP: Yes Published: No BVL-2309360, ASB2012-11484
58	[redacted]	1997	HR-001: 24-Month Oral Chronic Toxicity and Oncogenicity Study in Rats, Vol. 2 (Seite 501-1000) IET 94-0150 Vol. 2 GLP: Yes Published: No BVL-2309362, ASB2012-11485
59	[redacted]	1997	HR-001: 24-Month Oral Chronic Toxicity and Oncogenicity Study in Rats, Vol.3 (Seite 1001-1500) IET 94-0150 Vol. 3 GLP: Yes Published: No BVL-2309364, ASB2012-11486
60	[redacted]	1997	HR-001: 24-Month Oral Chronic Toxicity and Oncogenicity Study in Rats, Vol. 4 (Seite 1501-2051) IET 94-0150 Vol. 4 GLP: Yes Published: No BVL-2309366, ASB2012-11487
61	EPA	1980	Glyphosate; Submission of rat teratology, rabbit teratology, dominant lethal mutagenicity assay in mice. EPA 1980a/103601/103601-090/661A Washington (DC): United States Environmental Protection Agency, Office of Toxic substances.; www.epa.gov/pesticides/chemicalsearch/chemical/foia/cleared-reviews/reviews/.pdf (1980) 1-6 ASB2015-8547
62	EPA	1992	Determination of glyphosate in drinking water by direct-aqueous-injection HPLC, post column derivatization, and fluorescence detection. In: Methods for the determination of organic compounds in drinking water - Supplement II. EPA/600/R-92–129 Washington (DC): Environmental Monitoring Systems Laboratory, Office of Research and Development, United States Environmental Protection Agency. Available through NTIS (http://www.ntis.gov) ASB2015-8424
63	Eriksson, M., Hardell, L., Carlberg, M., Akerman, M.	2008	Pesticide exposure as risk factor for non-Hodgkin lymphoma including histopathological subgroup analysis Int J Cancer vol.123, 7 (2008) 1657-1663 GLP: No Published: Yes BVL-2309722, ASB2012-11614
64	European Commission	2010	Guidance document on pesticide residue analytical methods SANCO/825/00 rev. 8.1 ASB2015-8438
65	European Union	2008	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 Official Journal of the European Union, L 353/1 ASB2015-8591

Number	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not
66	European Union	2009	Directive 2009/128/ec of the European Parliament and of the council of 21 october 2009 establishing a framework for community action to achieve the sustainable use of pesticides Official Journal of the European Union, L 309/71 ASB2015-8588
67	European Union	2009	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 Official Journal of the European Union, L 309/1 ASB2015-8589
68	European Union	2013	Commission Regulation (EU) No 284/2013 of 1 March 2013 setting out the data requirements for plant protection products, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market ASB2015-8658
69	FAO	2000	Glyphosate, N-(phosphonomethyl)glycine. Specifications and evaluations for plant protection products. Rome: Food and Agriculture Organization of the United Nations ASB2015-8587
70	Flower, K.B., Hoppin, J.A., Lynch, C.F., Blair, A., Knott, C., Shore, D.L., Sandler, D.P.	2004	Cancer risk and parental pesticide application in children of agricultural health study participants Environmental Health Perspectives vol.112, 5 (2004) 361-635 GLP: No Published: Yes BVL-2309734, ASB2012-11620
71	[redacted]	2009	Micronucleus Test of Glyphosate TC in Bone Marrow Cells of the CD Rat by oral administration LPT 23917 GLP: Yes Published: No BVL-2309329, ASB2012-11479
72	Forgacs, A.L., Ding, Q., Jaremba, R.G., Huhtaniemi, I.T., Rahman, N.A., Zacharewski, T.R.	2012	BLTK1 Murine Leydig Cells: A Novel Steroidogenic Model for Evaluating the Effects of Reproductive and Developmental Toxicants GLP: No Published: Yes BVL-2309736, ASB2012-11621
73	Fox, V.	1998	Glyphosate acid: In vitro cytogenetic assay in human lymphocytes CTL/P/6050 ! SV 0777 GLP: Open (1) Yes (3) Published: No BVL-2154314, TOX2000-1995
74	[redacted]	1996	Glyphosate acid: Mouse bone marrow micronucleus test CTL/P/4954 ! SM 0796 GLP: Open (1) Yes (3) Published: No BVL-2154317, TOX2000-1996
75	Gasnier, C.; Dumont, C.; Benachour, N.; Clair, E.; Chagnon, M. C.; Séralini, G. E.	2009	Glyphosate-based herbicides are toxic and endocrine disruptors in human cell lines Toxicology (2009)

Number	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not
			GLP: No Published: Yes BVL-1872547, ASB2009-7384
76	Gehin, A., Guillaume, Y.C., Millet, J., Guyon, C., Nicod, L.	2005	Vitamins C and E reverse effect of herbicide-induced toxicity on human epidermal cells HaCaT: a biochemometric approach International Journal of Pharmaceutics vol.288, 2 (2005) 219-226 GLP: No Published: Yes BVL-2309760, ASB2012-11826
77	George, J., Prasad, S., Mahmood, Z., Shukla, Y.	2010	Studies on glyphosate-induced carcinogenicity in mouse skin: a proteomic approach J Proteomics vol.73, 5 (2010) 951-964 GLP: No Published: Yes BVL-2309766, ASB2012-11829
78	George, J.; Shukla, Y.;	2013	Emptying of intracellular calcium pool and oxidative stress imbalance are associated with the Glyphosate-induced proliferation in human skin keratinocytes HaCaT cells http://dx.doi.org/10.1155/2013/825180 GLP: No Published: Yes BVL-2716313, ASB2014-8034
79	Germany	1998	glyphosate (Monograph) 11 Dezember 1998 GLP: Open Published: Yes ASB2010-10302
80	Germany; Slovakia;	2015	Glyphosate: Renewal Assessment Report, Volume 1-3 revised ASB2015-1194
81	Greim, H.; Saltmiras, D.; Mostert, V.; Strupp, C.;	2015	Evaluation of carcinogenic potential of the herbicide glyphosate, drawing on tumor incidence data from fourteen chronic/carcinogenicity rodent studies DOI: 10.3109/10408444.2014.1003423 Crit Rev Toxicol, 2015; 45(3): 185–208 ASB2015-2287
82	Guilherme, S.; Santos, M. A.; Barroso, C.; et al.;	2012	Differential genotoxicity of Roundup formulation and its constituents in blood cells of fish (Anguilla anguilla): considerations on chemical interactions and DNA damaging mechanisms DOI 10.1007/s10646-012-0892-5 Ecotoxicology (2012) 21:1381–1390 ASB2014-7619
83	Guilherme, S.; Santos, M.A.; Pacheco, M.;	2014	Are DNA-damaging effects induced by herbicide formulations (Roundup® and Garlon®) in fish transient and reversible upon cessation of exposure? http://dx.doi.org/10.1016/j.aquatox.2014.06.007 Aquatic Toxicology 155 (2014) 213–221 ASB2015-8631
84	Guyton, K.Z.; Loomis, D.; Grosse, Y.; El Ghissassi, F.; Benbrahim-Tallaa, L.; Guha, N.; Scoccianti, C.; Mattock, H.; Straif, K.;	2015	Carcinogenicity of Tetrachlorvinphos, Parathion, Malathion, Diazinon, and Glyphosate http://dx.doi.org/10.1016/S1470-2045(15)70134-8 ! pp 490-491 www.thelancet.com/oncology (2015) 490-491 ASB2015-7076
85	Hardell, L., Eriksson, M.	1999	A case-control study of non-Hodgkin lymphoma and exposure to pesticides

Number	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not
			Cancer vol.85, 6 (1999) 1353-1360 GLP: No Published: Yes BVL-2309788, ASB2012-11838
86	Hardell, L., Eriksson, M., Nordstrom, M.	2002	Exposure to pesticides as risk factor for non-Hodgkin's lymphoma and hairy cell leukemia: Pooled analysis of two Swedish case-control studies page 1043-1049 Leukemia and Lymphoma, 2002 VoI. 43 5), pp. 1043-1049 GLP: No Published: Yes BVL-2309790, ASB2012-11839
87	Hardell, L.; Eriksson, M.	1999	A case-control study of non-hodgkin lymphoma and exposure to pesticides Cancer (1999) 1353-1360 GLP: No (2) Open (1) Published: Open (1) Yes (2) TOX1999-686
88	Hidalgo, C.; Rios, C.; Hidalgo, M.; Salvadó, V.; Sancho, J.V.; Hernández, F.	2004	Improved coupled-column liquid chromatographic method for the determination of glyphosate and aminomethylphosphonic acid residues in environmental waters. doi:10.1016/j.chroma.2004.02.044 PMID:15117086 Journal Chromatogr A, 1035(1):153–157 ASB2015-8423
89	Hoar Zahm, S.; Weisenburger, D. D.; Babbitt, P. A. et al.	1990	A case control study of non-Hodgkin's lymphoma and the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) in Eastern Nebraska. 349-356 Epidemiology 1990;1:349-356 ASB2013-11501
90	[redacted]	2008	Glyphosate Technical - Micronucleus Assay in Bone Marrow Cells of the Mouse 1158500 GLP: Yes Published: No BVL-2309339, ASB2012-11483
91	IARC	2015	Glyphosate. IARC Monographs - 112 ASB2015-8421
92	Jan, M.R.; Shah, J.; Muhammad, M.; Ara, B.;	2009	Glyphosate herbicide residue determination in samples of environmental importance using spectrophotometric method doi:10.1016/j.jhazmat.2009.04.003 Journal of Hazardous Materials 169 (2009) 742–745 ASB2015-8285
93	Jasper, R.; Locatelli, G. O.; Pilati, C. et al.	2012	Evaluation of biochemical, hematological and oxidative parameters in mice exposed to the herbicide Glyphosate-Roundup page 133-140 Interdiscip Toxicol. 2012; Vol. 5(3): 133–140 GLP: No Published: Yes BVL-2716432, ASB2014-9583
94	Jauhiainen, A.; Räsänen, K.; Sarantila, R.; Nuutinen, J.; Kangas, J.	1991	Occupational exposure of forest workers to Glyphosate during brush saw spraying work GLP: No (9) Open (32) Published: Open (6) Yes (35) BVL-1345073, MET9600092
95	[redacted]	1991	Mutagenicity test: Micronucleus test with Glyphosate, batch 206-JaK-

Number	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not
			25-1 12324 GLP: Open (7) Yes (29) Published: No (28) Open (8) BVL-1345016, TOX9552374
96	[redacted]	1991	Mutagenicity test: In vitro mammalian cell gene mutation test with Glyphosate, batch 206-JaK-25-1 12325 GLP: Open (7) Yes (30) Published: No (29) Open (8) BVL-1345007, TOX9552372
97	[redacted]	1993	Mutagenicity test: Micronucleus test with AMPA, batch 286-JRJ-73-4 13268 GLP: No (1) Open (7) Yes (29) Published: No (29) Open (8) BVL-1345045, TOX9300379
98	[redacted]	1993	Mutagenicity test: Ames salmonella test with AMPA, batch 286-JRJ-73-4 13269 GLP: Open (7) Yes (30) Published: No (29) Open (8) BVL-1345047, TOX9300378
99	[redacted]	1993	AMPA, batch 286-JRJ-73-4: Mutagenicity test: In vitro mammalian cell gene mutation test performed with mouse lymphoma cells (L5178Y) 13270 GLP: Open (7) Yes (30) Published: No (29) Open (8) BVL-1345046, TOX9300380
100	Johnson, P.D., Rimmer, D.A., Garrod, A.N., Helps, J.E., Mawdsley, C.	2005	Operator exposure when applying amenity herbicides by all-terrain vehicles and controlled droplet applicators nn Occup Hyg vol.49, 1 (2005) 25-32 GLP: No Published: Yes BVL-2309832, ASB2012-11859
101	Kachuri, L.; Demers, P. A.; Blair, A. et al.	2013	Multiple pesticide exposures and the risk of multiple myeloma in Canadian men DOI: 10.1002/ijc.28191 ! page 1846-1858 Int. J. Cancer: 133, 1846–1858 (2013) GLP: No Published: Yes BVL-2716322, ASB2014-8030
102	Karunanayake, C.P., Spinelli, J.J., McLaughlin, J.R., Dosman, J.A., Pahwa, P., McDuffie, H.H.	2011	Hodgkin Lymphoma and Pesticides Exposure in Men: A Canadian Case-Control Study Journal of Agromedicine vol.17, 1 (2011) 30-39 GLP: No Published: Yes BVL-2309844, ASB2012-11865
103	Kier, L. D.; Kirkland, D. J.	2013	Review of genotoxicity studies of Glyphosate and Glyphosate-based formulations page 284-315 Crit Rev Toxicol, 2013; 43(4): 283–315 GLP: No Published: Yes BVL-2716380, ASB2014-9587
104	Kier, L. D.; Stegeman, S. D.	1993	Mouse micronucleus study of AMPA EHL-90 170 / ML-90-404 ! MSL-13243 GLP: Open (8) Yes (31) Published: No (33) Open (6) BVL-1345044, TOX9552413

Number	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not
105	[redacted]	1983	A chronic feeding study of Glyphosate (Roundup technical) in mice 77-2061 ! (BDN-77-420) GLP: No (35) Open (64) Published: No (37) Open (62) BVL-1345024, TOX9552381
106	Kojima, H.; Katsura, E.; Takeuchi, Sh.; Niiyama, K.; Kobayashi, K.	2004	Screening for estrogen and androgen receptor activities in 200 pesticides by in vitro reporter gene assays using Chinese hamster ovary cells 2004/1036097 Environmental Health Perspectives Volume 112, Number 5, April 2004 GLP: No Published: Yes BVL-1923824, ASB2010-14389
107	Kojima, H.; Takeuchi, S.; Nagai, T.;	2010	Endocrine-disrupting Potential of Pesticides via Nuclear Receptors and Aryl Hydrocarbon Receptor Journal of Health Science, 56(4) 374–386 ASB2015-7815
108	Koller, V. J.; Fürhacker, M.; Nersesyan, A. et al.	2012	Cytotoxic and DNA-damaging properties of Glyphosate and Roundup in human-derived buccal epithelial cells DOI 10.1007/s00204-012-0804-8 Arch Toxicol (2012) 86: 805–813 GLP: No Published: Yes BVL-2716316, ASB2014-7618
109	Kreutz, L.C.; Barcellos, L.J.G.; de Faria Valle, S.; de Oliveira Silva, T.; Anziliero, D.; dos Santos, E.D.; Pivato, M.; Zanatta, R.;	2010	Altered hematological and immunological parameters in silver catfish (Rhamdia quelen) following short term exposure to sublethal concentration of glyphosate doi:10.1016/j.fsi.2010.09.012 Fish & Shellfish Immunology 30 (2011) 51e57 ASB2015-8279
110	[redacted]	2001	Carcinogenicity Study with Glyphosate Technical in Swiss Albino Mice Toxi: 1559.CARCI-M GLP: Yes Published: No BVL-2309396, ASB2012-11491
111	Kumar, S.; Khodoun, M.; Kettleson, E.M.; McKnight, C.; Reponen, T.; Grinshpun, S.A.; Adhikari, A.;	2014	Glyphosate-rich air samples induce IL-33, TSLP and generate IL-13 dependent airway inflammation Toxicology 325 (2014) 42–51 ASB2015-8276
112	Kwiatkowska, M.; Huras, B.; Bukowska, B.	2014	The effect of metabolites and impurities of Glyphosate on human erythrocytes (in vitro) page 34-43 Pesticide Biochemistry and Physiology 109 (2014) 34–43 GLP: No Published: Yes BVL-2716440, ASB2014-9603
113	Kyomu, M.	1995	HR-001: In vitro cytogenetics test IET 94-0143 GLP: Yes Published: No BVL-2309317, ASB2012-11475
114	Landgren, O., Kyle, R.A., Hoppin, J.A., Freeman, L.E.B., Cerhan, J.R., Katzmann, J.A., Rajkumar, S.V., Alavanja, M.C.	2009	Pesticide exposure and risk of monoclonal gammopathy of undetermined significance in the Agricultural Health Study DOI 10.1182/blood-2009-02-203471 GLP: No Published: Yes BVL-2309874, ASB2012-11875

Number	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not
115	[redacted]	1981	A lifetime feeding study of Glyphosate in rats - Data evaluation report 77-2062 GLP: No (1) Open (1) Yes (1) Published: No BVL-2154319, TOX2000-1997
116	[redacted]	1981	Lifetime feeding study of Glyphosate (Roundup technical) in rats 77-2062 ! BDN-77-416 GLP: No (1) Open (5) Published: No BVL-2309378, TOX2000-595
117	Lavy, T. L.; Cowell, J. E.; Steinmetz, J. R.; Massey, J. H.	1992	Conifer seedling nursery worker exposure to glyphosate GLP: Open Published: Open (5) Yes (4) BVL-2332597, TOX9650912
118	Lee, E.A.; Strahan, A.P.; Thurman, E.M.;	2002	Methods of Analysis by the U.S. Geological Survey Organic Geochemistry Research Group—Determination of Glyphosate, Aminomethylphosphonic Acid, and Glufosinate in water using online solid-phase extraction and High-Performance Liquid Chromatography/Mass Spectrometry 01–454 U.S. Department of the Interior, Lawrence, Kansas ASB2015-8239
119	Lee, W.J., Colt, J.S., Heineman, E.F., McComb, R., Weisenburger, D.D., Lijinsky, W., Ward, M.H.	2005	Agricultural pesticide use and risk of glioma in Nebraska, United States Occupational and Environmental Medicine vol.62 (2005) 786-792 GLP: No Published: Yes BVL-2309886, ASB2012-11882
120	Lee, W.J., Lijinsky, W., Heineman, E.F., Markin, R.S., Weisenburger, D.D., Ward, M.H.	2004	Agricultural pesticide use and adenocarcinomas of the stomach and oesophagus Occupational and Environmental Medicine 61 (9):743-749 vol.61, 9 (2004) 743-749 GLP: No Published: Yes BVL-2309888, ASB2012-11883
121	Lee, W.J.; Cantor, K.P.; Berzofsky, J.A.; Zahm, S.H.; Blair, A.;	2004	Non-Hodgkin's lymphoma among asthmatics exposed to pesticides DOI 10.1002/ijc.20273 Int. J. Cancer: 111, 298–302 (2004) ASB2015-8238
122	Lee, W.J.; Sandler, D.P.; Blair, A.; Samanic, C.; Cross, A.J.; Alavanja, M.C.R.;	2007	Pesticide use and colorectal cancer risk in the Agricultural Health Study doi:10.1002/ijc.22635 Int J Cancer. 2007 July 15; 121(2): 339–346 ASB2015-8228
123	Levine, S.	2012	EDSP assays and regulatory safety studies provide a weight of evidence that Glyphosate is not an endocrine disruptor page 128 ASB2014-9609
124	[redacted]	1983	CHO/HGPRT gene mutation assay with Glyphosate ML-83-155 ! 830079 GLP: Open (7) Yes (30) Published: No (31) Open (6) BVL-1345008, TOX9552369
125	[redacted]	1983	In vivo bone marrow cytogenetics study of Glyphosate in Sprague-Dawley rats ML-83-236 ! 830083

Number	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not
			GLP: Open (7) Yes (28) Published: No (29) Open (6) BVL-1345015, TOX9552375
126	[redacted]	1984	CHO/HGPRT gene mutation assay with Glyphosate - Amendment MSL-3212 ! 830079 ! ML-83-155 GLP: Open Published: No Z35243
127	Li, A. P.; Long, T. J.	1988	An evaluation of the genotoxic potential of Glyphosate Page: 537-546 ! L 361 GLP: No (10) Open (5) Published: Open (5) Yes (10) BVL-2146649, TOX9500253
128	Lioi, M. B.; Scarfi, M. R.; Santoro, A. et al.	1998	Genotoxicity and oxidative stress induced by pesticide exposure in bovine lymphocyte cultures in vitro Page: 13-20 Mutation Research 403 1998. 13–20. GLP: No Published: Yes BVL-2716170, ASB2013-9836
129	Lueken, A., Juhl-Strauss, U., Krieger, G., Witte, I.	2004	Synergistic DNA damage by oxidative stress (induced by H2O2) and nongenotoxic environmental chemicals in human fibroblasts Toxicology Letters vol.147, 1 (2004) 35-43 GLP: No Published: Yes BVL-2309896, ASB2012-11886
130	Manas, F., Peralta, L., Raviolo, J., Ovando, H.G., Weyers, A., Ugnia, L., Cid, M.G., Larripa, I., Gorla, N.	2009	Genotoxicity of AMPA, the environmental metabolite of glyphosate, assessed by the Comet assay and cytogenetic tests Ecotoxicology and Environmental Safety vol.72, 3 (2009) 834-837 GLP: No Published: Yes BVL-2309906, ASB2012-11891
131	Manas, F.; Peralta, L.; Raviolo, J.; Ovando, H. G.; Weyers, A.; Ugnia, L.; Gonzalez Cid, M.; Larripa, I.; Gorla, N.	2009	Genotoxicity of Glyphosate assessed by the comet assay and cytogenetic tests page 37-41 Genotoxicity of glyphosate assessed by the comet assay and cytogenetic tests GLP: No Published: Yes BVL-2309908, ASB2012-11892
132	Mañas, F.; Peralta, L.; Ugnia, L. et al.	2013	Oxidative stress and comet assay in tissues of mice administered Glyphosate and Ampa in drinking water for 14 days page 67-75 Journal of Basic & Applied Genetics GLP: No Published: Yes BVL-2716300, ASB2014-6909
133	McDuffie, H.H., Pahwa, P., McLaughlin, J.R., Spinelli, J.J., Fincham, S., Dosman, J.A., Robson, D., Skinnider, L.F., Ch	2001	Non-Hodgkin's lymphoma and specific pesticide exposures in men: cross Canada study of pesticides and health CanEpi 10:1155-1163 Cancer Epidemiol Biomarkers Prev vol.10, 11 (2001) 1155-1163 GLP: No Published: Yes BVL-2009742, ASB2011-364
134	[redacted]	1996	Glyphosate acid: One year dietary toxicity study in rats CTL/P/5143 ! PR 1012 GLP: Open (1) Yes (3) Published: No BVL-2154318, TOX2000-1998

Number	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not
135	Mink, P. J.; Mandel, J. S.; Sceurman, B. K. et al.	2012	Epidemiologic studies of Gyphosate and cancer: A review page 440-452 Regulatory Toxicology and Pharmacology 63 (2012) 440–452 GLP: No Published: Yes BVL-2716296, ASB2014-9617
136	Mladinic, M., Berend, S., Vrdoljak, A.L., Kopjar, N., Radic, B., Zeljezic, D.	2009	Evaluation of genome damage and its relation to oxidative stress induced by glyphosate in human lymphocytes in vitro Environmental and Molecular Mutagenesis vol.50, 9 (2009) 800-807 GLP: No Published: Yes BVL-2309942, ASB2012-11906
137	Mladinic, M., Perkovic, P., Zeljezic, D.	2009	Characterization of chromatin instabilities induced by glyphosate, terbuthylazine and carbofuran using cytome FISH assay Toxicol Lett vol.189, 2 (2009) 130-137 GLP: No Published: Yes BVL-2309944, ASB2012-11907
138	Monge, P., Wesseling, C., Guardado, J., Lundberg, I., Ahlbom, A., Cantor, K.P., Weideroass, E., Partanen, T.	2007	Parental occupational exposure to pesticides and the risk of childhood leukemia in Costa Rica Scandinavian Journal of Work Environment & Health vol.33, 4 (2007) 293-303 GLP: No Published: Yes BVL-2309948, ASB2012-11909
139	Monroy, C.; Cortes, A.; Sicard, D. et al.	2005	Cytotoxicity and genotoxicity of human cells exposed in vitro to glyphosate page 335-345 GLP: No Published: Yes BVL-2309950, ASB2012-11910
140	Motojyuku, M.; Saito, T.; Akieda, K.; Otsuka, H.; Yamamoto, I.; Inokuchi, S.;	2008	Determination of glyphosate, glyphosate metabolites, and glufosinate in human serum by gas chromatography-mass spectrometry. doi:10.1016/j.jchromb.2008.10.003 J Chromatogr B Analyt Technol Biomed Life Sci, 875(2):509–14. ASB2015-8160
141	Nakashima, K., Yoshimura, T., Mori, H., Kawaguchi, M., Adachi, S., Nakao, T., Yamazaki, F.	2002	Effects of pesticides on cytokines production by human peripheral blood mononuclear cells-fenitrothion and glyphosate Chudoku Kenkyu vol.15, 2 (2002) 159-165 GLP: No Published: Yes BVL-2309968, ASB2012-11919
142	Nedelkoska, T.V.; Low, G.K.C.;	2004	High-performance liquid chromatographic determination of glyphosate in water and plant material after pre-column derivatisation with 9-fluorenylmethyl chloroformate doi:10.1016/j.aca.2004.01.027 Analytica Chimica Acta 511 (2004) 145–153 ASB2015-8134
143	[redacted]	2002	Measurement of unscheduled DNA synthesis (UDS) in rat hepatocytes in vitro procedure with AMPA (Amino methyl phosphonic acid) IPL-R 020625 GLP: Yes Published: No BVL-2309480, ASB2012-11508
144	Niemann, L.; Sieke, C.; Pfeil, R.; Solecki, R.;	2015	A critical review of glyphosate findings in human urine samples and comparison with the exposure of operators and consumers

Number	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not
			DOI 10.1007/s00003-014-0927-3 J. Verbr. Lebensm., (2015) 10:3-12 ASB2014-11029
145	Nordström, M.; Hardell, L.; Magnuson, A.; Hagberg, H.; Rask-Andersen, A.	1998	Occupational exposures, animal exposure and smoking as risk factors for hairy cell leukaemia evaluated in a case-control study Page: 2048-2052 British Journal of Cancer (1998) 77(11), 2048-2052. GLP: No Published: Yes BVL-2716207, TOX1999-687
146	OECD	2002	Guidance notes for analysis and evaluation of chronic toxicity and carcinogenicity studies OECD Environment, Health and Safety Publications. Series on Testing and Assessment No. 35 and Series on Pesticides No. 14. ENV/JM/MONO (2002)19 GLP: No Published: Yes BVL-2716073, ASB2013-3754
147	OECD	2012	Guidance document 116 on the conduct and design of chronic toxicity and carcinogenicity studies, supporting test guidelines 451, 452 and 453 2nd edition ENV/JM/MONO(2011)47 ASB2015-8445
148	Omran, N. E.; Salama, W. M.;	2013	The endocrine disrupter effect of atrazine and glyphosate on Biomphalaria alexandrina snails DOI: 10.1177/0748233713506959 Toxicol Ind Health published online ASB2014-7614
149	Orsi, L., Delabre, L., Monnereau, A., Delval, P., Berthou, C., Fenaux, P., Marit, G., Soubeyran, P., Huguet, F., Milpied, N., Leporrier, M., Hemon, D., Troussard, X., Clavel, J.	2009	Occupational exposure to pesticides and lymphoid neoplasms among men: results of a French case-control study Occupational and Environmental Medicine vol.66, 5 (2009) 291-298 GLP: No Published: Yes BVL-2309992, ASB2012-11985
150	Paganelli, A.; Gnazzo, V.; Acosta, H. et al.	2010	Glyphosate-based herbicides produce teratogenic effects on vertebrates by impairing retinoic acid signaling page 1586-1595 Chem. Res. Toxicol. 2010, 23, 1586–1595 GLP: No Published: Yes BVL-2309994, ASB2010-11410
151	Pahwa, P. P.; Karunanayak, C. P.; Dosman, J. A. et al.	2011	Soft-tissue sarcoma and pesticides exposure in men results of a canadian case-control study page 1279-1286 JOEM, Volume 53, Number 11, November 2011 GLP: No Published: Yes BVL-2716393, ASB2014-9625
152	[redacted]	2012	Micronucleus test of Glyphosate TGAI in mice 120709 ! 485-1-06-4696 ! DR-0112-6927-003 ! 10001701-27-1 [redacted] GLP: Yes Published: No BVL-2715972, ASB2014-9277
153	Peixoto, F.	2005	Comparative effects of the Roundup and glyphosate on mitochondrial oxidative phosphorylation

Number	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not
			Chemosphere vol.61, 8 (2005) 1115-1122 GLP: No Published: Yes BVL-2310010, ASB2012-11994
154	Peluso, M.; Munnia, A.; Bolognesi, C.; Parodi, S.	1997	32P-Postlabeling detection of DNA adducts in mice treated with the herbicide Roundup page 55-59 Environmental and Molecular Mutagenesis 31:55±59 (1998) GLP: No (2) Open (3) Published: Open (1) Yes (4) BVL-2310014, TOX1999-318
155	Phaneuf, D.; Weber, J.-P.;	1988	Étude de l'exposition professionnelle des travailleurs forestiers exposés au glyphosate WA 240.5 Centre de Toxicologie du Québec ASB2015-7889
156	Rank, J.; Jensen, A. G.; Skov, B. et al.	1992	Genotoxicity testing of the herbicide roundup and its active ingredient glyphosate isopropylamine using the mouse bone marrow micronucleus test, Salmonella mutagenicity test, and Allium anaphase-telephase test Mutat. Res. (1992) 29-36 GLP: Open Published: Open Z82234
157	Richard, S.; Moslemi, S.; Sipahutar, H.; Benachour, N.; Seralani, G. E.,	2005	Differential effects of Glyphosate and Roundup on human placental cells and aromatase 0601494 GLP: No Published: No (6) Yes (2) BVL-1872374, ASB2009-9024
158	Rossberger, St.	1994	Glyphosat: DNA repair test with primary rat hepatocytes 931564 ! 94-03-28 ro GLP: Open (4) Yes (7) Published: No (6) Open (5) BVL-2327069, TOX9400697
159	[redacted]	2012	Glyphosate technical - Micronucleus assay in bone marrow cells of the mouse 1479200 ! TK0112981 Harlan Cytotest Cell Research GmbH (Harlan-CCR) GLP: Yes Published: No BVL-2716029, ASB2014-9333
160	Roustan, A.; Aye, M.; De Meo, M.; Di Giorgio, C.;	2014	Genotoxicity of mixtures of glyphosate and atrazine and their environmental transformation products before and after photoactivation Chemosphere 108 (2014) 93–100 ASB2014-8086
161	Ruder, A.M.; Waters, M.A.; Butler, M.A.; Carreón, T.; Calvert, G.M.; Davis-king, K.E.; Schulte, P.A.; Sanderson, W.T.; Ward, E.M; Connally, L.B.; Heineman, E.F.; Mandel, J.S.; Morton, R.F.; Reding, D.J.; Rosenman, K.D.; Talaska, G.	2010	Gliomas and farm pesticide exposure in men: The upper midwest health study DOI: 10.1080/00039890409602949 Archives of Environmental Health: An International Journal, 59:12, 650-657 ASB2015-8078
162	Schinasi, L.; Leon, M. E.;	2014	Non-Hodgkin lymphoma and occupational exposure to agricultural pesticide chemical groups and active ingredients: A systematic review and meta-analysis doi:10.3390/ijerph110404449 ASB2014-4819

Number	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not
163	Séralini, G. E.; Clair, E.; Mesnage, R.; Gress, S.; Defarge, N.; Malatesta, M.; Hennequin, D.; Spiroux de Vendomois, J.	2012	Long term toxicity of a Roundup herbicide and a Roundup-tolerant genetically modified maize Page: 4221-4231 Food and Chemical Toxicology 50 (2012) 4221–4231 GLP: No Published: Yes BVL-2716397, ASB2012-15514
164	[redacted]	1991	Assessment of acute toxicity of "Glyphosate technical" after intraperitoneal administration to rats 12322 GLP: Open (1) Yes (2) Published: No (1) Open (2) TOX9300330
165	Sorahan, T.;	2015	Multiple myeloma and Glyphosate use: A re-analysis of US Agricultural Health Study (AHS) data doi:10.3390/ijerph120201548 Int. J. Environ. Res. Public Health 2015, 12, 1548-1559 ASB2015-2284
166	[redacted]	1990	Chronic study of Glyphosate administered in feed to albino rats - Appendix 1-6 MSL 10495 ! ML-87-148 GLP: Open (48) Yes (44) Published: No (35) Open (57) BVL-1345021, TOX9300244
167	[redacted]	1997	HR-001: 18-Month Oral Oncogenicity Study in Mice IET 940151 GLP: Yes Published: No BVL-2309415, ASB2012-11493
168	[redacted]	1996	Combined chronic toxicity and carcinogenicity study with Glyphosate technical in Wistar rats TOXI-886/1996 ! ES-GPT-C.C-R ! TOXI 886.C.C-R GLP: No (2) Open (6) Yes (3) Published: No (8) Open (3) BVL-2309343, TOX9651587
169	[redacted]	1992	Glyphosate technical (FSG 03090 H/05, March 1990): Dominant lethal test in wistar rats 888-DLT ! TOXI-888/1992 ! ES-GPT-DLT GLP: Open (4) Yes (6) Published: No (7) Open (3) BVL-2327264, TOX9551102
170	[redacted]	1994	Glyphosate technical (FSG 03090 H/05 March 1990): Genetic toxicology - In vivo mammalian bone marrow cytogenetic test 890-MUT-CH.AB ! TOXI-890/1993 ! ES-GPT-MUT-CH.AB GLP: Open (4) Yes (7) Published: No (6) Open (5) BVL-2327261, TOX9400323
171	[redacted]	1993	Glyphosate technical (FSG 03090 H/05 March 1990): Mutagenicity-micronucleus test in swiss albino mice 889-MUT.MN ! TOXI-889/1993 ! ES-GPT-MUT-MN GLP: Open (4) Yes (5) Published: No (6) Open (3) BVL-2327258, TOX9551100
172	Takeuchi, S., Iida, M., Yabushita, H. et al.	2008	In vitro screening for aryl hydrocarbon receptor agonistic activity in 200 pesticides using a highly sensitive reporter cell line, DR-EcoScreen cells, and in vivo mouse liver cytochrome P450-1A induction by Propanil, Diuron and Linuron 2011/1262291 Chemosphere vol.74 (2008) 155-165 GLP: No Published: Yes BVL-2377232, ASB2013-6443

Number	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not
173	Thongprakaisang, S.; Thiantanawat, A.; Rangkadilok, N.; Suriyo, T.; Satayavivad, J.;	2013	Glyphosate induces human breast cancer cells growth via estrogen receptors page 129–136 GLP: No Published: Yes BVL-2716291, ASB2013-11991
174	Uchida, M.; Takumi, S.; Tachikawa, K.; Yamauchi, R.; Goto, Y.; Matsusaki, H.; Nakamura, H.; Kagami, Y.; Kusano, T.; Arizono, K.;	2012	Toxicity evaluation of glyphosate agrochemical components using Japanese medaka (Oryzias latipes) and DNA microarray gene expression analysis. J Toxicol Sci. 2012; 37(2):245-54 ASB2015-8590
175	Vainio, H. et al.	1983	Hypolipidemia and peroxisome proliferation induced by phenoxyacetic acid herbicides in rats Biochem. Pharmacol. (1983) 2775-2779 GLP: Open Published: Open Z31881
176	van de Waart, E. J.	1995	Evaluation of the ability of Glyfosaat to induce chromosome aberrations in cultured peripheral human lymphocytes (with independent repeat) 141918 GLP: No (2) Open (6) Yes (1) Published: No (5) Open (4) BVL-2146653, TOX9651525
177	Varona, M.; Henao, G.L.; Díaz, S.; Lancheros, A.; Murcia, A.; Rodríguez, N.; Alvarez, V.H.;	2009	Evaluación de los efectos del glifosato y otros plaguicidas en la salud humana en zonas objeto del programa de erradicación de cultivos ilícitos Biomedica 2009;29:456-75 ASB2015-8039
178	Vasiluk, L., Pinto, L.J., Moore, M.M.	2005	Oral bioavailability of glyphosate: Studies using two intestinal cell lines Environmental Toxicology and Chemistry vol.24, 1 (2005) 153-160 GLP: No Published: Yes BVL-2310112, ASB2012-12043
179	[redacted]	1982	Acute inhalation toxicity of Roundup formulation to male and female Sprague-Dawley rats - incl. Amendment No. 1, Date: 15.12.1982 810093 ! ML-81-201 GLP: Open (2) Yes (3) Published: No TOX2002-693
180	[redacted]	1983	Four-week study of 33-1/3% use-dilution of Roundup in water administered to male and female Sprague-Dawley rats by inhalation 830025 ! ML-83-015 GLP: Open (1) Yes (3) Published: No TOX2002-694
181	Waddell, B.L.; Zahm, S.H.; Baris, D.; Weisenburger, D.D.; Holmes, F.; Burmeister, L.F.; Cantor, K.P.; Blair, A.;	2001	Agricultural use of organophosphate pesticides and the risk of non-Hodgkin's lymphoma among male farmers (United States) doi:10.1023/A:1011293208949 PMID:11519759 Cancer Causes Control, 12(6):509–17 ASB2015-8037
182	Walsh, L. P.; McCormick, C.; Martin, C. et al.	2000	Roundup inhibits steroidogenesis by disrupting steroidogenic acute regulatory (StAR) protein expression page 769-776 Environ Health Perspect 108: 769–776 (2000) GLP: No Published: Yes

Number	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not
			BVL-2310118, ASB2012-12046
183	WHO	2006	IARC Monographs on the evaluation of carcinogenic risks to humans ASB2015-8291
184	Williams, G.M., Kroes, R., Munro, I.C.	2000	Safety evaluation and risk assessment of the herbicide Roundup and its active ingredient, glyphosate, for humans Regulatory Toxicology and Pharmacology vol.31, 2 (2006) 117-165 GLP: No Published: Yes BVL-2310132, ASB2012-12053
185	[redacted]	2009	Glyphosate Technical: Dietary combined chronic toxicity / carcinogenicity study in the rat SPL2060-0012 GLP: Yes Published: No BVL-2309391, ASB2012-11490
186	[redacted]	2009	Glyphosate Technical: Dietary carcinogenicity study in the mouse SPL 2060-0011 GLP: Yes Published: No BVL-2309412, ASB2012-11492
187	[redacted]	1980	Dominant lethal mutagenicity assay with technical Glyphosate in mice 401-064 ! IR-79-014 GLP: No (27) Open (9) Yes (1) Published: No (31) Open (6) BVL-1345017, TOX9552377
188	Wright, N.P.	1996	Technical glyphosate: Chromosome aberration test in CHL cells in vitro 434/015 GLP: Yes Published: No BVL-2309319, ASB2012-11476
189	Xie, L.T., Thrippleton, K., Irwin, M.A., Siemering, G.S., Mekebri, A., Crane, D., Berry, K., Schlenk, D.	2005	Evaluation of estrogenic activities of aquatic herbicides and surfactants using an rainbow trout vitellogenin assay Toxicological Sciences vol.87, 2 (2005) 391-398 GLP: No Published: Yes BVL-2310138, ASB2012-12056
190	Yoshioka, N.; Asano, M.; Kuse, A.; Mitsuhashi, T.; Nagasaki, Y.; Ueno, Y.;	2011	Rapid determination of glyphosate, glufosinate, bialaphos, and their major metabolites in serum by liquid chromatography–tandem mass spectrometry using hydrophilic interaction chromatography doi:10.1016/j.chroma.2011.04.021 Journal of Chromatography A, 1218 (2011) 3675–3680 ASB2015-8033
191	Yue, Y., Zhang, Y., Zhou, L., Qin, J., Chen, X.	2008	In vitro study on the binding of herbicide glyphosate to human serum albumin by optical spectroscopy and molecular modeling J Photochem Photobiol vol.B 90, 1 (2008) 26-32 GLP: No Published: Yes BVL-2310144, ASB2012-12059

Annex

Table A-5.5-1: Methods for the analysis of glyphosate

Sample matrix	Assay procedure	Derivati-sation	LOD	LOQ	Validation complete?	Cited outside section 1.3	Reference
Water	HPLC/MS (with online solid-phase extraction)	FMOC	0.08 µg/L	0.20 µg/L	yes	no	Lee et al., 2001, ASB2015-8239
Water	ELISA	no	0.05 µg/L	0.5 µg/L	yes	no	Abraxis, 2005, ASB2015-7847
Water	LC-LC-FD	FMOC	0.02 µg/L	0.10 µg/L	yes	no	Hidalgo et al., 2004, ASB2015-8423
Water	HPLC with post-column reaction and FD	OPA	6.0 µg/L	25 µg/L	yes	no	EPA, 1992, ASB2015-8424
Water	UV visible spectro-photometer (at 435 nm)	no	1.1 µg/L	not determined	no	no	Jan et al., 2009, ASB2015-8285
Soil	LC–MS/MS with triple quadrupole	FMOC	0.02 mg/kg	0.50 mg/kg	yes	no	Botero-Coy et al., 2013, ASB2015-7882
Dust	GC-MS-MID	TFA/ TFEtOH	0.0007 mg/kg	not determined	no	no	Curwin et al., 2005, ASB2012-11595
Air	HPLC/MS with online solid-phase extraction	FMOC	0.01 ng/m^3	not determined	no	yes (1.4.1)	Chang et al., 2011, ASB2015-7895
Fruits and vegetables	HILIC/WAX with ESI-MS/MS	no	0.0012 mg/kg	0.005 mg/kg	yes	no	Chen et al., 2013, ASB2014-8087
Field crops (rice, maize and soybean)	LC–ESI-MS/MS	no	0.007 - 0.12 mg/kg	0.10 mg/kg	yes	no	Botero-Coy et al., 2013b, ASB2015-7883
Plant vegetation	HPLC with single polymeric	FMOC	0.3 mg/kg	1.0 mg/kg	yes	no	Nedelkoska and Low, 2004,

Sample matrix	Assay procedure	Derivati-sation	LOD	LOQ	Validation complete?	Cited outside section 1.3	Reference
	amino column						ASB2015-8134
Serum	LC–MS/MS	no	0.01 µg/mL	0.20 µg/mL	yes	no	Yoshioka et al., 2011, ASB2015-8033
Urine	HPLC with post-column reaction and FD	OPA	1.0 µg/L	not determined	unknown	yes (1.4.1, 4.1.2, 4.1.5)	Acquavella et al., 2004, ASB2012-11528
Urine	ELISA	no	0.9 µg/L	not determined	no	yes (1.4.1, 4.1.2, 4.1.5)	Curwin et al., 2007, ASB2012-11597

LOD Limit of detection
LOQ Limit of quantification
ELISA Enzyme linked immunoassay
FMOC 9-Fluorenylmethyl chloroformate
GC-MS-MID Gas chromatography-mass spectrometry with multiple ion detection
HILIC Hydrophilic interaction liquid chromatography
HPLC/MS High performance liquid chromatography-mass spectrometry
LC-LC-FD Two dimensional liquid chromatography with fluorescence detection
LC–ESI-MS/MS Liquid chromatography with electrospray ionization-tandem mass spectrometry
LC–MS/MS Liquid chromatography-tandem mass spectrometry
OPA *o*-Phthaldialdehyde
TFA Trifluoroacetic acid
TFEtOH Trifluoroethanol
WAX Weak anion exchange (chromatography)

Table A-5.5-2: Occupational and para-occupational exposure to glyphosate

Industry, country, year	Job/process	Results	Comments/additional data by IARC	Comments/additional data by RMS	Reference
Forestry					
Canada, 1986	Signaller Operator Overseer Mixer	Arithmetic mean of air glyphosate concentrations: Morning, 0.63 µg/m^3 Afternoon, 2.25 µg/m^3 Morning, 1.43 µg/m^3 Afternoon, 6.49 µg/m^3 Morning, 0.84 µg/m^3 Afternoon, 2.41 µg/m^3 Morning, 5.15 µg/m^3 Afternoon, 5.48 µg/m^3	Air concentrations of glyphosate were measured at the work sites of one crew (five workers) during ground spraying 268 urine samples were collected from 40 workers; glyphosate concentration was above the LOD (15 µg/L) in 14%	Product used: not given Application rate: not given Only 5 operators in the study: two signaller, one operator, one overseer and one mixer Application equipment: not given Taken together, there is a lack of important information/explanations in this report. From the present point of view, this study would not be acceptable as an OECD guideline-conform operator exposure study. Nevertheless, a rough estimate is presented for the inhalatory exposure of the operators. Highest measured air concentration in the study (mixer): 10.5 µg/m^3 corresponding to an operator exposure of 0.105 mg/person/day[a]. This is well below the estimated inhalation exposure according to the German operator predictive model (high crop hand held scenario, 'worst case'[b]) of 1.26 mg/person/day[c] used for risk assessment in the context of product authorisation. Therefore, no risk is expected to arise from this glyphosate air concentration.	Centre de Toxicologie du Québec, 1988, ASB2015-7889
Finland, year NR	Workers performing silvicultural clearing (n = 5)	Range of air glyphosate concentrations, <1.25 - 15.7 µg/m^3 (mean, NR)	Clearing work was done with brush saws equipped with pressurized herbicide sprayers Air samples were taken from the workers' breathing zone (number of samples, NR) Urine samples were collected during the afternoons of the working week (number, NR) Glyphosate	Product used: Roundup, containing 360 g a.e.[d]/L glyphosate Application rate: not given with regard to treated area Spraying solution: mixture of 8% Roundup, 87% water and 5% of a commercial 'carrier liquid' (40% isopropylamine alcohol) Only five operators in the field study in August 1988 Effective working time 6 hours/day Only two values for glyphosate in air could be determined at the end of the spraying week, i.e. 2.8 µg/m^3 and 15.7 µg/m^3, (all other values <1.25 µg/m^3) Taken together, the number of participants is very low in this study and only two air concentrations could be measured at all. From the present point of view, this study would not be acceptable as an OECD guideline-conform operator exposure study. Nevertheless, a rough estimate is presented for the inhalatory exposure of operators. The air concentration of 15.7 µg/m^3 corresponds to an operator exposure of	Jauhiainen et al., 1991, MET9600092

Industry, country, year	Job/process	Results	Comments/additional data by IARC	Comments/additional data by RMS	Reference
			concentrations in urine were below the LOD (10 µg/L)	0.157 mg/person/day[a]. This is well below the estimated inhalation exposure according to the German operator predictive model (high crop hand held scenario, 'worst case'[b]) of 1.26 mg/person/day[c] used for risk assessment in the context of product authorisation. Therefore, no risk is expected to arise from this glyphosate air concentration.	
USA, year NR	Workers in two tree nurseries (n = 14)	In dermal sampling, 1 of 78 dislodgeable residue samples were positive for glyphosate The body portions receiving the highest exposure were ankles and thighs	Dermal exposure was assessed with gauze patches attached to the clothing and hand rinsing Analysis of daily urine samples repeated over 12 weeks was negative for glyphosate	Product used: Roundup, containing 370 g a.e.[d]/L glyphosate (1.4 kg glyphosate a.e.[d]/gal product) 9 operators, application by trigger spray equipment ('weeders'), 2 operators, application by tractor-drawn spray equipment (applicators) and 2 scouts who did not apply the product themselves Application rate: 0.01 kg product/ha for operators using triggers spray, spraying solution: 1:40 dilution of Roundup or 0.11 kg product/ha for operators using tractor-drawn spray equipment, spraying solution, approximately 1:250 dilution of Roundup Taken together, processing of dermal exposure data is not easily comprehensible in this study, at least some pieces of information/explanations are lacking. From the present point of view, this study would not be acceptable as an OECD guideline-conform operator exposure study. Nevertheless, a rough estimate is presented for the dermal exposure of operators. By far the highest extrapolated total skin exposure in the study amounted to 48,618 µg glyphosate a.e.[d]/day for an applicator/weeder (mixing/loading for all other weeders and application) wearing work clothing and gloves (during both tasks?). This is equivalent to 90.033 mg glyphosate a.e.[d]/kg product/day (0.54 kg product was handled per day) corresponding to 33.312 mg glyphosate a.e.[d]/kg a.e.[d]/day. Taking into account an application rate of 3.6 kg a.e/ha (which is very common for many crops glyphosate is used on) external exposure to 119.9 mg a.e.[d]/person/day would result for operators using the derived data of the study for application on 1 ha. The estimated total external skin exposure according to the German operator predictive model which is used for risk assessment in the context of product authorisation (high crop hand-held scenario for 1 ha, 'worst case', since there are no data for hand-held applications downwards under high crops in this	Lavy et al., 1992, ASB2012-11859

Industry, country, year	Job/process	Results	Comments/additional data by IARC	Comments/additional data by RMS	Reference
				model) makes up 152.8 mg a.e.[d]/person/day (gloves during mixing/loading) or 115.0 mg glyphosate a.e.[d]/person/day (gloves during mixing/loading and application). This is in the same order of magnitude as the above mentioned exposure values derived by the study. Therefore, no risk is expected to arise from this dermal glyphosate concentration.	
Weed control					
United Kingdom, year NR	Municipal weed control workers (n = 18)	Median, 16 mg/m^3 in 85% of 21 personal air samples for workers spraying with mechanized all-terrain vehicle Median, 0.12 mg/m^3 in 33% of 12 personal air samples collected from workers with backpack with lance applications	[The Working Group noted that the reported air concentrations were substantially higher than in other studies, but was unable to confirm whether the data were for glyphosate or total spray fluid.] Dermal exposure was also measured, but reported as total spray fluid, rather than glyphosate	Products used: Roundup Pro Bioactive containing 360 g glyphosate a.e.[d]/L (all-terrain vehicles with front-mounted sprayers) Total Herbicide ready-to-use (NOMIX), Hilite Herbicide ready-to-use (NOMIX) and Roundup Pro Bioactive (Monsanto) diluted with 'Lightning' to give a 40% solution, (controlled drop applicators = lances with spinning discs connected to knapsack container, lance handle with trigger) 6 operators with all-terrain vehicles, monitored in May 1998, 1:14 dilution of product applied, mixing/loading not included into monitoring PPE was worn and described in the report 12 operators with drop applicators, mixing/loading included (where no ready-to-use product), monitored in May - October 1999 Application: 15 cm above the ground PPE was normally worn and described in the report It has to be noted that all glyphosate air concentrations reported for operators using all-terrain vehicles for application in this study are much higher than the concentrations measured for operators using hand-held equipment. Furthermore, it is striking that these concentrations are also ways beyond the values recorded in all other studies summarised in this Table. Median air concentration of glyphosate reported in the study for vehicle application: 15.6 mg glyphosate a.e.[d]/m^3 hand-held applicator: 0.12 mg glyphosate a.e.[d]/m^3 In this context it has been noted that the calibrated range of air sampling was 6.7 - 133 µg/m^3, which is 1000 times lower than the data reported. (Similar to the lower calibrated range also the LOQ is reported in µg/m^3.) For these reasons, either the data are obtained with invalid calibration or there is a typo in the units (mg/m^3 instead of correctly µg/m^3).	Johnson et al., 2005, ASB2012-11859

Industry, country, year	Job/process	Results	Comments/additional data by IARC	Comments/additional data by RMS	Reference
				Due to the inconsistencies concerning determination of air concentrations during application listed above, no comparison of measured and estimated values has been carried out. Once the spray droplets generated during application deposited, operators could only be exposed to released glyphosate-containing vapours. On the basis of the vapour pressure for glyphosate (1.31×10^{-5} Pa at 25°C) the saturated vapour concentration can be calculated. Irrespective of the input parameters used, the resulting air concentration will not exceed 1 µg glyphosate/m^3. Dermal exposure was considerably higher for operators using vehicles than for those with hand-held equipment. Median values for potential dermal exposure (without hands) for: vehicle application: 2.0 mL spray solution/h hand-held application: 0.13 mL spray solution/h Median values for hand exposure for: vehicle application: 3.0 mL spray solution/h hand-held application (rather actual exposure in this case): 0.004 mL spray solution/h Median values for foot exposure for: vehicle application: not determined hand-held application (rather actual exposure in this case): 0.001 mL spray solution/h Assuming usual application conditions for many glyphosate-containing products[e] total external dermal exposure would amount to: 180 mg glyphosate a.e.[d]/person (in the case of vehicle application) or 5.0 mg glyphosate a.e.[d]/person (in the case of hand-held application) The above mentioned total external dermal exposure values are in the same order of magnitude compared with the estimated total external dermal exposure according to the German operator predictive model even if the only suitable but less exposing application scenario for operators is taken into account there (field crop tractor-mounted application, 20 ha, application rate of 3.6 kg a.e.[d]/ha[c] assumed, without considering mixing/loading (compare with study design above)): 147 mg glyphosate a.e.[d]/person/day (without personal protective equipment)	

Industry, country, year	Job/process	Results	Comments/additional data by IARC	Comments/additional data by RMS	Reference
				or 37.4 mg glyphosate a.e.[d]/person/day (with protective suit during application) The estimated total external dermal exposure according to the German operator predictive model (hand-held application in high crops ('worst case', since there are no data for hand-held applications under high crops in this model) 1 ha, application rate of 3.6 kg a.e.[d]/ha[c] assumed, without considering mixing/loading ('worst case', since ready-to-use formulations as well as those which had to be diluted were used), with protective gloves and suit during application) makes up 22.2 mg glyphosate a.e.[d]/person/day. This is in the same order of magnitude as the above mentioned exposure values derived by the study.	
Farming					
USA, 2001	Occupational and para-occupational exposure of 24 farm families (24 fathers, 24 mothers and 65 children). Comparison group: 25 non-farm families (23 fathers, 24 mothers and 51 children)	Geometric mean (range) of glyphosate concentrations in urine: Non-farm fathers, 1.4 µg/L (0.13–5.4) Farm fathers, 1.9 µg/L (0.02–18) Non-farm mothers, 1.2 µg/L (0.06–5.0) Farm mothers, 1.5 µg/L (0.10–11) Non-farm children, 2.7 µg/L (0.10–9.4) Farm children, 2.0 µg/L (0.02–18)	Frequency of glyphosate detection ranged from 66% to 88% of samples (observed concentrations below the LOD were not censored). Detection frequency and GM concentration were not significantly different between farm and non-farm families (observed concentrations below the LOD were not censored)	Product applied: not given, at least one out of seven various pesticides (e.g. chlorpyrifos, metolachlor, atrazine) must have been used by the study participants, one of which was glyphosate Application rate: not given Application equipment: not given, but most probably boom sprayers since treated crops were corn and soybean 24 "operators" (farm fathers) monitored including their wives and children, often farm fathers did not apply the plant protection product themselves, but had it applied by contractors in many cases. This had actually no influence on the urinary glyphosate concentrations of the farm fathers according to the study. LOQ for the method for glyphosate analysis is not validated (please see Table A-5.5-1) Taken together, there is a lack of important information in this study report, especially with regard to non-dietary exposure conditions. From the present point of view, this study would not be acceptable as an OECD guideline-conform operator exposure study. Nevertheless, a worst case estimate is presented for the systemic exposure of operators resulting from the use of glyphosate-containing products. The highest measured urinary concentration of glyphosate for farm fathers in this study amounted to 18 µg glyphosate a.e.[d]/L. Assuming a urine volume of	Curwin et al., 2007, ASB2012-11597

Industry, country, year	Job/process	Results	Comments/additional data by IARC	Comments/additional data by RMS	Reference
				2 L per day the systemically available amount of glyphosate would be at least 36 µg (rounded for 40 µg in case that not all of it had been excreted in urine the same day). Hence, a systemic dose of 0.00057 mg a.e.[d]/kg body weight would result for a 70 kg weighing operator. This systemic exposure would account for 0.57% of the proposed AOEL of 0.1 mg/kg bw/day. The above mentioned exposure would be fully covered by the estimated total systemic exposure of 0.0473 mg a.e.[d]/kg bw/day according to the German operator predictive model (field crop tractor-mounted scenario for 20 ha, application rate of 3.6 kg a.e.[d]/ha[c] assumed, without personal protective equipment) used for risk assessment in the context of product authorisation. This exposure would correspond to 47.3% of the AOEL. Therefore, no risk is expected to arise from the detected systemic exposure to glyphosate for operators.	
USA, year NR	Occupational and para-occupational exposures of 48 farmers, their spouses, and 79 children	Geometric mean (range) of glyphosate concentration in urine on day of application: Farmers, 3.2 µg/L (< 1 – 233 µg/(L) Spouses, NR (< 1 to 3 µg/L) Children, NR (< 1 to 29 µg/L)	24-hour composite urine samples for each family member the day before, the day of, and for 3 days after a glyphosate application. Glyphosate was detected in 60% of farmers' samples, 4% of spouses' samples and 12% of children's samples the day of spraying and in 27% of farmers' samples, 2% of spouses' samples and 5% of children's samples 3 days after	Product used: Roundup Ultra in most cases (other products used were not further specified) Application rate: not given Application of either glyphosate-containing products alone or in combination with up to two other pesticides (2,4-D and/or chlorpyrifos), no further details given 48 operators monitored including their wives and children Application by tractors partly with closed cabs equipped with boom sprayers Validation status of the method for glyphosate analysis is unknown (please see Table A-5.5-1) Taken together, there is a lack of information/explanations in this study report, especially with regard to pesticide application conditions. From the present point of view, this study would not be acceptable as an OECD guideline-conform operator exposure study. Nevertheless, a worst case estimate is presented for the systemic exposure of operators (please see also Niemann et al. (2015, ASB2014-11029). By far the highest measured urinary concentration of glyphosate for an operator on the day of application in this study amounted to 233 µg glyphosate a.e.[d]/L. Assuming a urine volume of 2 L per day the systemically available amount of glyphosate would be at least 466 µg (rounded for 500 µg in case that not all of it had been excreted in urine the same day). Hence, a	Acquavella et al., 2004, ASB2012-11528

Industry, country, year	Job/process	Results	Comments/additional data by IARC	Comments/additional data by RMS	Reference
				systemic dose of 0.0071 mg a.e.[d]/kg body weight would result for a 70 kg weighing operator. This systemic exposure would account for 7.1 % of the proposed AOEL of 0.1 mg/kg bw/day. The above mentioned exposure would be fully covered by the estimated total systemic exposure of 0.0473 mg a.e.[d]/kg bw/day according to the German operator predictive model (field crop tractor-mounted scenario for 20 ha, application rate of 3.6 kg a.e.[d]/ha[c] assumed, without personal protective equipment) used for risk assessment in the context of product authorisation. This exposure would correspond to 47.3 % of the AOEL. Even if the use of closed tractor cabins in the study reduced exposure this would be covered by the factor of 6.7 between measured and predicted exposure value. Therefore, no risk is expected to arise from the detected systemic exposure to glyphosate for operators.	

LOD, limit of detection; ND, not detected; NR, not reported

[a] Assuming a breathing volume of 1.25 m^3/h and a working day of 8 h

[b] Although all herbicidal applications are usually directed downwards the 'high crop hand-held scenario' according to the German operator exposure predictive model is taken into account as the 'worst case', since there are no data for hand-held applications under high crops available in this model.

[c] Taking into account an application rate of 3.6 kg acid equivalent/ha which is quite common

[d] a.e., corresponding to acid equivalent

[e] Usual application rate of 3.6 kg a.e./ha in 100 L of water, giving a concentration of 36 mg glyphosate a.e./mL in the spray solution

Renewal Assessment Report

October 2015

Glyphosate
Addendum 1 to RAR
Part Ecotoxicology

Assessment of
IARC Monographs
Volume 112 (2015):
Glyphosate

RMS: Germany

Version history (Addendum 1)

Date	**Reason for revision**
October 2015	First Draft Evaluation IARC Monograph, Ecotoxicology

Preface

The International Agency for Cancer Research (IARC) of the World Health Organization (WHO) evaluated the active substance glyphosate and concluded that glyphosate is “probably carcinogenic to humans (Group 2A)”, based on the studies available to IARC. The full report on glyphosate from the IARC monograph (Volume 112) has been made publicly available on 29 July 2015.

As Rapporteur Member State (RMS) for the European renewal of approval of the active substance glyphosate, Germany was commissioned by EFSA to evaluate the IARC Monographs Volume 112 on glyphosate. Subsequently, an addendum to the original draft Renewal Assessment Report (DRAR from 2015) regarding toxicology and metabolisms of glyphosate was provided by the BfR by 31 August 2015 taking into account new information from the IARC monograph (Volume 112).

While the addendum of BfR addresses the relevance of the information provided by IARC work for the evaluation regarding the classification as potentially carcinogenic to humans, here the newly submitted studies are evaluated regarding their significance for the risk of non-target organisms exposed to glyphosate according to intented uses of plant protection products containing this active substance.. The evaluation presented in this addenndumg ‘ecotoxicology’ refers in particular to the section 4.2.1 (b) (iii) “Genetic and related effects in non-mammalian systems *in vivo*" of the IARC Monograph.

Executive summary

In addition to the evaluation of the IARC monograph regarding the potential carcinogenity of the active substance glyphosate in terms of human health and classification aspects already provided with addendum 1'toxicology' by BfR, herewith we assess the significance of the studies addressing "Genetic and related effects in non-mammalian systems *in vivo*" for the environmental risk assessment of glyphosate.

Taking into account the results of existing reproduction studies in non-mammalian systems, the geno-toxicological effects observed in biomarker studies with the active substance glyphosate listed in the IARC monograph are considered not to be manifest on the population level of non-target organisms. As a consequence, unacceptable effects on the environment due to the genotoxic potential of glyphosate can be excluded. Thus, from the evaluation of the studies regarding genotoxic effects of glyphosate in non-mammalian systems as presented in the IARC monograph, no changes to the environmental risk assessment of the active substance glyphosate do arise compared to our previous assessment outcome.

However, the geno-toxicological effects observed in some studies with glyphosate formulations containing surfactants from the group of the POEA (polyoxyethylene-alkylamine) are considered to be relevant for the authorisation of glyphosate products in the European Member States. RMS highlighted already in the DRAR (December 2013) that Member States should take steps to ensure a safe use of glyphosate products, such as demanding further data on POEA-containing products or substituting POEA (polyoxyethylene-alkylamine) in plant protection products by less critical surfactants.

In addition to the evaluation of the information from the IARC monograph, the RMS reiterates in this addendum the knowledge regarding the effects of glyphosate and other broad spectrum herbicides on the populations of non-target species (especially insects and farmland birds), caused by an alteration of the food web. From the perspective of the RMS, the approval of the active substance glyphosate is associated with the default of assessing, minimizing and compensating this type of risk in the Member States.

Contents

1 Genotoxicity in the Environmental Risk Assessment

The eco-toxicological endpoints used to evaluate the risk of plant protection products‘ intended uses on non target organisms in environmental risk assessment schemes are so-called apical endpoints. These endpoints address adverse ecological effects in terms of the protection goals set out in the relevant legislation which is mainly to ensure the sustainability of populations of non-target organisms. In contrast, endpoints from mechanistic studies addressing effects on biochemical parameters on the chromosomal, enzymatic, or other cellular organization levels are considered only as supporting information in environmental risk assessment. Such studies can provide an indication of potential effects on the organism level. For decision-making, however, the relevance to the population level of the effects observed in such studies has to be assessed by means of apical studies covering the toxicological mechanisms and targets addressed in the mechanistic studies. To this effect, the results obtained in mechanistic genotoxicity studies reported in the IARC monograph have to be linked to regulatory relevant endpoints as well as to the predicted environmental exposure in order to characterize the risk for the protection targets.

Genotoxicity studies characterize toxicological effects of chemicals on the genetic material of organisms. The exposure of an organism to a genotoxic substance can result in an interaction of these chemicals with DNA and can subsequently lead to a structural damage of the genetic material by means of DNA strand breaks. DNA strand breaks can be analysed for structural damage in the neutral and the alkaline comet assay (Tice et al. 2000). Finally, genotoxicity can induce mutations resulting in adverse effects - including cancer in somatic cells and reproductive changes - when manifested in germ cells (Jha, 2008). Therefore, a genotoxic substances might have the potential for adverse effects towards populations of non-target organisms. Generally, genetic changes in parental organisms –e.g. in their gametes - can be passed to subsequent generations and can influence in principle the genetic diversity of populations. This in turn might affect the health, and the survival of the population of non-target organisms (Bickham et al. 2000).

The active substance glyphosate and its metabolite aminomethylphosphonic acid (AMPA) have been studied for genotoxic potential via comet assay in a wide variety of assays in non-mammalian systems *in vivo* and *in vitro*, respectively. To the following, the studies evaluated

by the IARC monograph are summarized and discussed regarding their relevance for the assessment of the risk for non-target organisms exposed to glyphosate according to the indented uses of the plant protection products.

.

2 Studies addressing the active substance glyphosate and the metabolite AMPA

In the IARC monograph, the following studies on the active substance glyphosate have been considered i.a.:

1. Akcha, F., Spagnol, C., & Rouxel, J. (2012). Genotoxicity of diuron and glyphosate in oyster spermatozoa and embryos. Aquatic Toxicology, 106, 104-113.
2. Alvarez-Moya, C., Reynoso Silva, M., Valdez Ramírez, C., Gómez Gallardo, D., León Sánchez, R., Canales Aguirre, A., & Feria Velasco, A. (2014). Comparison of the in vivo and in vitro genotoxicity of glyphosate isopropylamine salt in three different organisms. Genetics and molecular biology, 37(1), 105-110.
3. Guilherme, S., Santos, M. A., Barroso, C., Gaivão, I., & Pacheco, M. (2012). Differential genotoxicity of Roundup® formulation and its constituents in blood cells of fish (*Anguilla anguilla*): considerations on chemical interactions and DNA damaging mechanisms. Ecotoxicology, 21(5), 1381-1390.
4. Lopes, F. M., Junior, A. S. V., Corcini, C. D., da Silva, A. C., Guazzelli, V. G., Tavares, G., & da Rosa, C. E. (2014). Effect of glyphosate on the sperm quality of zebrafish *Danio rerio*. Aquatic Toxicology, 155, 322-326.
5. Moreno, N. C., Sofia, S. H., & Martinez, C. B. (2014). Genotoxic effects of the herbicide Roundup Transorb® and its active ingredient glyphosate on the fish *Prochilodus lineatus*. Environmental toxicology and pharmacology, 37(1), 448-454.

In addition, the following study on AMPA was considered:

a) Guilherme, S., Santos, M. A., Gaivão, I., & Pacheco, M. (2014). DNA and chromosomal damage induced in fish (*Anguilla anguilla* L.) by aminomethylphosphonic acid (AMPA)—the major environmental breakdown product of glyphosate. Environmental Science and Pollution Research, 21(14), 8730-8739.

Four out of the six studies cited in the IARC monograph (see above) have already been considered by the RMS in the original draft RAR (Volume3 CA-CPB-9Appendix, Studies a), c), e) for glyphosate and study a) for AMPA). For the purpose of this addendum, those studies were re-evaluated and considered together with the new studies. For a summary of the results of the individual studies, refer to table 2.1.

Table 2-1: Discussion of studies by the RMS with the active substance glyphosate in the section 4.2.1 (b) (iii) "non-mammalian systems in vivo" of the IARC Monograph

Study (Author/year)	**Subject**	**Evaluation by IARC**	**Comment RMS on IARC evaluation**	**Report in dRAR April 2015**	**Final conclusion of RMS, considering IARC evaluation**
Moreno et al., 2014.	Genotoxic effects of the herbicide Roundup Transorb and its active ingredient glyphosate on the fish *Prochilodus lineatus*	Time of exposure 6, 24, and 96h. For erythrocytes: *P* = 0.01 after 6 h *P* = 0.014 after 96 h; no significant increase after 24 h For gill cells: *P*=0.02 after 6 h at 2.4 mg/L	**Agreement**	**yes**	Glyphosate and the tested product caused damage to the DNA nucleoids of *P. lineatus* at concentrations higher than those predicted in the environment. Erythrocytes exposed to both concentrations of the formulated product (1 and 5 mg/L) and the active substance glyphosate (0.48 and 2.4 mg/L) showed DNA damage after 96 h. Nevertheless, the study shows slight deficiencies, as it is unclear why the negative control showed increase in damaged nucleotids in gill cells (24.3 after 6h and 45 after 96h). This observation is further seen in the positive control, when DNA damage increases after 96 h only. Further studies might demonstrate the sensitivity of the test system is time dependent. Therefore, further studies would be needed to determine if the changes on DNA strands are reproducible. Furthermore, effects observed in the given experimental setup are not transferable into reproductive success of fish as demonstrated by three regulatory studies, conducted according to internationally agreed test guidelines, where at comparable exposure concentrations not adverse effects on reproductive success could be observed.

Guilherme et al. (2012)	Differential genotoxicity of Roundup(®) formulation and its constituents in blood cells of fish (*Anguilla anguilla*): considerations on chemical interactions and DNA damaging mechanisms.	Time of exposure 1 and 3 days $P < 0.05$	**Agreement**	**yes**	The experiment was conducted using the commercial formulation Roundup® Ultra, distributed by Bayer Crop- Science (Portugal), containing isopropylammonium salt of glyphosate at 485 g/L as the active ingredient (equivalent to 360 g/Lor 30.8 % of glyphosate) and POEA (16%) as surfactant. Fish were exposed to equivalent concentrations of the Roundup product (58, 116 µg/L), glyphosate (17.9, 35.7 µg/L) and POEA (9.3, 18.6 µg/L) for 1 and 3 days. The comet assay was applied to blood cells, either as the standard procedure, or with an extra step involving DNA lesion-specific repair enzymes in an attempt to clarify DNA damaging mechanisms. Mean values of genetic damage indicator displayed significantly higher values in comparison to the control. Both components of the formulated product seem to contribute to the effect on genetic structure of the formulation Roundup® Ultra. Roundup® Ultra displayed levels of damage at both tested concentrations and exposure times. Nevertheless, fish exposed to the highest concentration of glyphosate for 3 days recovered from the damage detected after 1 day exposure. The ability of the test organisms to recover from the observed effects was evaluated by adding repair enzymes to the cells. At the highest tested concentration of glyphosate, a repair of DNA damage was seen. Hence, the observed time-related disappearance of DNA damage could be a result of the intervention of a DNA repair system. Effects observed in the given experimental setup are not directly transferable into reproductive success of fish as demonstrated by three regulatory studies, conducted according to internationally agreed test guidelines, where at comparable exposure concentrations not adverse effects on reproductive success could be observed.

Lopes et al. (2014)	Effect of glyphosate on the sperm quality of zebrafish *Danio rerio*.	After 96 h, DNA integrity was 78.3 ± 3.5%, significantly reduced from control (94.7 ± 0.9%) and 5 mg/L (92.6 ± 1.9%), *(P* < 0.05)	**Agreement**	**no**	The effect of the active substance glyphosate on sperm quality of the fish *Danio rerio* was investigated after 24 and 9 h of exposure at concentrations of 5 mg/L and 10 mg/L. No significant differences in sperm concentration were observed; however, sperm motility and the motility period were reduced after exposure to glyphosate to both exposure periods. The mitochondrial functionality and membrane and DNA integrity were also reduced at the highest concentration for both exposure periods. It should be noted that in this experiment glyphosate was applied at high concentrations, which do not relate to predicted environmental concentrations in the environment (based on modelling assumptions, the tested concentrations is exceeding the predicted concentrations by factor > 50).
Alvarez-Moya et al. (2014).	Comparisonof the in vivo and in vitro genotoxicity of glyphosateisopropyl-amine salt in three different organisms	Time of exposure, 10 days *P* < 0.001 with concentrations > 7µM	**Agreement**	**no**	Based on modelling assumptions, the concentrations expected in surface waters exposed to drift are approx. 0.1 mg a.i./L. No effects on DNA integrity were observed in vitro for *Oreochromis niloticus* erythrocytes at 0.0007 mM, approximately corresponding to concentration expected in the environment. In vivo responses above concentrations >7 µM do not relate to the predicted environmental concentrations and did not show dose–response relationship.

Guilherme et al. (2014)	DNA and chromosomal damage induced in fish (*Anguilla anguilla* L.) by aminomethylphosphonic acid (AMPA)-the major environmental breakdown product of glyphosate	Time of exposure, 1 and 3 days $P < 0.05$ after 1 day of exposure	**Agreement**	**yes**	Mean values of genetic damage indicator measured by comet assay in blood cells of *A. anguilla* exposed to 11.8 and 23.6 µg/L aminophosphoric acid showed significant increases after 1 day. After 3 days, no effects were observed for the tested lower concentration. Possibly, tested fishs had the capacity to use successfully DNA repair mechanisms. No significant alterations were found in erythrocytic nuclear abnormalities (ENA) assays following the first day of exposure; considering the 3-day exposure, a significant increase for the higher concentration of AMPA was observed. Based on modelling assumptions, the concentrations expected in surface waters exposed to drift are 0.041 mg AMPA/L. Effects observed in the given experimental setup are not transferable into reproductive success of fish as demonstrated the regulatory study, conducted according to internationally agreed test guidelines, , where at comparable exposure concentrations not adverse effects on reproductive success could be observed.
Akcha et al. (2012)	Genotoxicity of diuron and glyphosate in oyster spermatozoa and embryos.	Time of exposure, 1 h	**Agreement**	**yes**	Both the active ingredient glyphosate, as well as the formulation Roundup® showed no significant effect on the development oysters at the concentrations tested.

In the IARC monograph, the results of the studies listed above were summarized as follows:

"*In fish, glyphosate produced DNA strand breaks in the comet assay in sabalo (Moreno et al., 2014), European eel (Guilherme et al., 2012b), zebrafish (Lopes et al., 2014), and Nile tilapia (Alvarez-Moya et al. 2014) AMPA also induced DNA strand breaks in the comet assay in European eel (Guilherme et al.2014b)). A glyphosate-based formulation produced DNA strand breaks in numerous fish species, such as European eel (Guilherme et al., 2010, 2012b, 2Q14a Marques et al. 2014 , 2015), sabalo (Calvante et al. 2008; Moreno et a, 2014,2014), guppy (De Souza filho et al. 2013), bloch (Nawani et al., 2013), neotropical fish Corydoras paleatus (de Castilhos Ghisi & Cestari, 2013), carp (Gholami-Seyedkolaei et al., 2013), and goldfish (Cavas& Könen, 2007).*

AMPA, the main metabolite of glyphosate, induced erythrocytic nuclear abnormalities (kidney-shaped and lobed nuclei, binucleate or segmented nuclei and micronuclei) in European eel (Guilherme et al., 2014b). Micronucleus formation was induced by different glyphosate- based formulations in various fish (Grisolia, 2002; Cavas& Könen, 2007; De Souza Filho et al., 2013; Vera-Candioti et al., 2013)"

2.1 Discussion and Conclusion

The induction of DNA strand breaks in both somatic and germ cells are of paramount importance. If unrepaired or mis-repaired, such damage might have effects on the immediate fitness as well as on reproductive success of the exposed organisms (Jha, 2008). DNA strand breaks in germs cells might lead to stable effects cells leading to heritable changes in fertility and fecundity. The studies from the peer reviewed literature evaluated by RMS in the draft RAR from 2015 , however related to effects observed in somatic cells. Genetic damage indicators were measured by the standard (alkaline) comet assay in erythrocytes of fish after *in-vivo* exposure of the test organisms.

It could be shown that the active substance glyphosate can cause damage to the DNA molecule in the test organism fish, although at concentrations higher than those predicted in the environment (Moreno et al. 2014, Alvarez-Moya et al. 2014). However, no effects on DNA integrity were observed *in-vitro* to *Oreochromis niloticus* at 0.0007 mM, approximately corresponding to concentration expected in the environment (Alvarez-Moya et al. (2014). In contrast, Guilherme et al. (2012) detected DNA damage at environmentally relevant concentrations of the active substance glyphosate as well as the metabolite AMPA (Guilherme et al. 2014). Both studies evaluated the ability of the test organisms to recover by adding repair enzymes to the cells after exposure to glyphosate or AMPA. Guilherme et al. (2012) exposed fish to environmentally relevant concentrations of a Roundup formulation (58, 116 µg/L), glyphosate (17.9, 35.7 µg/L) and the surfactant system based on POEA (9.3, 18.6 µg/L) for 1 and 3 days. The comet assay was applied to blood cells, either as the standard procedure, or with an extra step involving DNA lesion-specific repair enzymes in order to address DNA damage and possibly reparing mechanisms. Blood cells exposed to both concentrations tested as well as both exposure times displayed significantly higher values levels of the genetic damage indicator compared to control assays. Failure to repair this DNA damage might lead to mutations, reduced fitness and might in the end affect reproductive success. Nevertheless, fish exposed to the highest concentration of the active substance glyphosate for 3 days recovered from the damage that had been detected after 1 day exposure. Results indicated that after 3 days of exposure, DNA damage could no longer be detected, a fact which might point to the ability of an organism to identify DNA damage and induce DNA repair mechanisms (Guilherme et al. 2012).

The reported effects from mechanistic studies (i.e. induced DNA damage) do not allow direct conclusions on potential adverse effects on the population level of non-target organisms - i.e.

on the protection item relevant for decision-making. In order to link those results to the assessment of the risk for non-target organisms exposed to PPP containing the acive substance glyphosate, it has to be assessed whether the observed effects are in principle covered by apical studies with survival, developmental or reproductive endpoints of suitable surrogate species of non-target organisms.

The effect of the active substance glyphosate on sperm quality of the fish *Danio rerio* was investigated at exposure regimens exceeding the predicted environmental concentrations in surface waters (Lopes et al. 2014). High concentrations of glyphosate reduced motility and the motility period of sperm in this study, which could - at environmentally realistic concentration - alter reproductive success. No significant differences in sperm concentration were observed.

In three regulatory studies, conducted according to internationally agreed test guidelines, the active substance glyphosate was assessed for its toxicity on reproduction of fish. In general, chronic NOEC values for fish ≥10 mg/L are indicative of low chronic toxicity. The chronic endpoint from the 255 day "fish full life-cycle" study with the fathead minnow was 25.7 mg a.s./L (DOC: 895-00020). Please refer to Point 1.4 of this report and to Point B 9.2.1. of the original draft RAR of 2015 for further information.

The risk assessment for the metabolite AMPA is based on the results from an "early life stage" study (DOC: 2310943/WL-2010-328), in which fish embryos were exposed for 33 days in a flow through system. The results demonstrate similarly low chronic toxicity to fish as for the parent substance glyphosate, with a NOEC value of 12 mg a.s./L, which was the highest concentration tested. There were no effects on the survival and the growth parameters of *Pimephales promelas* observed at the highest concentration tested.

In conclusion, no significantly increased risks to non-target organisms' populations compared to the previous risk assessment (DRAR from 2013 and 2015) have to be considered when taking into account the information from the IARC monograph. Even though the observed genotoxicity effects could in principle have negative effects on reproduction in fish, no such effects were observed in the available reproduction studies, falsifying the initial suspicion of potential negative effects arising from the genotoxicity studies. Consequently, no significant changes in population densities and no unacceptable effects on functioning of the ecosystem are expected.

3 Studies with glyphosate-containing formulations

In the IARC monograph, the following studies on different glyphosate-containing formulations marketed mainly under the name "Roundup" have been considered:

1) Akcha, F., Spagnol, C., & Rouxel, J. (2012). Genotoxicity of diuron and glyphosate in oyster spermatozoa and embryos. Aquatic Toxicology, 106, 104-113.
2) Cavalcante, D. G. S. M., Martinez, C. B. R., & Sofia, S. H. (2008). Genotoxic effects of Roundup® on the fish *Prochilodus lineatus*. Mutation Research/Genetic Toxicology and Environmental Mutagenesis, 655(1), 41-46.
3) Clement, C., Ralph, S., & Petras, M. (1997). Genotoxicity of selected herbicides in *Rana Catesbeiana* tadpoles using the alkaline single cell gel DNA electrophoresis (comt) assay. Environ Mol Mutagen, 29, 277-288.
4) Conners, D. E., & Black, M. C. (2004). Evaluation of lethality and genotoxicity in the freshwater mussel *Utterbackia imbecillis* (Bivalvia: Unionidae) exposed singly and in combination to chemicals used in lawn care. Archives of environmental contamination and toxicology, 46(3), 362-371.
5) De Souza Filho, J., Sousa, C. C. N., Da Silva, C. C., De Sabóia-Morais, S. M. T., & Grisolia, C. K. (2013). Mutagenicity and genotoxicity in gill erythrocyte cells of *Poecilia reticulata* exposed to a glyphosate formulation. Bulletin of environmental contamination and toxicology, 91(5), 583-587.
6) dos Santos, K. C., & Martinez, C. B. (2014). Genotoxic and biochemical effects of atrazine and Roundup®, alone and in combination, on the Asian clam *Corbicula fluminea*. Ecotoxicology and environmental safety, 100, 7-14.
7) Guilherme S, Santos MA, Barroso C, Gaivao I, PachecoM (2012b). Differential genotoxicity of Roundup(®) formulation and its constituents in blood cells of fish (*Anguilla anguilla*): considerations on chemical interactions and DNA damaging mechanisms. Ecotoxicology, 21(5):1381-90.
8) Guilherme, S., Gaivao, I., Santos, M. A., & Pacheco, M. (2010). European eel (*Anguilla anguilla*) genotoxic and pro-oxidant responses following short-term exposure to Roundup®-a glyphosate-based herbicide. Mutagenesis, 25(5), 523-530.
9) Guilherme, S., Santos, M. A., Gaivão, I., & Pacheco, M. (2014). Are DNA-damaging effects induced by herbicide formulations (Roundup® and Garlon®) in fish transient and reversible upon cessation of exposure?. Aquatic Toxicology, 155, 213-221.
10) Marques, A., Guilherme, S., Gaivão, I., Santos, M. A., & Pacheco, M. (2014). Progression of DNA damage induced by a glyphosate-based herbicide in fish

(*Anguilla anguilla*) upon exposure and post-exposure periods—Insights into the mechanisms of genotoxicity and DNA repair. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology, 166, 126-133.

11) MezaJoya, F. L., RamírezPinilla, M. P., & Fuentes-Lorenzo, J. L. (2013). Toxic, cytotoxic, and genotoxic effects of a glyphosate formulation (Roundup® SL-Cosmoflux® 411F) in the direct?developing frog *Eleutherodactylus johnstonei*. Environmental and molecular mutagenesis, 54(5), 362-373.
12) Mohamed, A. H. (2011). Sublethal toxicity of Roundup to immunological and molecular aspects of *Biomphalaria alexandrina* to *Schistosoma mansoni* infection. Ecotoxicology and environmental safety, 74(4), 754-760.
13) Muangphra, P., Kwankua, W., & Gooneratne, R. (2014). Genotoxic effects of glyphosate or paraquat on earthwom coelomocytes. Environmental toxicology, 29(6), 612-620.
14) Nwani, C. D., Nagpure, N. S., Kumar, R., Kushwaha, B., & Lakra, W. S. (2013). DNA damage and oxidative stress modulatory effects of glyphosate-based herbicide in freshwater fish, Channa punctatus. Environmental toxicology and pharmacology, 36(2), 539-547.
15) Piola, L., Fuchs, J., Oneto, M. L., Basack, S., Kesten, E., & Casabé, N. (2013). Comparative toxicity of two glyphosate-based formulations to *Eisenia andrei* under laboratory conditions. Chemosphere, 91(4), 545-551.
16) Poletta, G. L., Larriera, A., Kleinsorge, E., & Mudry, M. D. (2009). Genotoxicity of the herbicide formulation Roundup®(glyphosate) in broad-snouted caiman (*Caiman latirostris*) evidenced by the Comet assay and the Micronucleus test. Mutation Research/Genetic toxicology and Environmental Mutagenesis, 672(2), 95-102

The objective of the literature survey in the Renewal Assessment of the active substance glyphosate was the evaluation of scientific peer-reviewed open literature published within the last 10 years for the active substance glyphosate. Additionally, studies with glyphosate containing products have been evaluated. Eleven out of the 16 studies cited in the IARC monograph had already been considered by the RMS in the original draft RAR (2013). In this addendum, studies with different glyphosate containing formulations were re-evaluated and considered together with the new studies. For a summary of the results of the individual studies, refer to table 2.2.

Table 3-1:Discussion of studies with the different commercial glyphosate containing formulations in the section 4.2.1 (b) (iii) "non-mammalian systems in vivo" of the IARC Monograph

No.	Author/year	Study	Evaluation by IARC	Comment RMS on IARC evaluation	Study reported in dRAR April 2015	Final conclusion of RMS, considering IARC evaluation
1	Akcha et al. (2012)	Genotoxicity of diuron and glyphosate in oyster spermatozoa and embryos.	Time of exposure, 1 h	Agreement	yes	Out of the three bioassays conducted at the laboratory, only one assay revealed glyphosate to have an embryotoxic effect at concentrations of 2.5 µg L ($p < 0.001$) upwards. Taking into account the data from the three bioassays, the main effects ANOVA revealed significant differences between the assays ($p < 0.001$). with assay 2 differing from assays 1 and 3. Therfore no concluding evidence exists for both the active substance glyphosate, as well as the formulation Roundup® Express no have an effect on the development oysters at the concentrations tested.
2	Cavalcante et al., (2008)	Genotoxic effects of Roundup® on the fish *Prochilodus lineatus*.	Single dose tested only, for 6, 24 and 96h. $P < 0.05$ for both erythrocytes and bronchial cells	Agreement	No	No significant differences erythrocytes and bronchial cells compared to control were observed in the piscine micronucleus test. The comet assay increased rates of DNA damage in blood and hepatic cells, when the fish were exposed to 10 mg/L glyphosate formulation. Authors conclude that short-term exposure of the pesticide Roundup® is genotoxic to the bioindicator fish species *C. paleatus*, even at a relatively low concentration. However, this low concentration tested did not result in clastogenic and/or aneugenic effects.

3	Clement et al. 1997	Genotoxicity of select herbicides in *Rana eatesbeiana* tadpoles using the alkaline single-cell gel DNA electrophoresis (comet) assay.	P < 0.05, with 6.75 mg/L; P < 0.001 with 27 mg/L (with 108 mg/L, all died	Agreement	No	Tadpoles exposed to the lowest concentration of Roundup, 1.69 mg/l, exhibited no significant increase in DNA damage relative to the negative controls However, tadpoles exposed to 6.75 and 27 mg/l gave significant increases in DNA damage (P < 0.05 and 0.001, respectively). All tested concentrations were below the recommended application levels.
4	Conners, Black (2004)	Evaluation of lethality and genotoxicity in the freshwater mussel *Utterbaekia imbeeillis* (Bivalvia: Unionidae) exposed singly and in combination to chemicals used in lawn care	2.5 and 5 mg/L for 24 h tested NOEC, 10.04 mg/L	**Agreement**	No	Authors evaluated the lethal and genotoxic effects of chemicals used in lawn care on an early life stage of fresh water mussels (*Utterbackia imbecillis*). For the Parameter mortality LC50 Glyphosate formulation was determined to 18.3 mg/L. The combined toxicity of equitoxic and environmentally realistic mixtures to mussels was additive. No genotoxic significant responses were observed in mussels exposed to glyphosate.
5	De Souza Filho et al. (2013)	Mutagenicity and Genotoxicity in Gill Erythrocyte Cells of *Poecilia reticulata* Exposed to a Glyphosate Formulation	Glyphosate, 64.8%, m/v ((648 g/L) **P < 0.05**	**Agreement**	Yes	The 96h LC_{50} for Roundup®Transorb in the absence and presence of predator stress were 3.76 mg ae/L and 3.39 mg ae/L, respectively. The 10-day LC50 value for Roundup was significantly lower, 2.12 mg ae/L and 1.91 mg ae/L in the absence and presence of predator stress, respectively. Lower concentrations of Roundup (1, 2 and 3 mg ae/L) induced the formation of micronuclei (MN) in the erythrocytes in a concentration-dependent manner. Presence of predator stress seemed to increase the toxicity and genotoxicity of Roundup®; but these effects were not statistically significant.

6	dos Santos Martinez (2014).	Genotoxic and biochemical effects of alrazine and Roundup(®), alone and in combination, on the Asian clam *Corbicula fluminea*	Time of exposure, 96 h; Significant increase when atrazine (2 or 10 mg/L) was added to glyphosate ($P < 0.05$) No increase after exposure to atrazine or glyphosate	**Agreement**	**yes**	The study aimed to evaluate biochemical and genotoxic effects of the herbicides atrazine (ATZ) and Roundups (RD) separately, as well as their mixture, on the fresh water clam after 96 h exposure. Roundup did not increase DNA damage, but induced alterations in biochemical parameter related to oxidative stress (alterations in Superoxide dismutase activity and catalase activity). Exposure to ATZ and RD separately did not increase DNA damage.
7	Guilherme et al. (2010)	European eel (*Anguilla anguilla*) genotoxic and pro-oxidant responses following short-term exposure to Roundup® a glyphosate-based herbicide	$P < 0.05$ (Positive dose-response)	**Agreement**	yes	Micronuclei test (MN) and erythrocytic nuclear abnormalities (ENA) results did not significantly differ between studied treatments and control.Comet assay: significant effects indicating DNA damage in erythrocytes at concentrations of 58 and 116 µg/L. This paper reports physiological studies with a commercial formulation, which could not be taken into account as critical information for the assessment of the active substance glyphosate itself. Nevertheless it shows that commercial formulations of glyphosate might have elicit effects.

8	Guilherme et al. (2012)	Differential genotoxicity of Roundup® formulation and its constituents in blood cells of fish (*Anguilla anguilla*): considerations on chemical interactions and DNA damaging mechanisms.	Time of exposure, 1 and 3 day comet assay improved with the DNA- lesion-specific FPG and EndoIII With FPG $P < 0.05$; with comet assay alone, $P < 0.05$ at 116 µg/L	**Agreement**	yes	The experiment was conducted using the commercial formulation Roundup® Ultra, distributed by Bayer Crop-Science (Portugal), containing isopropylammonium salt of glyphosate at 485 g L-1 as the active ingredient (equivalent to 360 g L-1 or 30.8 % of glyphosate) and POEA (16 %) as surfactant. Fish were exposed to equivalent concentrations of Roundup (58, 116 µg/), glyphosate (17.9, 35.7 µg/L) and POEA (9.3, 18.6 µg/L), during 1 and 3 days. The comet assay was applied to blood cells, either as the standard procedure, or with an extra step involving DNA lesion-specific repair enzymes in an attempt to clarify DNA damaging mechanisms. Mean values of genetic damage indicator displayed significantly higher values in comparison with the control. Both components seem to contribute to the effect on genetic structure of the formulation. Both concentrations and exposure times displayed levels of damage. Nevertheless, fish exposed to the highest concentration of glyphosate for 3 days recovered from the damage detected after 1 day exposure. Moreover, the ability of the test organisms to recover was evaluated by adding repair enzymes to the cells after exposure to glyphosate. Interestingly, then at the highest concentration of glyphosate a repair of DNA damage was seen. Hence, the time-related disappearance of DNA damage could be a result of the intervention of a DNA repair system. The recovery phenomenon was not observe for POEA treatment or Roundup® Ultra treatment. Comparing the respective highest concentrations the surfactant displayed the most elevated levels of DNA damage among the study agents. Effects observed in the given experimental setup are not transferable into reproductive success of fish as demonstrated by three regulatory studies, conducted according to internationally agreed test guidelines.

9	Guilherme et al. (2014a).	Are DNA-damaging effects induced by herbicide formulations (Roundup· and Garlon®) in fish transient and reversible upon cessation of exposure?	Single dose tested only; Time of exposure:3 days; DNA damage, but not oxidative DNA damage, 14 days after exposure; P< 0.05	**Agreement**	yes	The study investigated the ability of fish to recover from the DNA damage induced by short-term exposures to Roundup® and Garlon® (triclopyr-based). Roundup®Ultra, distributed by Bayer CropScience (containing isopropylammonium salt of glyphosate at 485 g/L as the active ingredient (equivalent to 360 g/L or 30.8% w/v of glyphosate) and POEA (16% v/v) as surfactant). Exposure for 3 days, recovery for 1, 7 and 14 days (post-exposure period). Fish were exposed to 116 µg/L Roundup®, which is assumed to display a realistic worse case concentration. A decrease of the non-specific DNA damage induced by Roundup® after 14days in herbicide-free water could be demonstrated, while a complete recovery of DNA stability could not be shown. A recovery was observed when considering non-specific DNA damage on day 14 post-exposure. Effects of commercial formulations of glyphosate on responses like changes on chromosomal damage in the field of environmental risk assessment are currently considered as supporting information, especially since this evaluation aims at assessing the risk of non target organisms exposed to glyphosate. . Nevertheless, it is evident that further studies with the respective formulated product are needed to determine if the changes on biochemical traits are translated to sublethal long-term effects.

	Marques et al. (2014)	Progression of DNA damage induced by a glyphosate-based herbicide in fish (*Anguilla anguilla*) upon exposure and post-exposure periods Insights into the mechanisms of genotoxicity and DNA repair.	Time of exposure, 3 days $P < 0.05$	**Agreement**	yes	The study indicated that concentrations of 8.1 mg/ L and 16.3 mg/L commercial glyphosate containing formulation provoke non-significant induction of nuclear lesions; whereas at concentrations of 24.4 mg/L after 96h significantly higher nuclear lesions induction were observed. Roundup® showed to induce DNA damage (measured both as GDIFPG and GDIEndoIII) in hepatic cells of *A. anguilla* at two concnetrations of a glyphosate containing products . After transfer to herbicide-free water, fish were able to reverse the genetic damage.
	Meza-Joya et al. (2013)	Toxic, cytotoxic,and genotoxic effects of aglyphosate formulation (Roundup®SL-Cosmotlux®411F) in the direct-developing frog *Eleutherodactylus johnstonei*	Exposure to an homogenate mist in a 300 cm2 glass terrarium Time of exposure: 0.5, 1, 2, 4,8 and 24h $P < 0.05$	**Agreement**	**yes**	The study evaluates the toxic, cytotoxic, and genotoxic effects of a glyphosate formulation (RoundupVR SL–CosmofluxVR 411F) in the direct-developing frog *E. johnstonei* by estimating the median lethal application rate (LC50), median hemolytic application rate (HD50), and extent of DNA damage using the in vitro and in vivo Comet assays. Toxicity results indicated that the application rate [37.4 mg acid equivalent (a.e.)/cm2] equivalent to that used in aerial spraying (3.74 kg a.e./ha) is not lethal in male and female adult frogs, whereas neonates are highly sensitive. LC 50 24h = 3.1 (2.8–3.6) kg a.e./ha LC 50 48h = 1.8 (1.5–2.0) kg a.e./ha LC 50 96 h 1.2 (1.1–1.4) kg a.e./ha In vivo and in vitro exposure of *E. johnstonei* erythrocytes to the glyphosate formulation induced DNA breaks in a dose-dependent manner with statistically significant values ($P<0.05$) at all doses tested. DNA damage initially increased with the duration of exposure and then decreased, suggesting that DNA repair events were occurring during in vivo and in vitro exposures.

	Mohamed (2011)	Sublethal toxicity of Roundup to immunological and molecular aspects of *Biomphalaria alexandrina* to *Schistosoma mansoni* infection	10 mg/I. Single dose tested only; for 24 h. The percentage of damaged DNA was 21% vs 4% (control)	**Agreement**	No	Investigation on the cellular mechanisms of *Biomphalaria alexandrina* snails' hemocytes against sublethal concentration (10mg/L) of Roundup (48%Glyphosate) during7days. Obtained results indicated that treatment and/or infection led to significant increase (P<0.05) in total hemocytes count.
	Muangphra et al. 2014	Genotoxic effects of glyphosate or paraquat on earthworm coelomocytes	Epidermic exposure 48 h on filter paper; LC50: 251.50 µg/cm2 $P < 0.05$, for total micro-, bi-, and trinuclei frequencies at 0.25 µg/cm2; when analysed separately, micro- and trinuclei frequencies significantly differed from controls only at the LC50	**Agreement**	yes	The genotoxicity of glyphosate containing formulations reflected by chromosomal aberrations, DNA damage, and cytoskeleton damage as measured by pinocytic adherence activity in *P. peguana* earthworm coelomocytes is evaluated. Commercial herbicide formulations with active ingredient 36% (w/v)was used with no further specification. Glyphosate did not cause any significant DNA damage as compared with the controls (Table III) in the Comet assay. The LC50 of glyphosate at 48 h to *P. peguana* was 251.45 µg cm–2, respectively. The product did not cause clastogenic effects, but induced aneugenic effects on coelomcytes including a marked chromosomal loss during anaphase at high concentration (LC50).
14	Nwani et al. (2013)	Induction of micronuclei and nuclear lesions in *Channa punctatus* following exposure to carbosulfan, glyphosate and atrazine	Exposure continued for 35 days; blood and gill cells collected on day 1, 7, 14,21 28 and 35 $P < 0.01$, for blood and gill cells; DNA damage increased with time and concentration	**Agreement**	Yes	The induction of nuclear lesions was concentration and duration dependent with the record of highest frequency at 24.4 mgLL at 96 h.

13	Piola et al. 2013	Comparative toxicity of two glyphosate-based formulations to *Eisenia andrei* under laboratori conditions.	Epidermic exposure during 72 h (on filter paper) $P < 0.001$	**Agreement**	yes	Median lethal concentration showed that formulations can be multiple times more toxic than glyphosate itself, and differences among the toxicity of formulations exist,. Formulations at sublethal concentrations can cause weight loss, and effects on DNA and lysosomal damage. Results highlight the importance of eco-toxicological assessment not only of the active ingredients, but also of the different formulations.
8	Poletta et al. (2009).	Genotoxicity of the herbicide formulation Roundup (glyphosate) in broad-snouted caiman (*Caiman latirostris*) evidenced by the Comet assay and the Micronucleus test	In-ovo exposure; blood sampling at the time of hatching $P < 0.05$ in both Experiments 50-1000 µg/egg in experiment 1; 500 -1750 µg/egg in experiment 2	**Agreement**	No	The study evaluated the genotoxic potential of Roundup® in erythrocytes of broad-snouted caiman (*Caiman latirostris*) after *in ovo* exposure. Comet assay and the Micronucleus test revealed a concentration-dependent effect. No difference was found in the MN frequencies < 500 µg/egg. Under the experimental conditions set, no external malformations were observed in any of the caimans of the different groups. Nevertheless, results demonstrated biochemical alterations, as well as growth delay in caimans when oversprayed with the formulation Roundup®. Concerning developmental parameters, data obtained demonstrated that none of the treatments applied produced severe effect after 12 months

IARC summarized the findings from those studies as follows: "*Glyphosate-based formulations induced DNA strand breaks in other species, including caiman (Poletta et al., 2009), frog (Meza-Joya et al. 2013), tadpoles (Clements et al. 1997), and snail (Mohamed, 2011), but not in oyster (Akcha et al. 2012), clam (dos Santo& Martinez, 2014), and mussel glochidia (Conners & Black, 2004). In earthworms, one glyphosate-based formulation induced DNA strand breaks while two others did not (Piola et al., 2013; Muangphra et al., 2014), highlighting the potential importance of components other than the active ingredient in the formulation."*

3.1 Discussion and Conclusion

The monograph of IARC discussed in this addendum (IARC Monographs, Glyphosate, Volume 112.2 (2015)) cites numerous genotoxicity studies that use glyphosate-containing formulation in a wide range of assays. Numerous formulations containing glyphosate as the active substance are available, the most prominent marketed under the name Roundup®. However, the specific composition of the formulations - especially the employed surfactant systems - was not available for all the evaluated studies. Given the importance of specific surfactants belonging to the ethoxylated alkylamines -as e.g. POE-Tallowamine- in driving the toxicity of formulated glyphosate products, it is not possible to evaluate the studies regarding their relevance for the reassessment of the active substance glyphosate without any information on the so-called inerts in the tested formulations. It is likely that several studies using the formulation Roundup® might test a product with POEA (polyoxyethylene-alkylamine) (especially Roundup® Original). The results of these tests have limited relevance for effects of glyphosate containing products that do not contain POEA and for the active substance glyphosate itself. It should be considered that the lead formulation for the assessment of the active substance glyphosate in the European Union does also not contain POEA as surfactant. Therefore, the studies carried out with POEA-containing glyphosate formulations are considered as additional information for the assessment of the re-authorization of the active substance glyphosate.

Likewise, RMS considered it adequate to provide general background information to other EU Member States to facilitate the assessment of the risk arising from glyphosate-based formulations other than the lead formulation. Please refer for detailed information to the draft RAR Chapter B.9.11 “Surface active substances in glyphosate-based formulations”.

The monograph of IARC discussed in this addendum (IARC Monographs, Glyphosate, Volume 112.2 (2015)) cites peer-reviewed open literature reporting on tests that might likely have been performed with several different formulations marketed under the name Roundup®. The results of these tests demonstrates that such formulations can cause chromosomal damage in several non-mammalian systems. Positive results were reported in in-vivo studies in fish (Guilherme et al. 2010, 2012b, 2014; Marques et al. 2014; Calvante et al. 2008; De Souza Filho et al. 2013; Nwani et al. 2013, De Castilhos Ghisi et al. 2012), amphibians (Clements et al., 1997, Meza-Joya et al. 2013, Yadav et al. 2013) reptiles (Poletta et al. 2011) and snails (Mohamed, 2011). Moreover, fruit flies, worm and plant systems

showed increased susceptibility to chromosomal damage as a result of exposure to glyphosate containing products (Kale et al. 1993, Piola et al. 2013, Muanghra et al. 2014 and Rank et al. 1993, Dimitrov et al., 2006). However, no severe effects were observed in oyster (Akcha et al. 2012), clam (dos Santo& Martinez, 2014), and mussel glochidia (Conners & Black, 2004).

The commercial formulations under the name Roundup® showed a concentration-dependent effect on the genetic integrity of fish species by means of the comet assay. Increased frequency rates of DNA damage in blood cells of fish after short-term exposure of Roundup® has been documented at concentrations ≥24.4 mg/L (Nwani et al. 2013, Marques, et al. 2014, Calvante et al. 2008, Guilherme et al., 2012). Moreover, it was shown that environmentally relevant concentrations of Roundup® formulations can induce positive results in comet assays using the European eel as a test species (Guilherme et al. 2012 and 2014).
Guilherme et al. (2012b and 2014) detected DNA damage at environmentally relevant concentrations using the commercial formulation Roundup® Ultra, containing isopropylammonium salt of glyphosate as the active ingredient and POEA as surfactant and included the test substances glyphosate and POEA separately. Fish were exposed to environmentally relevant concentrations of Roundup® Ultra (58, 116 µg/L), glyphosate (17.9, 35.7 µg/L) and POEA (9.3, 18.6 µg/L) for 1 and 3 days. The comet assay was perfomed with blood cells, either as the standard procedure, or with an extra step involving DNA lesion-specific repair enzymes as to clarify DNA damaging as well as their possible repair mechanisms. Both concentrations Roundup® Ultra and exposure times displayed significantly higher values levels of the genetic damage indicator. In contrast to the active substance glyphosate, a recovery phenomenon was not observed for blood cells of fish exposed either to POEA treatment or Roundup® Ultra treatment. In a second study, Guilherme et al. (2014) exposed fish to 116 µg/L Roundup® Ultra – a concentration which is assumed to cover realistic worse case exposure. Genetic damage indicators revealed a decrease of the non-specific DNA damage, but not a complete recovery of DNA stability. In a study by (Marques et al. 2014) a Roundup® formulation induced DNA damage which was hovewer shown to be reversible when fish were allowed to recover in herbicide-free water.

Furthermore, among the peer reviewed publications, micronucleus test were performed (Guilherme et al. 2010, Calvante et al. 2008). Since these test are capable of quantifyming chromosome and/or genome mutations by determining frequencies of micronucleus abnormalities, a combination of the comet assay and the micronucleus test might be

recommended for genotoxicity testing, because only a small amount of induced DNA damage might lead to fixed mutations depending on e.g. cellular status, repair capacity, genetic background of the cells.. In the study of Calvante et al. (2008) the frequencies of micronucleus and other erythrocyte nuclear abnormalities (ENAs) were not significantly different between Roundup®-exposed fish and their respective negative controls. By contrast, De Souza Filho et al. (2013) showed for a different formulation and test species that induced formation of micronuclei in erythrocytes are possible. Both results demonstrate the demand to generate data for glyphosate containing products with surfactants individually for each product in the framework of product authorization.

Considering the wide use of products formulated with glyphosate and surfactants that might increase the toxicity of the active substance, it it important to understand the mechanisms behind the observed damages in the genetic material of non-target organisms. Comparing all eco-toxicological data available on the effects of the active substance glyphosate with studies using glyphosate-based formulation with POEA, formulated products might cause damage at environmental relevant concentrations and elicidet effects that were not directly reversible (as in the case of the active substance glyphosate alone, see Guillerme et al. 2012b)..

In order to finally conclude on the implication of the reported results on the protection targets, e.g. individuals, populations, communities of organisms exposed to glyphosate containing PPP in environmental relevant concentrations, results related to genetic damage in in-vitro systems with artificial exposure conditions currently need to be related to ecological effects such as changes in population growth or reproductive output. At present, the long term endpoints derived in reproduction studies are considered to supersede results from biomarker studies.

By examining the interactions between the response of non target organisms and chemical exposure, criteria may be established in order to identify early onset biomarkers indicative of toxic effects elicided by chemicals. Nevertheless, in the submitted and evaluated apical studies, population-related effects at low concentrations were attributable to the effect of POE-alkyl amines.

Finally RMS highlights that -besides regulatory defined frameworks and agreed protection targets in assessment of the risk deriving from intended uses of PPP in the EU-, observed effects leading to DNA damage have the potential to induce physiological alterations, e.g. energy metabolism shifts, changes on fitness and reproductive success, and in the long run

might lead to population relevant effects - even if the submitted apical studies did not detect effects in the tested non-target organisms. As relevant effects were reported in two studies with formulation containing POEA surfactant (e.g. Roundup®Ultra Guilherme et al., 2012, ASB2014-7619, Guilherme et al., 2014, ASB2015-8631) European Member States are stronlgy encouraged to consider the substitution of POEA in plant protection products with other less toxic surfactant systems. According to Point 7.1.7 of Regulation (EU) No 284/2013, the competent Authorities have to discuss case by case the need to perform supplementary studies with formulated products in addition to studies with the active substance alone. The RMS recommends to discuss at EU level and to agree on a strategy to address further the genotoxic potential of active substances and formulated PPP products to non-target organisms. In the framework of the renewal of approval of the active substance glyphosate, apical studies on reproduction of non-target organisms with the representative formulation as confirmatory information should be performed.

4 Data relevant to compare information from peer reviewed literature to regulatory demands

The aim of an environmental risk assessment is to prevent inacceptable effects on ecosystems. For this purpose exposure concentrations and eco-toxicological data are assessed. Ecological risks are estimated, by the relation of potential exposure and the possible effects (risk = toxicity to exposure ratio). In order to ensure a uniform approach in the EU, data requirements and the procedure are defined in various guidance documents, inter alia, the Guidance Document on Aquatic Ecotoxicology (EFSA Journal 2013;11(7):3290). The predicted environmental concentrations and further details of calculation for glyphosate acid in surface water (PEC_{sw}), arising as a consequence of drift, drainage and run-off, are calculated and are provided in RAR Volume 3 CA- B8, 2015. The PEC_{sw} values for glyphosate acid and the major metabolites were calculated using the FOCUS (2000) surface water models. Additionally, PEC_{SW} values were estimated for the metabolites AMPA and HMPA. As worst case covering all intended uses, PEC_{sw} were derived for pre-emergence application of glyphosate to various field crops and for post-weed emergence use of glyphosate to the soil and trunks trees representing the intended use in perennial crops.

For glyphosate, maximum PEC_{SW} at FOCUS Step 1 were 104 µg/L, both for the pre-emergence use in field crops and post-weed emergence use in perennial crops. Maximum PEC_{SW} of glyphosate at Step 2 for the intended pre-emergence use in field crops ranged from 18 to 23µg/L. For the intended post-weed emergence use in perennial crops, maximum PECSW of glyphosate at Step 2 were 39 µg/L. In general only a few studies have investigated toxic effects at environmentally relevant concentrations. A few positive results were obtained at concentrations greater than the predicted environmental predicted exposure for the active substance glyphosate (Moreno et al., 2014; Lopes et al., 2014; Alvarez-Moya et al., 2014). In the only peer reviewed study investigating the potential DNA damaging ability of glyphosate and using environmentally realistic concentrations in the experimental setup (Guilherme et al. (2012b) fish exposed to 36µg/L glyphosate for 3 days recovered from the damage detected after 1 day exposure.

The main eco-toxicological endpoints used in environmental risk assessment aims at addressing the likelihood of adverse ecological effects according to the protection goals set out in the relevant legislation. The relevant aspects that need to be protected according to environmental protection goals is set by risk managers in the EU, including suitable

protection units, e.g. individuals, populations, communities (e.g. Guidance Document on Aquatic Ecotoxicology (EFSA Journal 2013;11(7):3290). RMS wants to point out that the definition of protection goals regarding non-target organisms itself already implies that all types of adverse effects of a PPP on a defined subject of protection has to be considered. Genetic resources were identified as important ecosystem services in agricultural landscapes which are potentially affected by pesticides (EFSA Journal 2010; 8(10):1821)

The RMS believes that it is necessary to evaluate the effects on the diversity and abundance of non-target organisms at higher trophic level in the light of the current scientific knowledge in order provide the base for risk management to ensure that the protection goals with respect to non-target organisms and therefore biodiversity in general can be achieved.

Endpoints or ecological effects that are measured in ecotoxicity tests currently include mortality, reduction in growth, reproductive impairment, changes in numbers of species, bioaccumulation of residues in non-target organisms, with the aim of protecting the long-term conservation of populations of non-target organisms in space and time.

Thereby, other toxicity endpoints may be used if lines of evidence determine that they can be linked to assessment endpoints in a reasonable manner. The determination of qualitative or biochemical parameters on, chromosomal, enzymatic, or other cellular organization levels are considered as supporting information, because this information might to provide valuable knowledge on genetic disrupting mechanisms at an early stage before a manifestation at the apical level can be observed. Currently, it represents a useful addition for interpreting observed apical effects in higher reproduction or long term studies. Generally applying information on molecular or cellular levels would enable a shift in the practice of risk assessment away from the use of apical endpoints, towards the use of biological information generated with in-vitro methods provided that effects would lead to apical responses. Data on mutagenicity and carcinogenicity is not routinely considered as representative of an adverse ecological effect, but instead is predominantly used in human cancer risk assessment. However, the above paradigm might unjustifiable and the genetic ecotoxicology might be moved into quantitative risk assessment comparably with mutagenicity and carcinogenicity data in a human risk context.

Finally, the identification of a genetic disrupting substance is considered to be reflected in a population-related effect that is reported by reproductive endpoints. Currently the population-related effects are detected with Fish Short-Term Reproduction-Assay (OECD 229), Fish

Screening Test (OECD 230), Fish Sexual Development Test (OECD 234) and Fish Full-Life-Cycle Test (US EPA OPPT 850.1500). Biochemical studies are able to provide valuable supporting information on genetic disrupting mechanisms already at an early stage before it manifests in the organism at the apical level.

In the present European renewal assessment for the active substance glyphosate, three regulatory long-term studies were conducted according to internationally agreed test guidelines. The active substance glyphosate was assessed for its toxicity towards the reproduction capacity of fish. Additionally the metabolite AMPA was assessed in a chronic fish study. A summary of data for chronic toxicity of fish is shown in RAR Volume 3 CA- B9 Part Ecotoxicology and in the following table:

Table 4-1: Chronic toxicity studies with glyphosate acid and metabolites AMPA

Species	**Substance**	**Test design**	**NOEC**	**Reference**
Pimephales promelas	Glyphosate acid	255d	25.7 mg a.s./L	Review Report for the active substance Glyphosate (SANCO/6511/VI/99-final), Anonym, 95-00020
Brachydanio rerio	Glyphosate acid	168 h	1 mg a.s./L	Doc ID: 2310938 /RF-D62.16/99
Oncorhynchus mykiss	Glyphosate acid	85-day (60 days post-hatch	9.63 mg a.s./L	Doc ID 2310941 /1005.029.321
Pimephales promelas	AMPA	33-day (7days post-hatch	12 mg a.s./L	Doc ID 2310943 /WL-2010-328

The active substance glyphosate acid has low chronic toxicity to aquatic vertebrates. The chronic endpoint for the 255 day fish full life-cycle study with the fathead minnow was 25.7 mg a.s./L ((DOC: 895-00020).). In general, chronic NOEC values for fish ≥10 mg/L are indicative of low chronic toxicity. In this study spawning, eggs per female and eggs per spawn did not differ significantly between controls and fish exposed to concentrations of glyphosate as high as 25.7 mg/L. Percentage of live fry hatching in concentrations of glyphosate as high as 25.7 mg/L was not different from values observed in controls.

For the performance of a quantitative risk assessment for non-target organisms, a short-term study (DOC: 2310938 /RF-D62.16/99) was considered, which provides a NOEC=1mg/L in combination with an assessment factor of 10. In this study, fertilized spawn (*Danio rerio*) was exposed in a continuous flow system for 7 days to glyphosate. Exposure started in an early

life stage and is considered to potentially affect a higly sensitive stage of development. In this study, an increase in the mortality rate was observed at a concentration of 3.2 mg glyphosate/L (statistically significant at a concentration of 5.6 mg/L).

A fish early life-stage study with the active substance glyphosate demonstrates similarly low chronic toxicity to fish with a NOEC value of 9.63 mg a.s./L, which was the highest concentration tested (DOC: 2310941/1005.029.321). In this study, fertilized spawn (*Oncorhynchus mykiss*) were exposed in a continuous flow system for 85 days. The study was rated as valid and there were no significant effects on the embryo vitality, the survival rate and growth parameters observed.

The risk assessment for the metabolite AMPA is based on an early life stage study (DOC: 2310943/WL-2010-328), in which fish embryos were exposed for 33 days in a flow through system. The results demonstrate similarly low chronic toxicity to *Pimephales promelas*, with a NOEC value of 12 mg a.s./L, which was the highest concentration tested. There were no effects on the survival and the growth parameters observed at the highest concentration tested.

The calculated toxicity-exposure-ratio (TER) values taking into account the lowest endpoints derived from regulatory studies as well as endpoints derived from peer-reviewed literature are provided RAR Volume 3 CA- B9 Part Ecotoxicology Chapter B 9.2. The TER values for glyphosate acid exposure to aquatic organisms all exceed the Annex VI acceptability trigger of 10, indicating that glyphosate acid does not pose an unacceptable risk to aquatic organisms following application according to the proposed uses of the glyphosate containing lead formulation.

5 Contribution of co-formulants to the toxicity of glyphosate-based formulations

During the assessment of the peer-reviewed literature in the framework of the renewal of approval of the active substance glyphosate, a major difficulty is a clear differentiation between studies assessing the active substance glyphosate itself and such employing formulatied products with glyphosate as active substance and several co-formulants, often company confidential and therefore unknown to the public. Often, the formulation name "Roundup" is described as "glyphosate" subsuming even several different formulations. At a regulatory level for the renewal of an active substance assessment, information is evaluated on the active substance itself, glyphosate, and a representative lead formulation. The lead formulation for the assessment of glyphosate as active substance for plant protection products in the EU does not contain POEA (polyoxyethylene-alkylamine) as surfactant. Since several glyphosate-based products are formulated with POEA, RMS considered it adequate to provide general background information to other EU Member States to facilitate the environmental risk assessment arising from glyphosate-based plant protection products other than the lead formulation. For detailed information please refer to RAR Volume 3 CA- B9 Part Ecotoxicology Chapter B 9.11.

The DNA damaging potential of POEA is reported in the peer reviewed literature for several non-mammalian systems (Tsui and Chu 2003; Avigliano et al. 2014). Howe et al. (2004) tested the toxic potential of POEA to different species of amphibians. Guilherme et al. (2012b and 2014) detected DNA damage at environmentally relevant concentrations of the active substance glyphosate as well as for POEA in the test species fish. The experiment was conducted using the commercial formulation Roundup®Ultra (containing isopropyl-ammonium salt of glyphosate as the active substance and POEA as surfactant) and included the test substances glyphosate and POEA separately (see evaluation of the study in this addendum, chapter 3). In contrast to the active substance glyphosate a recovery phenomenon was not observed for the formulation Roundup® Ultra nor for the surfactant POEA itself. When analysing the results, the data demonstrate the highest levels of DNA damage in the treatments with the highest concentrations the surfactant. This results support the data presented by Tsui and Chu (2003) and Avigliano et al. (2014), indicating that the particular formulant POEA is far more toxic to aquatic organisms than the active substance glyphosate.

In conclusion, RMS highlights that POEA containing formulations can cause geno-

toxicological effects and concludes that existing information does not allow to exclude unacceptable effects on the population level following the intended uses of plant protection products containinig glyphosate and alkylamine ethoxylated co-formulants. Higher tier apical studies with such formulated products not always available. Therefore, EU Member States should pay particular attention when assessing and managing the risk for POEA-containing glyphosate products, e.g by requesting the generation of further data. Member Stated might also consider to substitute POEA in plant protection products by less critical surfactants.

6 Final Discussion

Studies addressing the active substance glyphosate and the metabolite AMPA

For a reasoned opinion on the reneval of approval of the active substance glyphosate, the results of biomarker studies can be used as supporting information, but do not supersede the results of apical tests at the level of organisms. Usually is not possible to predict whether results from biomarker test will be manifest in the populations of non-target organisms to be protected. Currently, in the assessment of the risk of non-target organisms exposed to the intended use of a plant protection product only studies with apical endpoints are employed, , as these do reflect closer the potential impact at population level, e.g. survival of individuals, their reproductive performance and the vitality of their offspring. An initial indication of a potential adverse effect on organisms which results from biomarker studies is currently superseded by studies related to the reproductive performance of such organisms. In available reproduction studies with the test species fish, no evidence of impairment as a result of possible damage to the genetic material of the non-target organisms as well as other harmful effects have been observed at environmental relevant concentrations of glyphosate. From the evaluation of the studies as reported in the IARC monograph no changes for the environmental risk assessment of the active substance glyphosate do arise as compared to the assessment of the RMS in the draft RAR from July 2015.

Studies with glyphosate-containing formulation

As the lead formulation for the assessment of the active substance glyphosate does not contain alkylamine ethoxylated surfactants (POEA), the studies carried out with POEA-containing glyphosate formulations are not considered relevant for the reneval of approval of the active substance glyphosate. However, RMS considered it adequate to provide general background information to other EU Member States to facilitate the assessment of the risk arising from glyphosate-based products other than the lead formulation. Please refer also to RAR Chapter B.9.11 “Surface active substances in glyphosate-based formulations” for detailed information. All (eco) toxicological data available give strong evidence that the toxicity of glyphosate-based formulation with POEA arises from the effects elicited by this type of surfactants. Similar to the risk assessment of the lead formulation, in the framework of the authorization of plant protection products formulated with POEA, fish reproduction studies were submitted. In these regulatory studies, population-related effects at low concentrations were attributable

to the effect of POEA, since the derived endpoints were significantly lower than the effects observed in studies with the active substance glyphosate alone. Moreover, effects of glyphosate products formulated with POEA were reported in two geno-toxicological studies as assessed in this addendum(Guilherme et al., 2012, ASB2014-7619, Guilherme et al., 2014, ASB2015-8631). Therefore, RMS highlighted that EU Member States might demand the generation of further data at the authorization of glyphosate-based products with surfactants. Moreover, on the basis of this data EU Member States are encouraged to consider the substitution of POEA in plant protection products.

7 Conclusions and recommendations

1) RMS highlighted that EU Member States might demand the generation of further data in the framework of the authorization of glyphosate-based products formulated with specific surfactants. Member States are encouraged to consider the substitution of alkylamine ethoxylates (POEA) in plant protection products with less toxic surfactants.

2) Data on genotoxicity measured in bioassays is not routinely considered as representative of an adverse ecological effect, but is predominantly used in human cancer risk assessment. Generally, understanding available information on molecular or cellular levels might in future help reducing the use of apical endpoints, towards biological information generated with in-vitro methods - provided that effects seen in in-vitro test do lead to apical responses.

3) RMS would support further activities in order to assess the outcome of genotoxic studies in environmental risk assessment. Guidance is needed in order to identify sensitive and validated biomarker models and to qualitatively and quantitatively relate respective information to regulatory endpoints.

4) The assessment of the possible risks of the use of plant protection products containing glyphosate by the RMS has revealed that no unacceptable impact on natural environment and groundwater might be expected only if the application of such products is accompanied by appropriate risk mitigation measures. As a consequence, RMS has requested in its draft RAR for the EU Member States that the approval of the

active substance glyphosate for the use in plant protection products in the EU is associated with default measures in order to minimizie and compensate the identified high risks for the populations of non-target organisms – especially insects and farmland bird species – arising from a disruption of the food web (also referred to as effects on biodiversity).

5) Irrespective of the environmental risks (especially food-web disruption) associated with the application of the active substance glyphosate, and from an eco-toxicological perspective, the mere substitution of the active substance glyphosate by other herbicidal chemicals is not considered to be appropriate by RMS, since alternative substances might not be more favourable in terms of environmental properties compared to the active substance glyphosate.

8 References relied on

1) Akcha, F., Spagnol, C., & Rouxel, J. (2012). Genotoxicity of diuron and glyphosate in oyster spermatozoa and embryos. Aquatic Toxicology, 106, 104-113.
2) Alvarez-Moya, C., Reynoso Silva, M., Valdez Ramírez, C., Gómez Gallardo, D., León Sánchez, R., Canales Aguirre, A., & Feria Velasco, A. (2014). Comparison of the in vivo and in vitro genotoxicity of glyphosate isopropylamine salt in three different organisms. Genetics and molecular biology, 37(1), 105-110.
3) Avigliano, L., Alvarez, N., Loughlin, C. M., & Rodríguez, E. M. (2014). Effects of glyphosate on egg incubation, larvae hatching, and ovarian rematuration in the estuarine crab Neohelice granulata. Environmental Toxicology and Chemistry, 33(8), 1879-1884.
4) Bickham, J. W., Sandhu, S., Hebert, P. D., Chikhi, L., & Athwal, R. (2000). Effects of chemical contaminants on genetic diversity in natural populations: implications for biomonitoring and ecotoxicology. Mutation research/Reviews in Mutation research, 463(1), 33-51.
5) Cavalcante, D. G. S. M., Martinez, C. B. R., & Sofia, S. H. (2008). Genotoxic effects of Roundup® on the fish Prochilodus lineatus. Mutation Research/Genetic Toxicology and Environmental Mutagenesis, 655(1), 41-46.
6) Clement, C., Ralph, S., & Petras, M. (1997). Genotoxicity of selected herbicides in Rana Catesbeiana tadpoles using the alkaline single cell gel DNA electrophoresis (comt) assay. Environ Mol Mutagen, 29, 277-288.
7) Conners, D. E., & Black, M. C. (2004). Evaluation of lethality and genotoxicity in the freshwater mussel Utterbackia imbecillis (Bivalvia: Unionidae) exposed singly and in combination to chemicals used in lawn care. Archives of environmental contamination and toxicology, 46(3), 362-371.
8) De Souza Filho, J., Sousa, C. C. N., Da Silva, C. C., De Sabóia-Morais, S. M. T., & Grisolia, C. K. (2013). Mutagenicity and genotoxicity in gill erythrocyte cells of Poecilia reticulata exposed to a glyphosate formulation. Bulletin of environmental contamination and toxicology, 91(5), 583-587.
9) Guidance Document on Aquatic Ecotoxicology (EFSA Journal 2013;11(7):3290

10) Guilherme, S., Gaivao, I., Santos, M. A., & Pacheco, M. (2010). European eel (*Anguilla anguilla*) genotoxic and pro-oxidant responses following short-term exposure to Roundup®—a glyphosate-based herbicide. Mutagenesis, 25(5), 523-530.

11) Guilherme, S., Santos, M. A., Barroso, C., Gaivão, I., & Pacheco, M. (2012b). Differential genotoxicity of Roundup® formulation and its constituents in blood cells of fish (*Anguilla anguilla*): considerations on chemical interactions and DNA damaging mechanisms. Ecotoxicology, 21(5), 1381-1390.

12) Guilherme, S., Santos, M. A., Gaivão, I., & Pacheco, M. (2014). Are DNA-damaging effects induced by herbicide formulations (Roundup® and Garlon®) in fish transient and reversible upon cessation of exposure?. Aquatic Toxicology, 155, 213-221.

13) Jha, A. N. (2008). Ecotoxicological applications and significance of the comet assay. Mutagenesis, 23(3), 207-221.

14) Lopes, F. M., Junior, A. S. V., Corcini, C. D., da Silva, A. C., Guazzelli, V. G., Tavares, G., & da Rosa, C. E. (2014). Effect of glyphosate on the sperm quality of zebrafish *Danio rerio.* Aquatic Toxicology, 155, 322-326.

15) Marques, A., Guilherme, S., Gaivão, I., Santos, M. A., & Pacheco, M. (2014). Progression of DNA damage induced by a glyphosate-based herbicide in fish (Anguilla anguilla) upon exposure and post-exposure periods—Insights into the mechanisms of genotoxicity and DNA repair. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology, 166, 126-133.

16) Meza-Joya, F. L., Ramírez-Pinilla, M. P., & Fuentes-Lorenzo, J. L. (2013). Toxic, cytotoxic, and genotoxic effects of a glyphosate formulation (Roundup® SL–Cosmoflux® 411F) in the direct-developing frog Eleutherodactylus johnstonei. Environmental and molecular mutagenesis, 54(5), 362-373.

17) Mohamed, A. H. (2011). Sublethal toxicity of Roundup to immunological and molecular aspects of Biomphalaria alexandrina to Schistosoma mansoni infection. Ecotoxicology and environmental safety, 74(4), 754-760.

18) dos Santos, K. C., & Martinez, C. B. (2014). Genotoxic and biochemical effects of atrazine and Roundup®, alone and in combination, on the Asian clam Corbicula fluminea. Ecotoxicology and environmental safety, 100, 7-14.

19) Moreno, N. C., Sofia, S. H., & Martinez, C. B. (2014). Genotoxic effects of the herbicide Roundup Transorb® and its active ingredient glyphosate on the fish Prochilodus lineatus. Environmental toxicology and pharmacology, 37(1), 448-454.

20) Muangphra, P., Kwankua, W., & Gooneratne, R. (2014). Genotoxic effects of glyphosate or paraquat on earthworm coelomocytes. Environmental toxicology, 29(6), 612-620.

21) Nwani, C. D., Nagpure, N. S., Kumar, R., Kushwaha, B., & Lakra, W. S. (2013). DNA damage and oxidative stress modulatory effects of glyphosate-based herbicide in freshwater fish, *Channa punctatus*. Environmental toxicology and pharmacology, 36(2), 539-547.

22) Piola, L., Fuchs, J., Oneto, M. L., Basack, S., Kesten, E., & Casabé, N. (2013). Comparative toxicity of two glyphosate-based formulations to *Eisenia andrei* under laboratory conditions. Chemosphere, 91(4), 545-551.

23) Poletta, G. L., Larriera, A., Kleinsorge, E., & Mudry, M. D. (2009). Genotoxicity of the herbicide formulation Roundup®(glyphosate) in broad-snouted caiman (Caiman latirostris) evidenced by the Comet assay and the Micronucleus test. Mutation Research/Genetic toxicology and Environmental Mutagenesis, 672(2), 95-102.

24) Tsui, M. T., & Chu, L. M. (2003). Aquatic toxicity of glyphosate-based formulations: comparison between different organisms and the effects of environmental factors. Chemosphere, 52(7), 1189-1197.