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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 ... 7
1.1 Identification of the agent ... 7
1.2 Production and use ... 7
1.2.1 Production ... 7
1.2.1.1 Manufacturing process ... 7
1.2.2 Uses ... 7
1.2.2.1 Agriculture ... 7
1.3 Measurement and analysis ... 7
1.4 Occurrence and exposure ... 8
1.4.1 Exposure ... 8
1.4.1.1 Occupational exposure ... 8
1.4.1.2 Community exposure ... 8
1.4.2 Exposure assessment ... 9

2 Studies of Cancer in Humans ... 10
2.1 Cohort studies ... 10
2.2 Case–control studies on non-Hodgkin lymphoma, multiple myeloma, and leukaemia ... 15
2.3 Case–control studies on other cancer sites ... 22
2.4 Meta-analyses ... 25
2.5 Categorization of evidence from studies in humans ... 25
2.5.1 Contribution of co-formulants to the toxicity of glyphosate-based formulations ... 25
2.5.2 Conclusions on the classification of the evidence relevant to carcinogenicity from studies in humans into the IARC-categories ... 27

3 Cancer in Experimental Animals ... 29

4 Mechanistic and Other Relevant Data ... 46
4.1 Toxicokinetic data ... 46
4.1.1 Introduction ... 46
4.1.2 Absorption ... 46
4.1.3 Distribution ... 46
4.1.4 Metabolism and modulation of metabolic enzymes ... 46
4.1.5 Excretion ... 46
4.2 Mechanisms of carcinogenesis ... 47
4.2.1 Genetic and related effects ... 47
4.2.2 Receptor-mediated mechanisms ... 69
4.2.3 Oxidative stress, inflammation, and immunosuppression ... 74
4.2.3.1 Oxidative stress ... 74
4.2.3.2 Inflammation and immunomodulation ... 79
4.2.4 Cell proliferation and death ... 87
4.3 Data relevant to comparisons across agents and end-points ... 87
4.4 Cancer susceptibility data ... 87
4.5 Other adverse effects ... 88

5 Summary of Data Reported ... 89

5.1 Exposure data 89
5.2 Human carcinogenicity data 89
5.3 Animal carcinogenicity data 90
5.4 Mechanistic and other relevant data 93
5.5 Further conclusions and recommendations 94

6 References relied on 95

Annex 115

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 [redacted] (2001, ASB2015-8239) and [redacted] (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 [redacted] (2004, ASB2012-11528), [redacted] (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 ([redacted] 1988, ASB2015-7889; [redacted] 1992, TOX9650912; [redacted] 2005, ASB2012-11859, and [redacted] 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 [redacted] (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
[redacted] 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.
[redacted] 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.
[redacted] 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.
[redacted] 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
[redacted] 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.
[redacted] 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,

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		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 [redacted] (2015, ASB2015-2284), below		kidney, bladder, prostate and of melanoma, all lympho-haematopoietic cancers, NHL and leukaemia. Interpretation of multiple myeloma is limited.
[redacted] 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 [redacted] 2005a, ASB2012-11605 (see above).	See [redacted] 2005a, ASB2012-11605
[redacted] 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.
[redacted] 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.

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			even clearly below 1. Agreement with the limited power of the study.		
[redacted] 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
[redacted] 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.
[redacted] 2015, ASB2015-2284	Glyphosate and multiple myeloma, re-analysis of AHS data; data of the study of [redacted], 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

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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 ([redacted] 2001, ASB2015-8037 and [redacted] 1990, ASB2013-11501).

Five studies reported no increased risk of non-Hodgkin lymphoma and/or leukaemia or multiple myeloma. ([redacted] 1990, TOX2003-999; [redacted] 1992, ASB2015-7885; [redacted] 2012, ASB2012-11865; [redacted] 2004a, ASB2015-8238, and [redacted] 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 ([redacted] 2013, ASB2014-7523; [redacted] 1999, ASB2012-11838; and [redacted] 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 [redacted] (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: [redacted] 2003, ASB2012-11606; [redacted] 2005a, ASB2012-11605; [redacted] 2008, ASB2012-11614; [redacted] 2002, ASB2012-11839; [redacted] 2001, ASB2011-364, and [redacted] 2009, ASB2012-11985.

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

2 of the 6 studies used for the analysis of non-Hodgkin lymphoma reported no increased risk of non-Hodgkin lymphoma ([redacted] 2005a, ASB2012-11605 and [redacted] 2009, ASB2012-11985).

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

Finally, IARC referred in a publication in Lancet ([redacted] 2015, ASB2015-7076) to 3 studies ([redacted] 2003, ASB2012-11606; [redacted] 2001, ASB2011-364, and [redacted] 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
[redacted] 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.
[redacted] 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.
[redacted] 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
[redacted] 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)
[redacted] 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
[redacted] 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
[redacted]	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

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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)
[redacted] 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 [redacted] 1999, TOX1999-686, ASB2012-11838 and [redacted], 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 [redacted] 1999, TOX1999-686, ASB2012-11838 and [redacted], 1998, TOX1999-687 in this addendum).	Yes, page 530 and 535	See Table 2.2-2
[redacted] 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
[redacted] 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
[redacted] 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

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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
[redacted], 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
[redacted], 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)
[redacted] 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
[redacted] 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

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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
[redacted] 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 [redacted] 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
[redacted] (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.
[redacted] (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.

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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.
[redacted] (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
[redacted] (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.
[redacted] (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

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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
[redacted] (2002, ASB2012-11839) This study pools data from [redacted] (1999, ASB2012-11838) with data from [redacted] (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

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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 [redacted] (ASB2011-364), significant in the [redacted] (based on 29 cases) (ASB2012-11614) and not unequivocal in [redacted] (2003, ASB2012-11606) (a further study with data from the AHS in 2005 by [redacted] (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 ([redacted], 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
[redacted] 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
[redacted] 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
[redacted] 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
[redacted] 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
[redacted] 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
[redacted] 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

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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
[redacted] 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 [redacted] (2002, ASB2012-11839) and [redacted] (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

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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 [redacted] (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 ([redacted] 2003, ASB2012-11606; [redacted] 2005, ASB2012-11605; [redacted] 2008, ASB2012-11614; [redacted] 2002, ASB2012-11839; [redacted] 2001, ASB2011-364; [redacted] 2009, ASB2012-11985), which qualified according to the set criteria. Although one of these ([redacted] 2005, ASB2012-11605) is a prospective cohort study, it was not ranked higher. And one study ([redacted] 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 [redacted] (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; [redacted] 2003, ASB2012-11600, and [redacted] 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. ([redacted] 2009, ASB2009-7384; [redacted] 2005, ASB2009-9024; [redacted], 2007, ASB2009-9018, and [redacted] 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 [redacted] 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 ([redacted] 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 [redacted] 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 ([redacted] 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.

ED_001592_00000069-00031

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%, [redacted]	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%, [redacted]	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%, [redacted]	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%, [redacted]	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%, [redacted]	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
[redacted] 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

ED_001592_00000069-00032

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

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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%, [redacted]	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%, [redacted]	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%, [redacted]	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

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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%, [redacted]	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%, [redacted]	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%, [redacted]	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

ED_001592_00000069-00035

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%, [redacted]	in m/f)				
[redacted] TOX2000-1998, P24, 95.6%, [redacted]	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
[redacted] 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
[redacted] 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

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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 as reported by [redacted] (1997, ASB2012-11493), another positive result was obtained (Table 3-4). The IARC working group did report but not evaluate this study. 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).

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 tumors adenoma 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. A Cochran-Armitage trend test was performed, with p-values reported in a separate row.**

Dose	**male**	
(mg/kg bw)	N	adenoma
0	50	0
165	50	0 (1.000)
838	50	0 (1.000)
4348	50	2 (0.495)
Trend test (p-value)		0.0078

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 (Table 3-5). 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		0.5284	0.0496	0.3207

Dose	male			
(p-value)				

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 Stout and Ruecker (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 [redacted] (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 ([redacted] 2008, ASB2012-12059 and [redacted] 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 ([redacted] 2008, ASB2015-8160 and [redacted] 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 ([redacted] 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 [redacted] (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. [redacted] (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 ([redacted] 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 ([redacted] 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 ([redacted] 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 ([redacted] 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 ([redacted] 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 ([redacted] 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 ([redacted] 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 ([redacted] 2012, ASB2014-7619, [redacted] 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	Evaluated 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
[redacted] 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.
[redacted] 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	
[redacted] 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.
[redacted] 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	
[redacted] 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	
[redacted] 1995, ASB2012-11475	No	Chinese hamster lung	Chromosomal aberrations	–	–	-S9: 62.5 -	GLP, TG 473	p. 345-351	

ED_001592_00000069-00053

Reference	Evaluated 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
		cells (Chromosomal damage)				500 µg/mL +S9: 250 - 1000 µg/mL Purity: 95.7%			
[redacted] 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).
[redacted] 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).
[redacted] 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	
[redacted] 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	
[redacted] 1988, TOX9500253	Yes	Hamster, Chinese CHO-K1BH4 ovary,	*Hprt* mutation	–	–	-S9: 2 - 22.5 mg/mL +S9:	NR	p. 338	Not entirely clear from the original study report which dose level was actually the highest under activation conditions.

ED_001592_00000069-00054

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
also reported in RAR, TOX9552369, Z35243		cell line (Mutation)				5 - 22.5 or 25 mg/mL Purity: 98.7%			
[redacted] 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.
[redacted] 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
[redacted] 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.

ED_001592_00000069-00055

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
[redacted] 2009b, ASB2012-11906	Yes	Human lymphocytes (DNA damage)	DNA strand breaks, standard and hOGG1 modified comet assay	+	+	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). No indication of pH or osmolality control. Results not reported separately for replicate cultures. Authors state that no clear dose-dependent effect was observed.
[redacted] 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 .
[redacted] 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.
[redacted] 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.,	Yes	Buccal	DNA strand	+	NT	10-2000 µg/mL	NR	p. 404	$P \leq 0.05$ (20 µg/mL)

ED_001592_00000069-00056

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
2012, ASB2014-7618		carcinoma TR146 (DNA damage)	breaks, comet assay			Purity: 95%			No indication of pH or osmolality control. Results not reported separately for replicate cultures. No clear dose-response effect. Higher activity of formulation than pure a. s.
[redacted] 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.
[redacted] 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.
[redacted] 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.
[redacted] 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.

ED_001592_00000069-00057

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
									Test guideline deleted by now.
[redacted] 1995, ASB2012-11477	No	B. subtilis H17, M45 (DNA damage/repair)	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).

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)	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.

ED_001592_00000069-00058

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
						*p<0.05		
[redacted] 1994, TOX9400323	No	Mouse, Chromosome aberration test, bone marrow	Glyphosate, 96.8 % oral, 2 x 0-5000 mg/kg bw (24 h interval), sampled 24 h after second dose	Negative	GLP, OECD 475 (1984)	*No. of aberrations per 250-250-500 metaphases (male/female/total)* Control: 12/10/22 5000 mg/kg: 10/11/21 PosControl: 139*/155*/294* *p<0.05	5 animals per sex. 50 metaphases/animal examined. *Mitotic index (%) (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*	p. 358
[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)	p. 359, 374 ff.

ED_001592_00000069-00059

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
							% MNPCE [mean±SD (range)]: *0.02±0.02 (0.0-0.07)*	
[redacted] 2012, ASB2014-9333	No	Mouse, Micronucleus test, bone marrow	Glyphosate, 96.3 % oral, 1x 0 or 2000 mg/kg bw, sampled after 24 and 48 h	Negative	GLP, OECD 474 (1997)	*MN/2000 PCE [mean±SD, (range)]:* 24h, Control: 3.2±3.6 (0-8) 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	7 males per group (Control and PosControl: 5 males each). 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)	p. 359. 375 ff.
[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								
[redacted] 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*	Consistent with OECD 475 (1984): 6 animals per sex and sampling time. Ca 50 metaphases/animal examined. Slides were coded and scored "blind".	p. 358, 383

ED_001592_00000069-00060

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
						* p < 0.05	Original study reported in RAR as Li, 1983 (TOX9552375).	
[redacted] 1993, Z82234	Yes	Mouse, Micronucleus test, bone marrow	Glyphosate isopropylamine salt, purity not stated i.p., 1x 0, 100, 150 or 200 mg/kg bw sampled after 24 and 48 h	Negative	No GLP, no reference to TG	*% MNPCE (mean±SD):* 24h, Control: 0.27±0.11 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	Consistent with OECD 474 (1983): Mostly 5 animals per sex and dose and sampling time. 1000 PCE scored/animal. Slides were scored randomly. PCE/NCE: no effect.	p. 385, 388f.
[redacted] 997, 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
[redacted] 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

ED_001592_00000069-00061

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
[redacted] 1999, ASB2012-11482	No	Mouse, Micronucleus test, bone marrow	Glyphosate, 95 % i.p., 2x 0, 187.5, 375 or 562.5 mg/kg bw (24 h interval), sampled 24 h after second dose	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) 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	5 animals per sex and dose. 1000 PCE and 1000 NCE scored per animal. PCE/NCE: no effect (but PosControl). MN/1000 NCE: no effect (but PosControl). *$LD50_{i.p.}$=750 mg/kg*	p. 358, 367 ff.
[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] 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),	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)	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.*	p. 358. 364 ff.

ED_001592_00000069-00062

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
			sampled 24 h after second dose			62.5 mg/kg: 0.6 (0-3)/0.0 (0) PosControl: 23.0* (8-30)/12.2* (7-26) *p<0.01	PCE/NCE no effect. Historical control: ca. 3 MN/1000 PCE	
[redacted] 2010, ASB2014-9284	No	Mouse, Micronucleus test, bone marrow	Glyphosate, 98 % i.p., 2x 0, 125, 250 or 375 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: 0.4 (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	5 animals per sex and dose. 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	p. 358. 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
[redacted] 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
[redacted] 1997, Z59299	Yes	Mouse DNA adduct (8-OHdG by LC/UV),	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	3 male animals per group, at least 3 independent repeat experiments	p. 386

ED_001592_00000069-00063

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
		kidney				4 h: approx. 0.5 moles 8-OHdG/10^5 moles dG 24 h: approx. 0.4 moles 8-OHdG/10^5 moles dG*		
[redacted] 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
[redacted] 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
[redacted] 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
[redacted] 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
[redacted] 2013, ASB2014-	No	Mouse comet assay,	Glyphosate (96%) Drinking water, 14 days, 0, 40 or	+	No GLP, no	Tail moment (mean ± SEM): Control: 2.98±1.08	6 animals per group	p. 404

ED_001592_00000069-00064

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
6909		blood cells	400 mg/kg bw per day; sampled after treatment period		reference to TG	40 mg/kg bw per day: 8.54***±7.82 400 mg/kg bw per day: 9.06***±5.15	sex of animals not clear	
[redacted] 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 [redacted] (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

ED_001592_00000069-00065

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
[redacted] 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	
[redacted] 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).
[redacted] 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 $P < 0.01$

ED_001592_00000069-00066

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		
[redacted] 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.
[redacted] 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
[redacted] 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
[redacted]	Yes	Mouse	Analytical grade	Positive	No GLP,	MNE/1000 analysed cells:	5 animals per group	p. 422, 434

ED_001592_00000069-00067

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 [redacted] 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)

ED_001592_00000069-00068

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		
[redacted] 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

ED_001592_00000069-00069

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 ([redacted] 2009, ASB2009-7384; [redacted] 2005, ASB2009-9024; [redacted] 2007, ASB2009-9018 and [redacted] 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 [redacted] (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). [redacted] (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, [redacted] (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
[redacted] 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.
[redacted] 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.
[redacted] 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

ED_001592_00000069-00071

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.
[redacted] 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.
[redacted] 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.
[redacted] 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

ED_001592_00000069-00072

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.
[redacted] 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.
[redacted] 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.
[redacted] 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.
[redacted] 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
[redacted] 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

ED_001592_00000069-00073

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.
[redacted] 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
[redacted] 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.

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4.2.3 Oxidative stress, inflammation, and immunosuppression

4.2.3.1 Oxidative stress

Human cells *in vitro*, data on glyphosate:

[redacted] (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.

[redacted] (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.

[redacted] (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.

[redacted] (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.

[redacted] (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:

[redacted] (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.

[redacted] (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:

[redacted] (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.

[redacted] (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.

[redacted] (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.

[redacted] (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:

[redacted] (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.

[redacted] (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:

[redacted] (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.

[redacted] (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.

[redacted] (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.

[redacted] (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.

[redacted] (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 [redacted] (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 [redacted] (2010, ASB2012-11829) cannot be supported.

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

[redacted] (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 ([redacted] 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 ([redacted] 1979, ASB2015-8535) and a glyphosate formulation (but not glyphosate) ([redacted] 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:

[redacted] (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 [redacted] (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 [redacted] (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 [redacted] (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 [redacted] (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 [redacted] (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 [redacted] (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 ([redacted] 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 [redacted] (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 [redacted] (1992, TOX9551954) are in agreement with the negative finding for effects on the immune system of the study of [redacted] (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 [redacted] (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
[redacted] 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 [redacted] (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 [redacted] 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.
[redacted] 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.

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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.		
[redacted] 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.
[redacted] 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.
[redacted] 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

ED_001592_00000069-00086

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.
[redacted] 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.

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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 ([redacted] 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 [redacted] (2001, 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 ([redacted] 1988 (ASB2015-7889), [redacted] 1992 (TOX9650912), [redacted] 2005 (ASB2012-11859) and [redacted] 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 ([redacted] 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.

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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 Charles River Laboratories (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 ([redacted] 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 show clearly 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 ([redacted] 2012, ASB2014-7619, [redacted] 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.