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The Carcinogenic Hazard of Glyphosate: BfR's "Weight of Evidence Approach"*

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Abstract

The contradictory assessment of the carcinogenic properties of glyphosate is a focal point of the controversy related to the future fate of this herbicidal active ingredient. The International Agency for Research on Cancer (IARC) evaluated that glyphosate is "probably carcinogenic to humans". In contrast, the European Food Safety Authority (EFSA) and the German Federal Institute for Risk Assessment (BfR), to which the assessment was commissioned, concluded that glyphosate is unlikely to pose a carcinogenic hazard to humans. A classification as "probably carcinogenic to humans" would have serious consequences, because in principle it would exclude the future marketing of glyphosate according to EU directive 1107/2009. While this controversy was carried out almost exclusively in the media, the intention of this paper is to return to a scientific debate. Both sides (IARC and BfR/EFSA) recognise a significant increase of tumour incidences in a total of seven carcinogenicity studies in mice and rats. However, based on a weight of evidence approach, BfR and EFSA provided five reasons for dismissing these carcinogenic effects. Here, these five reasons are critically assessed. Following BfR's call for a science-based discussion, this institution is challenged to rebut the points raised here with concrete arguments or to admit their correctness.

1. Introduction

In a recently published article in this journal, von Mühlendal and Otto (2016) state, "The controversies surrounding glyphosate will be revived during the second half of 2017 at the latest, but one can barely expect that there will be important new arguments." This can be agreed, because the arguments are already on the table, as are the experimental data. The problem, however, is that the dispute is not based on concrete facts, because both sides – proponents and opponents of a continued approval of this herbicidal active ingredient – are carrying out the controversy almost exclusively in the media (one exception is the paper by Portier et al. 2016). The current paper is an attempt to foster the objective discussion that Germany's Federal Institute for Risk Assessment (BfR) has repeatedly asked for. At the same time BfR cannot be saved from the reproach of having created additional confusion by mixing up risk and hazard. In May 2016, for instance, officials of this institution tried to create the impression that the herbicide's categorization as "probably carcinogenic to humans" by the International Agency for Research on Cancer (IARC) did not go far enough: "Thus, the IARC only did a first step of the assessment of health risks which the BfR, the European authorities as well as the JMPR1 completed by taking into consideration the expected exposure to glyphosate originating from agricultural use (BfR 2016, translation by P.Cl.). However this announcement conceals the fact that BfR and the European authorities did not complete IARC's "first step", but dismissed it and made a 180° turn. According to them it is unlikely that glyphosate poses a carcinogenic hazard ("the EU peer review experts, with only one exception, concluded that glyphosate is unlikely to pose a carcinogenic hazard to humans and the evidence does not support classification with regard to its carcinogenic potential"; EFSA 2015, S.2). This means that the "second step", i.e. the risk assessment of glyphosate, was not performed on the basis of a "probably carcinogenic to humans" classification, but on the basis of a judgment that glyphosate would only cause general toxicity.

[1] Joint Meeting on Pesticide Residues of the FAO/WHO

There follows a brief review of the applicable European legislation for authorizing pesticide active ingredients. This is also done to correct von Mühlendal and Otto's (2016) opinion that the BfR and the European Food Safety Authority (EFSA) "should have explicitly restricted themselves to an assessment of the risks". Thereafter the data from carcinogenicity studies as contained in the 4.322-page Renewal Assessment Report will be presented. Since 24 November 2015 the final version of this document has been freely available on EFSA's website. An analysis follows of the "weight of evidence approach" used by BfR and EFSA, which formed the basis of their conclusion that glyphosate does not pose a carcinogenic hazard. The article closes with an appeal to the BfR to enter into the objective science-based discussion initiated in this article.

2. The EU Pesticide Regulation 1107/2009

Annex II of Regulation (EC) 1107/2009 plays a key role in the discussion about the possible carcinogenic potential of glyphosate. Paragraph 3.6.3 states: "An active substance … shall only be approved, if, on the basis of assessment of carcinogenicity testing carried out in accordance with the data requirements … and other available data and information, … reviewed by the Authority, it is not or has not to be classified, in accordance with the provisions of Regulation (EC) No 1272/2008, as carcinogen category 1A or 1B". This statement is followed by an "unless", which describes particular circumstances which would allow an approval. As a general rule, however, a classification into category 1A or 1B would result in a ban, making a risk assessment obsolete. For category 1B compounds, the classification as a presumed human carcinogen is mainly based on evidence in animals. This would apply for glyphosate. As a side remark it should be noted that category 1A and 1B classification also exists for mutagenicity and reproductive toxicity.

Therefore, for the reporting authority (the BfR) and the EFSA, the first task is to make a hazard evaluation (an evaluation of the properties of the compound). If no ban is pending because of the principal reasons (see above), a risk assessment will follow, and, inter alia, an acceptable daily intake (ADI) for humans will be deduced. Furthermore, it is logical that the risk assessment for a suspected carcinogenic compound (category 2) would be significantly different as compared to that carried out for more harmless compounds. In other words, without an appropriate evaluation of the hazard inherent in a chemical substance, a correct risk assessment is impossible.

The data needed for a category 1B classification is laid down in Regulation (EC) 1272/2008. The Regulation (Annex 1, Section 3.6.2.2.3b) defines "sufficient evidence of carcinogenicity" as a causal relationship between an agent (i.e. its administration in a suitable animal experiment, P.Cl.) "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." Furthermore, additional factors have to be taken into consideration, which will be discussed further below in the paragraph "Weight of Evidence Approach".

3. Carcinogenicity Studies of Glyphosate – the Data

The available carcinogenicity studies on glyphosate are described in the Renewal Assessment Report (RAR, RMS Germany 2015a) and its Addendum (RMS Germany 2015b), both produced by the BfR. The draft CLH Report (Dossier, BAuA 2016) represents a

more clearly structured summary with almost identical contents, which also was written by the BfR, and was submitted in spring 2016 to the European Chemicals Agency (ECHA). From this document it is clear that a total of 2 carcinogenicity studies in rats and 5 studies in mice demonstrated significantly increased tumour incidences after glyphosate administration. More precisely, 11 significantly increased incidences for 6 different tumour types were identified in these 7 studies. These were haemangiosarcoma, malignant lymphoma, and renal tumours in mice and pancreas carcinoma, liver adenoma, and C-cell adenoma of the thyroid in rats.

Here, we focus on the ECHA dossier which – as mentioned above – will lead to a revival of the controversy surrounding glyphosate during the second half of 2017 at the latest. However, the data presented in the dossier are congruent with those of the RAR. For the sake of clarity, our analysis will concentrate on one tumour type – the malignant lymphoma in mouse studies. An analysis with similar results could for instance also be presented for the renal tumours observed in the mouse studies. The data are summarized in Table 1. Information about statistical significance has been derived from the ECHA dossier (BAuA 2016).

Table 1: Incidences of malignant lymphoma in males of mouse carcinogenicity studies of glyphosate; number of animals (n) = 50 per group and sex, except for the study of 2009 (n=51) and 1983 (n=48-50); p-values<0.05 are considered significant. It should be noted that with a one-tailed error probability (i.e. testing only for a significant increase of the incidence) the calculated p-value would be divided in half; for pair-wise comparisons the p-values displayed refer to the high dose-group; for the trend test, the value refers to the entire study. In cases of trend tests, the Cochran-Armitage-trend test was used. Data from the ECHA dossier (BAuA 2016).

Year of study	Mouse strain	Doses (mg/kg body wt.)* Tumour incidence	Statistical method and p-value, all non-trend tests were pairwise comparisons
2009	Crl:CD1	0 – 71 – 234 – 810 0 – 1 – 2 – 5	Chi-Square-Test, p = 0.067 Trend-Test, p = 0.0037
2001	HsdOLA:MF1	0 – 15 – 151 – 1460 10 – 15 – 16 – 19	Z-Test, p = 0.002 Fisher's Exact Test, p = 0.077 Trend-Test, p = 0.0655
1997	Crj:CD1	0 – 165 – 838 – 4338 2 – 2 – 0 – 6	Fisher's Exact Test, p = 0.269 Trend-Test, p = 0.0085
1993	CD1, not further specified	0 – 100 – 300 – 1000 4 – 2 – 1 – 6**	Fisher's Exact Test, p = 0.741 Trend-Test, p = 0. 0760
1983	CD1, not further specified	0 – 157 – 814 – 4841 2 – 5 – 4 – 2***	No information, called significant in the narrative.

*dietary administration, doses were calculated from concentration in food, food intake and bodyweight

**according to ECHA-Dossier only incidences of lymph nodes with macroscopic changes

*** sum of lymphoreticular neoplasms, malignant lymphoma not specified

As can be seen, significantly increased tumour incidences were shown in 3 of the 5 mouse studies, although with different statistical methods – in some cases with trend-tests, in other cases with pair-wise comparisons. A dedicated guidance is available for the conduct and design of rodent chronic and carcinogenicity studies (OECD 2012). It contains a decision tree (flow diagram) for the statistical analysis. This flow diagram explicitly recommends the use of the Cochran-Armitage Trend Test or Poly-k-Test for the statistical analysis of incidence data of tumours and other pathological findings. In addition this OECD guidance emphasizes concerning the use of trend tests or pair-wise comparisons: "Significance in either kind of test is sufficient to reject the hypothesis that chance accounts for the result." – OECD 2012, p. 116). Last but not least, this OECD guidance argues that in carcinogenicity tests, a one-sided test may be more appropriate, because it is not expected that the test substance would decrease tumour incidence (OECD 2012, p. 133). Using a one-sided test, the error probability would be divided in half and another 4 comparisons shown in Table 1 would become statistically significant.

To summarize, it can be put on record that in conformity with the applicable guidance (OECD 2012), 3 out of 5 mouse carcinogenicity studies exhibited a significant increase in malignant lymphoma. It should be remembered that according to Regulation (EC) 1272/2008 it is considered as "sufficient evidence of carcinogenicity" (to classify for a category 1B carcinogen) if statistically significant carcinogenic effects are shown – inter alia – in 2 or more studies in one species conducted in different laboratories. In spite of the existing data comprising statistically significant increases of tumour incidences in more than 2 studies, in another species (rats) and in further tumour types, the BfR and the group of experts at the EFSA concluded that no carcinogenic effect of glyphosate can be seen. This gives reason to have a closer look at the arguments of the authorities.

4. The "Weight of Evidence Approach"

In the documents of BfR and EFSA as well as in the ECHA dossier, the existence of a statistically significant increase in the incidence of malignant lymphoma is acknowledged. However, with reference to Regulation (EC) 1272/2008, it is pointed out that additional factors have to be taken into consideration. These factors and additional considerations lead the aforementioned institutions to the conclusion that, based on a comprehensive evaluation (weight of evidence approach), glyphosate should not be considered as carcinogenic in spite of a statistically significant increase in the incidence of malignant lymphoma in 3 studies. Their reasoning relates to the following points:

1. Contradicting statistical results.
2. Inconsistencies as related to the dose-response-relationship.
3. The possibility of a confounding effect of excessive toxicity at test doses (Regulation (EG) No. 1272/2008, Annex II, Item 3.6.2.2.6).
4. A suspected infection with oncogenic viruses in the mouse study of 2001.
5. Historical control data.

One further point (point 6) can be added with regard to the handling of mechanistic evidence as identified by the IARC. The validity of these arguments is examined below. Table 2 (see page 13) summarizes the arguments of BfR/EFSA as compared to those of the author of this article.

4.1. Contradicting Statistical Results

In the final discussion of the tumour findings, the ECHA dossier emphasizes: "Mostly, but not always, trend tests revealed statistical significance but pairwise comparisons failed to detect a significant difference relative to the control group" (BAuA 2016, p.93). This statement ignores a number of aspects which invalidate the argument of contradicting statistical results.

First of all it should be stressed that one of the studies (dating from 1993) must be considered invalid, while the data of another (dating from 1983) are not specified with regard to malignant lymphoma. If this inadequacy is taken into account, for malignant lymphoma the argument is invalid that statistical significance is seen "mostly, but not always", when using trend tests. As can be derived from Table 1, for the remaining studies, those of 1997, 2001 and 2009, a significant increase in incidence was identified.

Why is the study of 1993 totally unacceptable and the one of 1983 insufficient with regard to malignant lymphoma? In a footnote to the respective table in the reports of the authorities it is mentioned that in the study of 1993, the microscopic assessment was restricted only to those lymph nodes that exhibited macroscopic abnormalities. This is a totally unacceptable way of evaluation. Even if it were acceptable, it would still be wrong to calculate the tumour incidence as the number of animals with malignant lymphoma out of the total number of animals of the respective study group. Instead the number of animals with malignant lymphoma out of the number of animals with macroscopically changed lymph nodes should have been calculated. Therefore the study of 1993 is unusable with regard to malignant lymphoma. Concerning the study of 1983, "(u)nfortunately, malignant lymphoma was not mentioned as a particular pathological entity", but the dossier submitter claims that "it can be reasonably assumed" that malignant lymphoma were reported as 'lymphoreticular neoplasia' (BAuA 2016, p.71). Because of this uncertainty in nomenclature the study of 1983 is ill-suited to be used as an argument that there are contradictions in statistical significance.

However, the supposed contradictions of the statistical results appear to be constructed anyway. It is commonplace that different statistical procedures are more or less appropriate, depending on the data structure. Trend tests, not pair-wise comparisons, are explicitly recommended in the flow-chart of the OECD guidance for the assessment of tumour incidences. In addition, if further recommendations given by the OECD guidance are taken into account, there is no contradiction in the statistical results between the studies of 1997, 2001 and 2003 at all.

First of all, this relates to the consideration already mentioned above that one-sided tests may be more appropriate for carcinogenicity studies. Using this approach, statistical significance is detected for both methods (trend tests and pair-wise comparisons) in the studies of 2001 and 2009; only for the study of 1997 is statistical significance limited to the trend test.

Another important aspect is the fact that these 3 studies have been performed in 3 different strains of mice (see Table 1). It is well known that different strains can react quite differently to the administration of a test substance. The fact that a significant increase of malignant lymphoma was seen in all three strains of mice underscores the robustness of the effect.

Finally, the dialectics between statistical and biological significance (relevance) need to be considered – something that was emphasized several times by the BfR too in the ECHA dossier. However, this duality was used asymmetrically by the BfR: i.e. statistical significance

was questioned, based on an alleged lack of biological relevance. However, the OECD guidance explicitly points to the fact that this applies also the other way round ("Similarly, declaring a result non-significant … should not be interpreted as meaning the effect is not biologically important..." OECD 2012, p.118). And there is plenty of biological relevance for glyphosate:

- there are two mechanism providing plausible explanations for the carcinogenicity of glyphosate;
- there is limited epidemiological evidence suggesting an association with cancer;
- there is a clear dose-response relationship in the studies of 2001 and 2009.

More specifically, the IARC assessed that for glyphosate, there is "strong evidence" for two carcinogenic mechanisms (genotoxicity and oxidative stress), and the limited epidemiological evidence (for Non-Hodgkin lymphoma, NHL) points to the lymphatic system as a particular target. The BfR even agrees that there is limited evidence for NHL. Shouldn't the finding of malignant lymphoma in the mouse studies then attract particular attention?

4.2 Inconsistencies as Related to the Dose-Response Relationship

In the ECHA dossier it is pointed out that the results are inconsistent as related to the dose-response relationship. As proof of this claim, reference is made to the incidence of malignant lymphoma in the studies of 1993, 1997 and 2009 (see Table 1), and it is indicated that (a) different tumour frequencies were seen for the control groups of 1993 and 1997 and (b) similar tumour frequencies were observed at different doses when comparing the studies of 2009 and 1997 (BAuA2016, p. 71). This conclusion is based on a major error. It is assumed that in all three studies the same strain of mice was used, because the acronym CD-1 appears in all three strain designations. However, for a long time it has been known that due to a genetic drift, major differences in a reaction can occur between different sub-strains. In particular, this applies to outbred stocks (commonly called strains) routinely used in toxicity studies and carcinogenicity bioassays. Moreover, even within the same sub-strain of an outbred stock, huge variability is possible (Festing 2016). This is why, when using data from the historical control database, it is necessary to restrict the data to studies using animals of same strain, from the same laboratory, collected within the last 5 years (OECD 2012, p. 135). Yet in the ECHA dossier, results of studies from 2009 were compared with results from 1993 and 1997 – too wide a timescale to be valid – and three sub-strains of mice were simply lumped together.

4.3 Excessive Toxicity in the High Dose-Groups

In the ECHA dossier and even more so in the EFSA conclusion (EFSA 2015), significant carcinogenic effects are dismissed with the justification that these were so-called high-dose effects which occurred only above a "limit dose" of 1,000 mg/kg body weight, as defined by the OECD. But this argument does not endure scrutiny. First of all it needs to be emphasized that the clearest dose-response relationship was seen in the study of 2009, the top dose of which was only 810 mg/kg. In the study of 2001, the top dose animals received 1,460 mg/kg body weight – which is only marginally above the assumed "limit dose", in particular if one takes into consideration that, according to the RAR, 80% of glyphosate is excreted unresorbed. Furthermore, a fixed "limit dose" is defined in OECD Guideline No. 452 describing the conduct of chronic toxicity studies (OECD 2009a), but not in Guideline No. 451, describing the conduct of carcinogenicity studies (OECD 2009b). In addition, the 1,000

mg/kg limit is not mandatory, but a recommendation: "a top dose not exceeding 1000 mg/kg body weight/day may apply" (OECD 2012, p. 66). Last but not least, excessive toxicity is an untenable claim when the criteria of Regulation (EC) 1272/2008 are applied: In none of the 3 studies (1197, 2001, 2009) was survival rate affected, nor were any excessive histopathological changes typical for excessive toxicity seen, such as cell necrosis (RMS Germany 2015a). The excessive toxicity claimed by the BfR refers to a 15% decrease in body weight gain in high-dose groups (RMS Germany 2015b, p. 2). However, for the study of 1997 there is a clear relationship between the reduced body weight gain and a reduced food consumption (RMS Germany 2015b). This not surprising in a situation where 1 kilogram food contains 40g of glyphosate, but this has nothing to do with excessive toxicity. For the other two studies, no food consumption data are available in the RAR. The unaffected lifespan of the high-dose groups, the lack of (excessive) histopathological changes, and the association between a reduced body weight gain and a reduced food consumption, are clear evidence that the contention of excessive toxicity is wrong.

4.4 Infection with Oncogenic Viruses

In the mouse carcinogenicity study of 2001 using the HsdOLA:MF1 strain, a significantly increased incidence of malignant lymphoma in glyphosate-treated mice was observed, but as compared to CD-1 mouse strains, there was also a high rate of spontaneous tumours of this type. In BfR's and EFSA's documents, it is sometimes claimed that the observed spontaneous incidence of malignant lymphoma could be or was caused by oncogenic viruses. To understand the following discussion, it is important to know that the mouse strain HsdOLA:MF1 belongs to the group of "Swiss" mice, similar to the situation with CD-1 mice, where numerous sub-strains exist.

The work of Wogan and Pattengale (1984) cited in the ECHA dossier emphasizes that (a) almost all spontaneous or treatment-induced lymphomas in mice contained oncogenic viruses; that (b) many other species, including humans, are carriers of oncogenic viruses; and that synergistic effects are possible between these viruses and chemical compounds concerning carcinogenicity (BAuA, p.72). However, it is interesting how the authorities handled the concrete situation in case of the study from 2001. Referring to a few publications, the final version of the RAR (as submitted by RMS Germany in March 2015) discusses whether the high tumour incidence in this study might be influenced by oncogenic viruses. Later, in the EFSA conclusion it was written that "the study was re-considered … as not acceptable due to viral infections" (EFSA 2015, p. 10). Later, in the ECHA dossier it was disclosed that the non-acceptance of the study was based on the remark of an employee of U.S. EPA during a telephone conference. At the same time it was admitted in the ECHA dossier that the study report did not contain indications of an impaired health status of the animals or of a viral infection, and that "the actual basis of EPA's decision is not known" (BAuA, p.72). Besides admitting this, the ECHA dossier refers to Tadesse-Heath et al. (2000), according to whom, "widespread" infections with oncogenic viruses have led to high, but remarkably variable incidences of tumours of the lymphoreticular system (BAuA, p.72).

However, this is incorrect. The word "widespread" does not occur at all in the publication. Instead, the authors refer to the investigation of mice from one source, which were studied in two different laboratories, and emphasize at the end of their publication that they investigated only one population concerning a highly expressive phenotype and that there are different

inbred strains and outbred stocks of "Swiss-Webster" mice in the U.S. and Europe which cannot be considered as identical. In other words, the results should not be generalized.

4.5. Historical Control Data

Historical control data, in comparison to the actual study data, were discussed in much detail by the BfR in their Addendum to the RAR (RMS Germany 2015b), as well as in the ECHA dossier. However, such a discussion can only benefit from those details if they are correct. But this was not the case. First of all it needs to be pointed out that the OECD guidance (OECD 2012) and other key documents (ECHA 2015, IARC 2006) emphasize that the concurrent control group of the actual study is always the most important point of reference, and that historical control data should only be used if the concurrent control group data are appreciably "out of line" with data from other studies.

In the summary and the discussion of the chapter on carcinogenicity the BfR claims that the tumour incidences found in glyphosate-treated animals "fell within their historical control range" (BAuA, p. 93). In reality however, the control group date of the 2001 study support the finding of a true glyphosate effect. The historical control data for the incidence of malignant lymphoma ranged from 6-30% with a mean value of 18.4% (data from 5 studies with a total of 250 animals between 1996 and 1999). The incidence of the high-dose group of the 2001 study with its 38% was not only above the mean but even above the range. As the BfR itself noted, the historical control data were unusable for the study of 2009. Only for the 3rd study, that of 1997 the incidence of the high-dose males (12%) was within the range of historical control data (3.85-19.23%). The question arises what the "within their historical control range" refers to. As an additional problem the OECD guidance discourages the use of arithmetic means, standard deviations and ranges as reference figures, because these can be biased by rogue outliers. Instead it is recommended to use the median and the interquartile range. However, such reference figures cannot be found in the documents written by the BfR.

Finally one can ask why there should be a need at all to use historical control data for such a robust effect as the statistically significant increase of malignant lymphoma in three different mouse strains.

4.6 Mode of action

Part of the plausibility of an effect is that it can be explained how it comes about. If there is evidence for one or more mechanisms of action, more weight will be put on the finding of statistically significant tumour incidences than when such evidence is lacking. This applies just as much to a substance with only one rat and one mouse study as for glyphosate, where a total of 11 statistically significant increases of tumour incidences were identified in 7 different studies with two different species. For glyphosate, the IARC determined "strong evidence" for genotoxic effects and oxidative stress as modes of action for carcinogenicity (IARC 2015). With regard to the molecular structure, the BfR did not see any potential for the formation of reactive oxygen species from glyphosate; however, it recognized as a possible mechanism the fact that reactive oxygen species could be generated due to an uncoupling of mitochondrial oxidative phosphorylation caused by glyphosate. In this regard, BfR and IARC are in line. However, this agreement was surprisingly contradicted in the Addendum to the RAR (RMS Germany 2015b) and the ECHA dossier (BAuA 2016). There the BfR concludes that "from the sole observation of oxidative stress and the existence of a plausible

mechanism for induction of oxidative stress … alone, genotoxic or carcinogenic activity in humans cannot be deduced for glyphosate" (RMS Germany 2015b, p. 78). This is surprising because if a true "weight of evidence approach" were used, one should expect that all aspects of an issue would be subjected to a holistic consideration. If doubt existed whether a significantly increased tumour incidence represents a true effect when evaluating carcinogenicity studies, the recognition of a valid mechanism of action should result in the removal of this doubt. The BfR, however, decouples these two strings of the "weight of evidence" (carcinogenicity studies and mechanistic evidence) completely and denies them separately.

A similar approach can be seen concerning genotoxicity. In contrast to the IARC, the BfR concludes that glyphosate has no genotoxic potential. This assessment is based on studies submitted by the industry as required by legislation and includes 16 tests in Salmonella typhimurium (AMES test) which all came out negative. In parallel, almost all genotoxicity tests published in the peer-reviewed literature – the majority of which demonstrated genotoxic effects – were dismissed by the BfR as insufficient. The reasons given by BfR for categorizing so many of over 80 published studies as insufficient varied. In some cases it was justified. However, the generalization used by the BfR to dismiss those studies that were subjected to peer review is not comprehensible. In strong contrast, the BfR had no problem accepting the 17 tests performed in bacterial systems (16 of them were AMES tests) submitted by industry. This should not have happened, because of the antibacterial properties of glyphosate. It is known that glyphosate was patented as a broad-spectrum antibiotic (U.S. patent number 7771736) and an "antimicrobial substance" (U.S. patent number 20040077608). The AMES test is considered inappropriate for genotoxicity testing of antibiotics (Luijten et al. 2016), and glyphosate is an antibiotic. Where was the critical assessment of the BfR in this case?

5. Conclusion

The EFSA and the collaborating authorities are obliged to perform a hazard evaluation before a risk assessment can be applied within the framework of the approval of a pesticide active ingredient. If an active ingredient is classified as "probably carcinogenic" (category 1A and 1B), in principle it cannot be approved according to Regulation (EC) 1107/2009. The scientific database contained in the reports supports a categorization of glyphosate as a category 1B carcinogen, according to Regulation (EC) 1272/2008. However, BfR and EFSA offer five arguments within a "weight of evidence approach" in an attempt to justify dismissing the finding of significantly increased tumour incidences. A thorough analysis shows that these five arguments are untenable.

I request that BfR engage in the objective, science-based discussion that it has repeatedly called for, and that it either refute the five points raised in this critique or admit their correctness.

6. Acknowledgements

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Annex:

Table 2: Summary of the critique of the „weight of evidence approach" by BfR and EFSA (referring to malignant lymphoma in mouse studies). The critical assessment relates tot he studies of 1997, 2001 and 2009, because – as detailed in the text – the studies of 1983 and 1993, as related to malignant lymphoma were only of limited use (1983) or completely useless (1993).

Issue	Opinion by BfR and EFSA	Critique of the Opinion
Contradicting statitistical results	Trend-tests were mostly (but not always) significant, however for pairwise comparisons there were no significant differences.	Trend-tests are explicitly recommended for the assessment of tumour incidences by the applicable OECD guidance. Even pairwise comparisons result in statistical significance if one-sided tests as recommended by the same guidance are used.
Inconsistencies concerning the dose-response-relationship	Different tumour incidences in the control groups and similar tumour incidences at different dosages in the different studies.	This is invalid, because it ignores the fact that different strains of mice were used in the different studies.
Excessive toxicity in high-dose groups	An increase in tumour incidences occurred only after exceeding the „limit-dose" of 1,000 mg/kg and excessive toxicity was observed.	A significant increase was also seen at 810 mg/kg. A „limit-dose" is not defined in OECD Guideline 451 (Carcinogenicity). Excessive toxicity was not seen in any of the studies. The reduced body weight is due to reduced food consumption (as a consequence of the high glyphosate concentration in the test diet).
An infection with oncogenic viruses makes the study of 2001 unusable	According to EFSA the study is not acceptable because of a viral infection; infections with oncogenic viruses are widespread in the strain of mice used.	According to the ECHA-dossier there is no proof for this claim made in the EFSA-Conclusion. In the publication, that is cited as alleged evidence for widespread infections by oncogenic viruses in the particular mouse strain, the term widespread is not used. To the contrary the authors emphasized, that they only presented results from two laboratories with mice from the same breeder.
Tumour incidences as related to historical control data	The tumour incidences of glyphosate-treated animals were in the range of historical control data.	For the study of 1997 OECD-recommendations for historical control data (HCD) are violated, for the 2001 study the HCD actually support the tumour finding, and no usable HCD are available for the study of 2009.
No conclusive evidence for a carcinogenic mode of action	From the "sole" observation of oxidative stress and a plausible mechanisms for its formation a carcinogenic action cannot be deduced.	Because of statistically significant increases in tumour incidences in three independent studies and epidemiological evidence, although limited, for tumours of the lymphatic system it is incorrect to speak of a „sole" observation of oxidative stress.

Imprint

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