PAN Germany
Pestizid Aktions-Netzwerk e.V.

The 31 August 2015 Addendum to the Renewal Assessment Report on Glyphosate

A critical analysis

by Peter Clausing, PAN Germany

Hamburg - November 2015

Introduction

In the European Union (EU) the approval for glyphosate expires at the end of 2015.[1] The re-approval for the EU market crucially depends on whether or not glyphosate poses a carcinogenic hazard (as an "intrinsic property" of the compound). If the European Commission classifies it as a "presumed human carcinogen", then it cannot be approved as an active ingredient of pesticides, unless human exposure is "negligible", according to EU legislation in force (Regulation 1107/2009; Annex II, 3.6.3).

The International Agency for Research on Cancer (IARC) has classified glyphosate as "probably carcinogenic to humans" (IARC Monographs, Volume 112). This classification was based on "limited evidence" in humans and "sufficient evidence" in experimental animals as well as "strong evidence" for two mechanisms of action associated with carcinogenicity, namely genotoxicity and the ability to induce oxidative stress.

The EU's own assessment is expected to be published by the European Food Safety Agency (EFSA) on 12 November 2015. This assessment will be based on the Renewal Assessment Report (RAR) finalised by the German authorities on 31 March 2015 (subsequently called RAR of March 2015), and an Addendum to this Report finalised by the same authorities on 31 August 2015 (subsequently called Addendum). In this Addendum, Germany as the Rapporteur Member State (RMS) has reviewed its earlier assessment of carcinogenicity in the light of the publication of the IARC monograph on 29 July 2015.

This Addendum was leaked and made public by public television station MDR on 20 October 2015[2]. It shows that Germany as the RMS acknowledges the evidence of carcinogenicity and supports IARC's evaluation. However, it still concludes the very opposite, i.e. that "no hazard classification for carcinogenicity is warranted for glyphosate according to CLP criteria (Addendum, p. iii)."

The present analysis documents that this conclusion is not only in contradiction with the IARC classification but also with the very data the RMS is presenting in its own report.

Legislative background

In the EU, an active ingredient of pesticides is classified as a carcinogen 1B ("presumed human carcinogen"), if there is "sufficient evidence" from experiments "to demonstrate animal carcinogenicity" (Regulation on classification, labelling and packaging [CLP] 1272/2008, Annex I; 3.6.2.1).

The term 'sufficient' has been adopted from the IARC (cf. CLP Regulation 1272/2008, Annex I; 3.6.2.2.3). and is defined as follows: "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" (emphasis added).

This regulation further states that the "(c)lassification of a substance as a carcinogen is a process that involves two interrelated determinations: evaluations of strength of evidence and consideration of all other relevant information to place substances with human cancer potential into hazard categories" (3.6.2.2.2.) and goes on to state that "(s)trength of evidence involves the enumeration of tumours in

[1] The final vote for an extension until 30 June 2016 is pending.
[2] http://www.mdr.de/fakt/fakt-glyphosat-bfr-bewertung102.html

human and animal studies and determination of their level of statistical significance" (Annex I; 3.6.2.2.3.).

The statistical significance of tumour increases is determined using the Cochran-Armitage- Trend Test (subsequently called Trend Test). While there are other statistical methods, this test is explicitly recommended by the applicable OECD guidance (OECD 2012, p. 123). The IARC used this test in its monograph for the evaluation of animal studies. The RMS used it in its Addendum to re-evaluate the study reports submitted by industry.

Finally, Regulation 1272/2008 states: "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" (Annex I; 3.6.2.2.4). These "other factors" include two considerations which play a particular role in the RMS' argumentation: "tumour type and background incidence", evaluated through the use of historical control data and "the possibility of a confounding effect of excessive toxicity at test doses" (Annex I; 3.6.2.2.6).

Last but not least, it is important to note that any carcinogen classification based on Regulation 1272/2008 is a hazard characterisation, not an assessment of risk. It is unrelated to the expected uses of the active substance and exposure of humans to it.

Carcinogenicity studies in rodents revisited

Mouse studies

In its Addendum, the RMS recognises that five valid long-term feeding studies in mice demonstrate a significant increase in tumours related to glyphosate exposure. This is a radical departure from the RAR of March 2015 in which the RMS reported only one mouse study (from 2001) as showing a significant increase in the incidence of tumours, in this case malignant lymphoma (see Table 1).

In the RAR of March 2015 the RMS argued that even the finding in the 2001 mouse study was irrelevant because the specific study was conducted in a mouse strain (Swiss albino) that is characterized by a high spontaneous incidence of malignant lymphoma, and that the other mouse studies which employed CD-1 strains (studies from 1993, 1997, 2009) did not show any significant increase in the incidence of malignant lymphoma.

Furthermore, in the RAR of March 2015, renal tumours were observed in three studies but not identified as treatment-related, as well as haemangiosarcoma in two studies (see Table 1).

It should be noted that all five study reports were written before the publication of the OECD guidance recommending the Trend Test in 2012. However, even in the preceding guidance, the Trend Test is mentioned, and when discussed with the other statistical method, i.e. pairwise comparisons, this guidance explains that "(s)ignificance in either kind of test is sufficient to reject the hypothesis that chance accounts for the result" (emphasis added, OECD 2002, p. 62). Therefore, bearing in mind both old and new guidance, already in March 2015 it was inappropriate for the RMS to evaluate these studies as showing no carcinogenic effect.

In its Addendum, the RMS concedes that "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" (emphasis added, Addendum p. 37).

Table 1: Significant increase in tumour incidence in male mice (indicated by +) using pairwise testing (RAR of March 2015) compared with the Cochran Armitage Trend Test (Addendum). Since 2012, this trend test is the method of statistical evaluation recommended by the OECD.

Year	**Top dose** (mg/kg bw)	**Renal tumours**		**Haemangiosarcoma**		**Malignant lymphoma**	
		BfR March	**BfR August**	BfR March	**BfR August**	BfR March	**BfR August**
1983	4.841	-	**+**				
1993	1.000			-	**+**	-	**-**
1997	4.843	-	**+**	-	**+**	-	**+**
2001	1.460	-	**+**			+[@)]	**-*)**
2009	810					-	**+**

bw = body weight; [@)]statistically significant based on the pairwise Z-test as performed by the authors of the study report; ***)** close to statistical significance (p=0.0655)

In the same Addendum, after applying the Trend Test, the RMS reports a significantly increased incidence of one or even several tumour types for male mice in each of the five studies. Surprisingly, the RMS dismisses all these findings and concludes that they are unrelated to treatment (Addendum, p.90-93). The RMS goes on to argue that "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" (Addendum, p. iii).

What consideration of biological significance does the RMS offer in the Addendum?

Historical control data

The RMS argues that the significantly elevated tumour incidences are all irrelevant because they are covered by historical control data**.** To fully understand the futility of this argument it is necessary to keep in mind the recommendations given by the applicable guidance (OECD 2012) on this issue.

For historical control data this Guidance No. 116 (OECD 2012) states on p. 135 (emphasis added): "In any discussion about historical control data, it should be stressed that the concurrent control group is always the most important consideration in the testing for increased tumour rates. The historical control data can, though, be useful provided that the data chosen are from studies that are comparable with the study being investigated. It is widely recognized that large differences can result from disparities in factors such as pathology nomenclature, strain, husbandry, pathologists. It has been suggested that historical control data should only be used if the concurrent control data are appreciably 'out of line' with recent previous studies and that only historical data collected over the last 5 years should be used."

In an extremely strong violation of these important principles, the RMS presents historical control data of 51 studies collated by Charles River Laboratories between 1987 and 1996 (year of study initiation). Good practice would have been to use historical control data for the same strain of mice, used within the same laboratory, collected over the last 5 years prior to the study, and ideally assessed by the same study pathologist.

Details of the Charles River pool of historical control data compared to the date of the actual studies, as far as available, are presented in Table 2. The RMS uses these data as its crucial argument in an attempt to invalidate findings of significantly increased tumour incidences.

Table 2: Study data (as far as available) of the five valid mouse carcinogenicity studies used in the RAR as compared to the Charles River historical control pool.

Study	Strain	Study initiation	Study location
Charles River Historical Control data	*Crl:CD-1(ICR)BR*	*Between 1987 and 1996*	*Not disclosed in Addendum. Pool of 51 studies total is suggestive of data from various laboratories*
1983	CD-1, substrain not mentioned in RAR of 2015	Before 1983	Not disclosed in RAR of 2015
1993	CD-1, substrain not mentioned in RAR of 2015	Before 1993	Not disclosed in RAR of 2015
1997	Crj:CD-1	1995	Institute of Environmental Toxicology, Tokyo, Japan
2001	Swiss Albino	1997	Rallis Research Ctr., Rallis, India
2009	Crl:CD-1(ICR)BR	2005	Harlan Laboratories, Shardlow, UK

As it can be derived from Table 2:

- The significant findings of the study from 1983 are dismissed with "historical" data collected after the study was conducted.
- The significant findings of the studies from 1997 and 2001 using the strains Crj:CD-1 and Swiss Albino, respectively, are dismissed with historical data from Crl:CD-1(ICR)BR
- The significant findings of the study from 2009 are dismissed with historical data from a period ending more than 7 years before study initiation

Possible further mismatches cannot be assessed due to the lack of details in RAR and Addendum[3].

It should be noted that for the study from 2001 valid historical control data (same strain, same laboratory) were available which actually confirmed the validity of the observed significant increase of the incidence of malignant lymphoma as described in detail in the RAR of March 2015 (Volume 3, B 6.5.2.). There, it is stated (emphasis added): "The incidence was statistically significantly elevated as compared to the actual control groups in this study, was above the mean values of the (relatively small) historical control and, for males, outside the historical control range." Concealing this fact and using an absolutely inappropriate data base the RMS now bluntly states the untruth by saying: "Also in the study with Swiss mice, which have considerably higher background incidences for malignant

[3] The gaps highlighted in this assessment underscore the importance that all study reports used for regulator decisions are made publicly available as demanded by civil society (e.g. http://www.pan-europe.info/sites/pan-europe.info/files/public/resources/campaigns/pesticides/2015_10_29ngo_letter_glyphosate.pdf)

lymphomas, the observed incidences were within the historical control range." (Addendum p.92, emphasis added).

A similar contradiction can be found within the RAR of March 2015 itself, related to the study from 2009. There, it was described that historical control data were requested from the laboratory that conducted this study, but the historical control data supplied were unusable. In Volume 3 it is stated: "However, the quality and regulatory value of the historical control data is very much compromised ..." (RAR of March 2015, p. 509). In contrast, in Volume 1 of it is stated that the observation of "slightly higher incidences in top dose males" (in fact a significant increase) was dismissed, because this was "… fully covered by historical control data" (RAR of March 2015, p. 65).

In relation to the significant increase in haemangiosarcoma, the RMS simply states: "The background incidences for haemangiosarcoma in male CD-1 mice provided by Charles Rvier Laboratories … were up to 6/50 (12%) … Therefore the observed incidences for haemangiosarcoma were spontaneous and unrelated to treatment" (Addendum, p. 92). This means, the RMS considers the significantly increased incidence in the study of 1997 with Crj:CD-1 mice as insignificant, because of a background incidence observed in Crl:CD-1(ICR)BR that was "up to 12%" without specifying how many of the 51 studies exhibited such a high incidence. Besides the deficiency of comparing different strains, it should be noted that the OECD recommends to use the median and interquartile ranges (OECD 2012, p. 135). By using the arithmetic mean and the simple range of historical data (Addendum, p. 91) the RMS did not follow the recommendation of the OECD.

In summary, the RMS' argument that a high background incidence invalidates the significant findings of the five mouse carcinogenicity studies is based on an inappropriate use of data. In addition, the presentation of data is insufficient and contradictory between different parts and versions of the RAR.

Excessive toxicity

Another argument used in the Addendum to dismiss the significant findings of animal carcinogenicity is "excessive toxicity" (p. ii) or "high-dose phenomenon" (p.36). Again, it is worth comparing the argument of the RMS with the recommendations given by the applicable Guidance and Guidelines.

The RMS refers to a top dose of 1,000 mg/kg that should not be exceeded in animal studies. Here it should be noted that a top dose of 1,000 mg/kg is mentioned in the OECD Guideline for Chronic Toxicity Studies (OECD 2009b), but not in the OECD Guideline for Carcinogenicity Studies (OECD 2009a). In other words, no top dose limit is defined for carcinogenicity studies, although they may be limited to 1,000 mg/kg when combined with a chronic toxicity study.

The RMS also refers to a recommendation that depression of body weight gain (as an indication of toxicity) should not exceed 10% as compared to the control group. Referring to the studies from 1983 and 1997, it argues that "excessive toxicity" has had a confounding effect here based on the observation that "the body weight gain was decreased by more than 15% compared to controls, but mortality/survival was not affected" (Addendum, p. ii).

First, it should be noted that the exact wording of the OECD Guidance No. 116 is that "the top dose should ideally provide some signs of toxicity such as slight depression of body weight gain (not more than 10%), without causing e.g., tissue necrosis or metabolic saturation". There is no mention of "necrosis" or "metabolic saturation" in the summaries of the long-term studies in mice presented in

the RAR of March 2015. Also, in the light of biological variability, a 15% depression of body weight is a moderate difference as compared to the ideal of “not more than 10%”.

More importantly, for the study from 1997 it is documented in the RAR of March 2015 that the observed decrease in body weight gain was related to a decrease in food consumption. In fact, the reduction of food consumption and the depression of body weight gain were even greater in the females of this study which did not exhibit any significant increase of any tumours. In addition, it is well-known that body weight and spontaneous tumour incidences are positively correlated (cf. OECD 2012, p. 133-134). In other words, if the body weight is reduced due to lower food consumption, it may result in a lower incidence of tumours, which means that the increased tumour incidences observed in the high dose group of the 1997 study could have been even higher if the body weight gain had not been reduced as compared to the control.

Finally it should be noted that a significant increase of tumour incidences was also observed in studies with top doses of 1000 mg/kg (study from 1993) and 810 mg/kg (study from 2009).

In conclusion, the argument of “excessive toxicity” has no factual basis in the studies reported.

Rat studies

In addition to the mouse studies, the RMS evaluated nine valid rat carcinogenicity studies. Through the application of the Trend Test statistically significant increases in tumour incidences in male rats were detected in the Addendum in two of these studies which had not been identified in the RAR of March 2015. This relates to pancreatic carcinoma in a study from 1981 and liver cell adenoma in a study from 1990. The study from 1990 also showed an incidence close to statistical significance for the combination of adenoma and carcinoma.

The RMS dismisses the finding of a significant increase in the incidence pancreatic carcinoma because this is “considered incidental” (Addendum, p. 92) without any further explanation. With regard to the positive trend for liver cell adenoma in male rats the RMS confirms this finding originally described by the IARC (2015), commenting that “IARC also noted lack of evidence for progression” (Addendum, p. 92).

Mechanistic evidence to assess the biological significance of the findings in animal studies

In this analysis, a brief consideration will be given to oxidative stress as a mechanism for carcinogenic effects. Genotoxicity as another mechanism has been discussed elsewhere (Clausing 2015).

Evidence of oxidative stress as a mechanism of carcinogenicity was completely omitted in the RAR of March 2015, while the Addendum states that “the majority of studies on oxidative stress in section 4.2.3 of IARC Monographs Volume 112 can be confirmed”. The Addendum continues to acknowledge: “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” (Addendum, p. 79).

However, in spite of this “support” the RMS concludes that “from the sole observation of oxidative stress and the existence of a plausible mechanism … alone, genotoxic or carcinogenic activity in humans cannot be deduced for glyphosate or glyphosate-based formulations” (Addendum, p. iv).

It needs to be emphasised that the issue here is not the question whether carcinogenic activity in humans can or cannot be "deduced". The issue is – according to the RMS – that "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" (Addendum, p. iii). While the "statistical significance" has clearly been shown for more than just one study and in some studies for more than just one tumour type, oxidative stress and genotoxicity in somatic cells provide the mechanistic evidence which underscores the "biological relevance" the RMS is calling for.

As noted above, any carcinogen classification under EU law is a hazard classification with a risk assessment to be followed. Therefore it is a futile to state – as the RMS did – that, "(i)n 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" (Addendum, p. iii).

Conclusion

According to the EU regulations in force, a compound will be classified as a carcinogen category 1B ("presumed human carcinogen") if the evidence shows "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" (Regulation 1272/2008, p. 104).

In case of glyphosate, five mouse studies and two rat studies have been identified that show a statistically significant increase in tumour incidences. In addition, mechanistic evidence exists as documented and analysed in the IARC monograph demonstrating the biological significance of these findings in relation to humans.

The RMS therefore has an amount of evidence available that vastly exceeds the requirements of the applicable legislation. The RMS's invalidation of this factual basis is based on an inappropriate application of the relevant OECD guidance and EU legislation. A revision of the assessment of the carcinogenic hazard posed by glyphosate appears unavoidable.

Acknowledgements

The valuable contributions and discussions of Franziska Achterberg and Angeliki Lysimachou are gratefully acknowledged.

References

Addendum: Renewal Assessment Report. Glyphosate Addendum I to RAR, 31 August 2015. http://www.mdr.de/fakt/fakt-glyphosat-bfr-bewertung100.html

Clausing, P. (2015): The Glyphosate Renewal Assessment Report. An Analysis of Gaps and Deficiencies. http://www.pan-germany.org/download/Glyphosat-Studie_Campact_PAN_korrigiert.pdf

IARC (2015): IARC monograph No. 112. Glyphosate. http://monographs.iarc.fr/ENG/Monographs/vol112/index.php

OECD (2002): OECD Environment, Health and Safety Publications. Series on Testing and Assessment No. 35 and Series on Pesticides No. 14. Guidance Notes for Analysis and Evaluation of Chronic

Toxicity and Carcinogenicity Studies.
http://www.oecd.org/officialdocuments/displaydocument/?cote=env/jm/mono(2002)19&doclanguage=en

OECD (2009a): OECD Guideline for the Testing of Chemicals No. 451, Carcinogenicity Studies.
http://www.oecd-ilibrary.org/environment/test-no-451-carcinogenicity-studies_9789264071186-en;jsessionid=16odl65wcetn2.x-oecd-live-03

OECD (2009b): OECD Guideline for the Testing of Chemicals No. 452, Chronic Toxicity Studies.
http://www.oecd-ilibrary.org/environment/test-no-452-chronic-toxicity-studies_9789264071209-en;jsessionid=16odl65wcetn2.x-oecd-live-03

OECD (2012): Guidance Document 116 on the Conduct and Design of Chronic Toxicity and Carcinogenicity Studies, Supporting Test Guidelines 451, 452 and 453, 2nd Edition Series on Testing and Assessment No. 116.
http://www.oecd.org/officialdocuments/displaydocument/?cote=ENV/JM/MONO(2011)47&doclanguage=en

RAR of March 2015: Renewal Assessment Report Glyphosate Volume 3; Annex B.6 Toxicology and metabolism, dated 31 March 2015.

Regulation Regulation on classification, labelling and packaging [CLP] 1272/2008: http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32009R1107&from=EN

Regulation 1107/2009: http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32009R1107&from=DE

Hamburg, November 2015

Dr. Peter Clausing, Pestizid Aktions-Netzwerk (PAN) e.V.

Nernstweg 32
D-22765 Hamburg
Tel. +49 (0)40-3991910-0
peter.clausing@pan-germany.org, +49-176 7801 2705
www.pan-germany.org

A healthy world for all.
Protect humanity and the environment from pesticides. Promote alternatives.