



The EFSA Conclusion on the Peer Review of the Glyphosate Risk Assessment

A Reality Check

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Background

On November 12th 2015, the European Food Safety Authority (EFSA) published its “Conclusion on the peer review of the pesticide risk assessment of the active substance glyphosate, *EFSA Conclusion*, (EFSA 2015) and subsequently released the final Renewal Assessment Report.

In general, the *EFSA Conclusion* concerning the carcinogenic hazard of glyphosate reflects the assessment made by the Reporting Member State (RMS) Germany and, therefore many aspects of the critique of the RMS' Addendum 1 published earlier (Clausing 2015) also applies to *EFSA's Conclusion*.

Carcinogenicity studies in rats and mice are crucial for the decision whether glyphosate is classified as carcinogenic or not. In the EU, an active ingredient of pesticides is classified as a carcinogen 1B (“presumed human carcinogen”), if apart from evidence in humans, 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: “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”.

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

Therefore, the evaluation of the different mouse and rat carcinogenicity studies plays a prominent role in the *EFSA Conclusion*, as it determines whether there is *sufficient* evidence from animal experiments to demonstrate carcinogenicity. EFSA's negligence of the significant increases of tumour incidence demonstrated in five mouse studies, is the foundation of the equivocal classification of glyphosate as not carcinogenic.

Consequently, the analysis presented here is focused on these mice studies.

EFSA's evaluation of tumour incidences in mouse studies is not based on evidence

To facilitate comprehension, the five mouse carcinogenicity studies discussed in more detail below are summarized in Table 1. This table compares the results of the original assessment made by the RMS in the Renewal Assessment Report (RAR, submitted on 31 March 2015, column “March”) and the results of the re-assessment which as presented in Addendum 1 to the RAR (RAR-ad, submitted on 31 August 2015, column “August”).

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

Year	Top dose (mg/kg bw)	Renal tumours		Haemangiosarcoma		Malignant lymphoma	
		March	August	March	August	March	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)

EFSA’s Conclusion deviates completely from the facts presented Table 1 and ignores numerous other pieces of evidence as detailed in the Open Letter signed by 96 Scientists (Portier et al. 2015). The issues concerning the five mouse studies will be discussed point by point below.

The study of 2001 - p. 10 of the EFSA Conclusion

This study is the only one where the EFSA initially accepted that it “showed a statistically significant increased incidence of malignant lymphoma” (the mouse strain Swiss albino was used in this study). However, the EFSA subsequently dismissed this observation, because the increased incidence of malignant lymphomas

- (a) “occurred at a dose level exceeding the limit dose of 1000 mg/kg bw per day recommended for the oral route of exposure in chronic toxicity and carcinogenicity studies”;
- (b) “was not reproduced in four other valid long term studies in mice”;
- (c) “The large majority of the experts had considered it highly unlikely that glyphosate would present carcinogenic potential due to the generally recognised high background incidence of malignant lymphomas in this strain”; and
- (d) “The study was re-considered during the second experts’ teleconference (TC 117) as not acceptable due to viral infections that could influence survival as well as tumour incidence – especially lymphomas”.

These EFSA claims are false because of the following reasons:

Claim (a): Dose level exceeding the limit dose

This false claim is discussed in detail together with the considerations on the high dose level of the other studies (see Section 4.)

Claim (b): Incidence of malignant lymphomas not reproducible in other studies

This is a false claim.

As it can be seen in Table 1 a significant increase in malignant lymphoma was identified in the majority of the studies (3 out of 5) when the appropriate statistical method is used.¹ In addition to the statistical significance *per se* the higher incidence of malignant lymphoma increased dose-dependently in two of the three studies (Table 2).

Table 2: Percent incidence of malignant lymphomas in male mice in the three studies with statistical significance. Note: CD-1 mice were used in the studies of 1997 and 2009, and Swiss albino mice in the study of 2001.

Year	Control	Low-Dose	Mid-Dose	High-Dose
1997	4 %	4 %	0 %	12 %
2001	20 %	30 %	32 %	38 %
2009	0 %	2 %	4 %	10 %

Claim (c): No carcinogenic potential due to high background incidence

This is false claim.

Under the given conditions a high background incidence does not matter (see below). Furthermore, a significant increase in malignant lymphomas was also observed in the two studies that used other mouse strains (Crj:CD-1, Cri:CD-1(ICR)BR) which have a clearly lower background incidence.

According to applicable OECD guidance “it should be stressed that the concurrent control group is always the most important consideration in the testing for increased tumour rates.” (OECD 2012, p. 135). This approach is also supported by virtually all existing guidelines and recommendations (Portier et al. 2015, p.5). In addition, for the 2001 study this finding is even supported by the historical control data presented in the RAR. As stated there, the observed significantly increased incidence of malignant lymphoma in high dose group males was not only above the mean value of historical controls, but even “outside the historical control range” (Volume 1, p.63).

In addition, referring to Sher (1974) and Roe and Tucker (1974), it is stated in the RAR that “these historical control rates were still lower than what was seen in the study by [name blacked-out]”, i.e. the study of 2001 (Volume 1, p.63). Only one of the three papers (Tadesse-Heath et al. 2000) reports a spontaneous incidence of malignant lymphoma higher than that observed in the high dose of the 2001 study. In addition, it needs to be emphasized that these publications do not present valid historical controls (see Section 5.)

Claim (d): Study not acceptable due to viral infections

This is a false claim.

The wording used in the *EFSA Conclusion* (“study was ... not acceptable due to viral infections ...”) is suggestive of a proven viral infection in this study, which supposedly contributed to the high spontaneous rate of malignant lymphoma. However, this is not true. In

¹ Since 2012, the OECD explicitly recommends the use of the Cochran-Armitage-Trend Test (OECD 2012, p. 123)

the RAR (Volume 1, p.63) it is clearly stated: “No information is available on possible abundance of such viruses in the mouse colonies from which animals used in the glyphosate studies were obtained.” Instead, reference is made to the paper by Tadesse-Heath et al. (2000) who in general “emphasized the contribution of widespread infections with murine oncogenic viruses to the high, remarkably variable incidence of tumours of the lymphoreticular system.” RAR (Volume 1, p.63)

There is no evidence that the mice used in the study of 2001 were infected by oncogenic viruses and the EFSA statement cited above is clearly misleading.

Lack of statistical significance in pair-wise comparison tests – p. 11 of the EFSA Conclusion

A “lack of statistical significance in pair-wise comparison tests” is claimed in the *EFSA Conclusion* as evidence for lack of carcinogenicity in the mouse studies listed in Table 1 of this document.

This claim is misleading.

The problem with this claim is two-fold.

First of all, for the study of 2001, there is actually a statistical significance in the pair-wise comparison test, additionally supported by historical control data and dose dependency as outlined above.

Secondly, the statement in the *EFSA Conclusion* gives the impression that a pair-wise comparison is the most relevant statistical method, which is not in line with applicable guidance. The European Union uses the OECD framework of testing guidelines for the safety assessment of chemicals and pesticides. Therefore, OECD guidance No. 116 (OECD 2012) is the relevant guidance. This guidance contains a “decision tree for common statistical procedures” (p. 123), which explicitly and unequivocally points to the Cochran-Armitage-Trend test (or Peto analysis/Poly-k-test in case of different survival rates between groups) as the method of choice for the analysis of tumour incidences. In this decision tree pair-wise comparisons are not even mentioned for the assessment of tumour rates. Unlike the pair-wise test that compares each exposure group to the control, the Cochran-Armitage-Trend test detects a linear trend, which, if significant, indicates an increasing risk of carcinogenicity with increasing exposure. In addition, for pair-wise comparisons and trend tests in general, both Guidance No. 116 (OECD 2012, p. 116) as well as Guidance No. 35 (OECD 2002, p. 62) state: “Significance in either kind of test is sufficient to reject the hypothesis that chance accounts for the result.”

In other words, for these two important reasons, significant effects detected by the Cochran-Armitage-Trend test (see Table 1) should fully account for the assessment of carcinogenicity. Therefore, the claim of “(n)o evidence of carcinogenicity ... due to lack of statistical significance in pair-wise comparison tests” (EFSA 2015, p.11) has no scientific basis.

Lack of consistency in multiple animal studies – p. 11 of the EFSA Conclusion

This conclusion is not founded by evidence.

As it can be easily derived from Table 1, it was possible to replicate the following findings:

In an extremely strong violation of important principles for the use of historical control data as defined in OECD guidance 116, RMS and EFSA attempted to invalidate the above mentioned significant carcinogenic effects. When looking at the evidence it turns out that this attempt has failed.

Recommendations

The fact that statistically significant increases in tumour incidences were seen in five independent, valid mouse studies carried out at different times and different laboratories, there is, according to CLP 1272/2008 [Annex II, 3.6.2.2.3.(b)], sufficient animal evidence demonstrating that glyphosate is a presumed human carcinogen. Therefore, classification of glyphosate in Category 1B is warranted.

Commissioner Andriukaitis of the Directorate General on Health & Food Safety and the Standing Committee on Plants, Animals, Food and Feed are urged to disregard the flawed *EFSA Conclusion* on glyphosate and to correct this conclusion appropriately.

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