The Glyphosate Renewal Assessment Report

An Analysis of Gaps and Deficiencies

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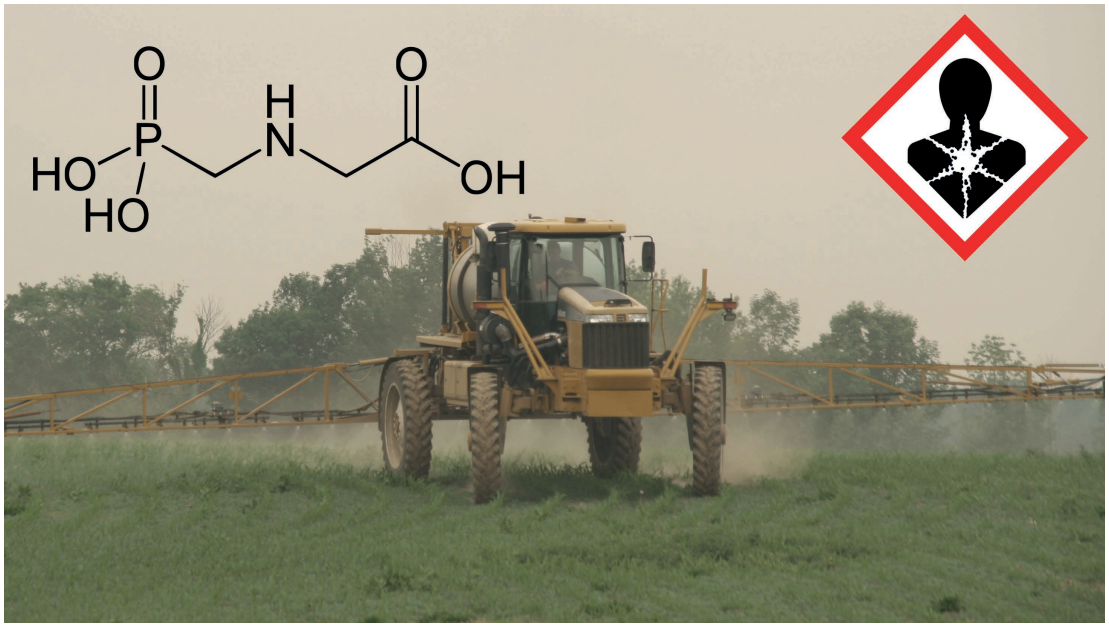

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Peter Clausing earned his Ph.D. in agriculture and became a toxicologist by postgraduate studies. From 1993 to 1996 he researched as a postdoctoral fellow at the FDA's National Center for Toxicological Research in Jefferson, AR, USA. From 1997 to 2001 he worked as a toxicologist and Head of the Department of Toxicology at a European Contract Research Organization. From 2001 until retirement in 2010 he worked as Study Director and Project Toxicologist in the Department of Toxicology of a large pharmaceutical company. Dr. Clausing has published more than 30 scientific papers in peer-reviewed journals.

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Zusammenfassung

Der Bewertungsbericht zur Wiedergenehmigung (Renewal Assessment Report, RAR) ist die Grundlage für die gesetzliche Entscheidung, ob Glyphosat in der Europäischen Gemeinschaft künftig zugelassen bleibt. Der RAR soll die Ergebnisse der behördlichen Bewertung des Dossiers liefern, zu dessen Einreichung der Antragsteller, d.h. die Industrie (in vorliegenden Fall die Glyphosat Task Force, GTF) verpflichtet war. Die hier vorliegende Analyse liefert wichtige Belege für ernsthafte Mängel des RAR in kritischen Bereichen der Risikobewertung. Die wichtigsten Beispiele werden für die Bereiche Gentoxizität und Kanzerogenität vorgelegt. Die Mängel betreffen die Vernachlässigung und fehlerhafte Beschreibung wichtiger wissenschaftlicher Veröffentlichungen, fehlende Anwendung der gültigen statistischen Analysemethoden auf die von der Industrie eingereichten Daten und falsche Behauptungen bezüglich historischer Kontrolldaten, um so wichtige Ergebnisse aus Krebsstudien an Mäusen verwerfen zu können.

Insbesondere fehlt die Berücksichtigung von Belegen zu den Mechanismen für die krebserregende Wirkung von Glyphosat, d.h. oxidativem Stress und Gentoxizität. Über oxidativen Stress in Bezug auf Krebsverursachung schweigt sich der Bericht völlig aus und nahezu ein Drittel aller Publikationen zur Gentoxizität von Glyphosat finden keine Erwähnung. Hinzu kommt, dass mindestens ein wichtige Studie zur Gentoxizität vom BfR verzerrt und mit falschen Zahlen dargestellt wurde.. Ferner vermittelt der Umgang mit einer wichtigen Krebsstudie an Mäusen, die von der Industrie durchgeführt wurde (d.h. fehlende Anwendung vom statistischen Methoden nach dem neuesten Stand, falsche Behauptungen über historische Kontrolldaten), den Eindruck, dass dies absichtlich so geschehen ist.

Die vorliegende Analyse belegt Auslassungen und die Verdrehung von Fakten im Teil „Toxikologie und Metabolismus“ das RAR-Entwurfs, der bei der EFSA eingereicht wurde. Das Bundesinstitut für Risikobewertung (BfR), dass für diesen Teil des RAR zuständig war, trägt die volle Verantwortung für diese Lücken und Mängel. Die Bundesregierung behauptet, dass das Bundesinstitut für Risikobewertung (BfR) „ ... alle vom Antragsteller vorgelegten Originalstudien sowie die in wissenschaftlichen Zeitschriften publizierten Studien detailliert geprüft und qualitätsgerecht bewertet“ hat. Die hier vorliegende Analyse widerspricht dieser Behauptung, indem belegt wird, dass von der Behörde wichtige Ergebnisse übersehen und zahlreiche Publikationen nicht berücksichtigt wurden. Die Schwächung der Beweislage durch drei verschiedene Herangehensweisen, d.h. Nichtbeachtung von Studien, fehlerhafte Analysen und Verdrehung von Tatsachen, nährt den Verdacht, dass Absicht im Spiel war.

Es sollte beachtet werden, dass diese Einschätzung auf der Basis einer Durchsicht der RAR-Version vom 31.3.2015 getroffen wurde, einer Version, die öffentlich nicht zur Verfügung steht.

Executive summary

The Renewal Assessment Report (RAR) is the basis for the legal decision whether approval should be granted for future use of glyphosate within the European Community. The RAR is supposed to provide the results of the assessment made by the authorities of a dossier that the applicant, i.e. the industry (in our case the Glyphosate Task Force, GTF), is obliged to submit. The analysis at hand provides evidence for serious deficiencies of the RAR in crucial areas of risk assessment. The most important examples are provided in the areas of genotoxicity and carcinogenicity. The deficiencies include neglect and wrong description of

important scientific publications, lack of applying up-to-date statistical analyses to the data provided by industry and false statements about historical control data used to dismiss important results from carcinogenicity studies in mice.

Specifically consideration of mechanistic evidence for glyphosate's carcinogenic effects, i.e. oxidative stress and genotoxicity is missing or insufficient. The report remains mute about oxidative stress as related to genotoxicity and almost one third of the scientific literature on genotoxicity is missing. In addition at least one important study on genotoxicity received a false and distorted description by the BfR. Furthermore, the handling of an important mouse carcinogenicity study by industry (i.e. not applying state-of- the-art statistical methods and wrong claims about historical control data) give the impression that this was done deliberately.

The current analysis demonstrated omissions and distortion of facts in the "Toxicology and Metabolism" part of the draft RAR that was submitted to the EFSA. The *Bundesinstitut für Risikobewertung* (Federal Institute for Risk Assessment, BfR) which was in charge of this part of the RAR has full responsibility for these gaps and deficiencies. The German Government claims that the BfR , performed a "detailed, quality-assured examination of all … original studies and the studies published in the scientific literature". The present analysis contradicts this statement by providing evidence that the agency overlooked or ignored important findings and that numerous publications from the scientific literature have not even been taken into consideration. The weakening of evidence by three different processes, i.e. neglect of studies, failure of analysis and distortion of facts, nourishes the suspicion that this was done on purpose.

It should be noted that this assessment is based on a review of the 31 March 2015 version of the RAR which is publicly not available.

Introduction

To understand the background of the present analysis, a short description of the approval procedure for pesticides is given below. In addition, what lead us to perform this analysis is briefly described.

In the European Community the legislation for the marketing of pesticides is laid down in Regulation [EC] No. 1107/2009. According to this regulation the active ingredients of pesticides, in our case glyphosate, have to be re-approved every 10 years. For this re-approval industry ("the applicant") is obliged to submit a dossier which contains all new information about the pesticide that had accumulated during the 10-year-period prior to submission of the dossier. In case of glyphosate the applicant was a consortium of corporations – the Glyphosate Task Force (GTF). This dossier compiled by the GTF includes a volume on "Toxicology and Metabolism" – the basis for the safety assessment of the pesticide to be performed by the authorities. The "Toxicology and Metabolism"-part of the dossier consists of so-called regulatory studies and studies published in the scientific literature. Regulatory studies follow defined study designs according to the OECD guidelines and have to be performed or commissioned by the applicant. While the study reports are confidential and only disclosed to the authorities, summaries of these reports are presented in the dossier which is publicly available[1]. In addition, the applicant is obliged to perform a literature search covering the last 10 years prior to the submission of the dossier. All relevant studies from this literature search have to be included in the dossier and for those considered not relevant the reason why the particular study has been excluded to be described.

The industry is allowed to select a country, the so-called Reporting Member State (RMS), where the dossier is to be assessed. This is to say that the authorities of the RMS are generating the (Renewal) Assessment Report. The final version of this report is submitted to the European Food Safety Authority (EFSA) which, based on a peer review makes a proposal to the European Commission (Directorate General for Health and Food Safety) concerning the (further) marketing approval of the pesticide.

In case of the renewal procedure for glyphosate Germany was selected by the GTF as the RMS. While the *Bundesinstitut für Verbraucherschutz* (BVL, Federal Office for Consumer Protection and Food Safety) had the overall responsibility, the *Bundesinstitut für Risikobewertung* (BfR) became responsible for the assessment of the human safety aspects, i.e. the Toxicology and Metabolism).In December 2013 the first draft of the Renewal Assessment Report (RAR) of glyphosate[2] was finalized and made available for public commenting in April/May 2014.. According to the BfR all public comments were included in an updated version of the RAR which has not been officially published. According to available information (Greim et al. 2015, BfR press release of 02 April 2015) at least two updates of the RAR were produced, one as of 29 January 2015 and one as of end of March 2015. The end-of-March- version was submitted by the RMS (i.e. Germany) to the EFSA in early April. Whereas scientists close to industry seem to have had access to the 29 January 2015 version (cf. Greim et al. 2015), civil society was denied this access even after inquiry, and therefore unable to evaluate what of the civil society submissions from the commenting phase (April/May 2014) was integrated into the report, and how they were integrated.

By coincidence the 31 March 2015 version became available to the author of this report, making possible this analysis. The identified gaps and deficiencies helped the BfR to come to

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1 http://registerofquestions.efsa.europa.eu/roqFrontend/ListOfQuestionsNoLogin?1

2 This version can be requested at http://dar.efsa.europa.eu/dar-web/provision

a different conclusion than WHO's International Agency for Research on Cancer (IARC) in the crucial areas of genotoxicity and carcinogenicity. When the IARC announced on 20 March 2015 (IARC 2015a) that it classified glyphosate as "probably carcinogenic to humans" the strong discordance to BfR's assessment became obvious. This motivated NGOs, including PAN Germany to have a second look at the draft RAR of 18 December 2013. Already from this revisit, contradictions and omission became clear which resulted in a press release on 15 April 2015 and another one on 30 July 2015. In its replies to these press releases, the BfR always referred to an ominous inclusion of civil society comments without addressing the issues raised by the press releases. After the version of the RAR-draft which was submitted to the EFSA by coincidence became available, it was possible to follow-up on these questions. The present document contains an in-depth analysis of the chapters on genotoxicity and carcinogenicity of the draft RAR dated 31 March 2015 and some considerations regarding reproductive toxicity.

Genotoxicity

Genotoxicity is a term to describe damage to DNA and its intracellular structures, the chromosomes, caused by chemical substances. If the DNA/chromosomes of egg cells or sperm cells are damaged, this false genetic information can be inherited to future generations. But this damage can also occur in various other cells of the body, because our cells are permanently dividing to replace old cells that have died. DNA/chromosomes have a crucial role to ensure that cell division is properly controlled. Due to genotoxic damage, uncontrolled (continuous) division of cells can occur, resulting in the growth of tumors.

A battery of tests is available to assess the genotoxic potential of pesticides, i.e. to evaluate whether a pesticide is capable of damaging entire chromosomes or small fractions of the DNA. The applicable EU legislation (Commission Regulation [EU] No 283/2013) follows a two-tiered approach. Starting with in vitro tests, i.e. tests in bacteria and cell lines (two or three different tests have to be performed). In vivo testing of animals is only required if no effects were found in these regulatory in vitro tests. This approach is in line with common text book knowledge, e.g. Hoffmann (1996, p. 278) who wrote that "(a)ny agent that causes a reproducible positive response in any of these assays … may be considered genotoxic:"

For two reasons genotoxicity is an important part of the risk assessment of pesticides. First of all, evidence of genotoxicity essentially precludes approval of a pesticide, although exceptions are possible (see Annex II of Regulation [EC] No 1107/2009). Secondly, evidence of specific forms of genotoxicity, in particular an increase of so-called micronuclei (indicative of DNA-damage) are considered a valid biomarker for cancer risks (Bonassi et al. 2011).

While the BfR came to the conclusion "that there is no in vivo genotoxicity and mutagenicity potential of glyphosate or its formulations to be expected under normal exposure scenarios" (RAR Volume 1, 31 March 2015 revision, p. 57), the International Agency for Research on cancer (IARC) concluded: "There is strong evidence that glyphosate causes genotoxicity." The IARC's conclusion is based on "studies that gave largely positive results in human cells in vitro, in mammalian model systems in vivo and in vitro, and studies in other non-mammalian organisms." (IARC 2015b, p.77).

This is a perplexing difference in the assessment by the two authoritative bodies. However, the following will illustrate the pattern how the literature on glyphosate is presented by the GTF and how the BfR misses to scrutinize it:

As required by Regulation [EC] No 1107/2009, the GTF provided the results of a literature search for scientific papers published since the year 2000. A total of 48 scientific publications

on genetic toxicology of glyphosate or glyphosate-based formulations are listed in Table 6.4-29 (Volume 3, pp. 395-399). Whereas 14 of these publications provide no evidence of genotoxicity, 31 of them describe genotoxic effects (results of 3 publications were inconclusive according to the GTF). On the other hand, the overwhelming majority of the regulatory studies3 did not show genotoxic effects. Obviously this is a contradiction which was "resolved" by applying certain criteria for relevance and reliability to the publications in the scientific literature. While it is essential to ensure relevance and reliability of scientific data, there is a suspicion that the criteria used in the RAR to assess the quality of scientific findings at least in part were used by industry to disqualify unpleasant studies. According to the Table 6.4-29 of the RAR 25 of the 31 publications have one or more deficiencies based on these formal criteria. Is this reason enough to dismiss these findings? According to the EFSA guidance on Submission of scientific peer-reviewed open literature (EFSA 2011), "relevance criteria should not be too restrictive", and "the fact that a study may not be conducted in accordance with Good Laboratory Practice (GLP) does not imply that the study is irrelevant" (p. 13). Therefore, one could wonder, if not the sheer number of scientific studies with genotoxic effects should be reason enough to follow the "precautionary principle" as recommended by Directive (EU) No. 1107/2009.

The suspicion that relevance and reliability criteria are applied in a distorted way, is nourished by the fact that some of the disqualified studies are discussed in detail, while valid studies with no remarks are just listed in the table mentioned without focusing on the findings. The handling of the paper by Koller et al. (2012) is of particular interest. It is one of the 3 publications[4] which received no negative remarks concerning reliability and relevance in Table 6.4-29 of the RAR. Koller et al. (2012) followed an established and internationally recognized protocol (Fenech 2007) and had paid attention to every detail regulators could ask for:

- they used both, glyphosate-based formulations and glyphosate as active ingredient;
- they used a positive control;
- they obviously made their assessment on coded slides (i.e. they Followed the protocol of Fenech, 2007);
- and, importantly, they showed that genotoxicity occurred at concentrations several times lower than cytotoxicity.

One would have expected that this paper received particular attention from the BfR. But this was not the case in the RAR. It is just listed in the table and is not discussed in the evaluating text. It is remarkable that, while in the RAR dose ranges are listed in the tables reporting regulatory studies, the table reporting studies published in the open literature on or after 2000 (Table 6.4.-29 in the RAR) only the maximum dose is mentioned, i.e. a dose of minor relevance for hazard identification, if effects are also seen at lower doses. For instance, in the case of the study published by Koller et al. (2012) the RAR states a dose of 200 mg glyphosate/liter. This was the highest dose for testing cytotoxicity. It remains intransparent from this table that Koller et al. (2012) identified genotoxic effects at a tenfold

3 Studies not publicly available that have been conducted by or on behalf of industry, following official (OECD) test guidelines.

4 The other two are by De Souza Filho et al. (2013) and Mañas et al. (2013)

lower concentration, i.e. 20 mg glyphosate/liter.[5] Most importantly, in the RAR reference is made to the results of single cell gel electrophoresis (SCGE) only. It is generally acknowledged that SCGE is a rather supplementary endpoint for genotoxicity. However, Koller et al. (2012) also discovered significant effects in the micronucleus test, a core test for genotoxicity. This was seen at a glyphosate concentration of 20 mg/liter for both the active ingredient (95% purity) and the glyphosate-based formulation (Roundup Ultra Max). It is difficult to believe that these results were left out without any purpose.

What role did the BfR play? According to the German Ministry of Agriculture (letter dated 29 June 2015, signed by Peter Bleser, its Parliamentary State Secretary), the BfR made a "detailed, quality-assured examination of all (…) original studies and the studies published in the scientific literature. For all chapters [of the RAR – P.Cl.] the BfR made its own assessment". The Koller et al.-paper and further examples described below raise doubts about the correctness of this statement. In the best case it was sloppy work by the BfR, in the worst case the BfR applied the same "selective view" as the industry. The BfR itself claimed in its FAQs of 24 July 2015 that "all the sources which formed the basis of the assessment report have … exclusively been assessed by staff of the BfR", and "the BfR did not rely on the summaries provided by the industry."

In the same FAQs the BfR claimed to have made its own literature search. It is almost impossible to distinguish in the RAR what literature was collected by the industry, what came from public commenting in spring 2014 and what was added by BfR's own literature search. However, even with these additions in the updated versions of the draft RAR, resulting in a total of 48 studies it turns out that additional 21 publications which were identified by the IARC (Table 1) were absent in the RAR[6], i.e. almost one third of the publications was missed by the BfR.

Table 1: Papers published after 1999, i.e. the reporting time of the current RAR, concerning genotoxic effects listed in the IARC monography, but missing in the RAR (for full reference see the IARC 2015b)

Author(s)	Year of Publication	Effect ? + = yes; - = no@
Akcha	2012	-
Conners & Black	2004	-
De Castilhos Ghisi & Cestari	2013	+
Dos Santos & Martinez	2014	-
Frescura et al.	2013	+
Gholami-Seyedkolaei	2013	+
Guilherme et al.	2014a	+

[5] Erroneously it was stated in the version published on 28 September. "Worse, while all results of this study seem to be valid, the table compiled by the GTF, and according to the BfR amended by the BfR presents only the less important results and even at a wrong dose! While the RAR states that effects were seen at 200 mg glyphosate/liter, Koller et al. (2012) identified genotoxic effects at a tenfold lower concentration, i.e. 20 mg glyphosate/liter."

[6] Based on analyses of the publicly available drafts, PAN Europe published a report in September 2014, describing scientific literature missing the RARs of seven pesticides, including glyphosate (PANE 2014).

Guilherme et al.	2014b	+
Lopes et al.	2014	+
Lueken et al.	2004	(-)/(+)
Marques et al.	2014	+
Mesa-Joya et al.	2013	+
Mohamed	2011	+
Munagphra et al.	2014	-/+
Nwani et al.	2013	+
Paz-y-Miño et al.	2011	-
Piola et al.	2013	-/+
Siddiqui et al.	2012	+
Vera-Candioti et al.	2013	+
Wang et al.	2012	(+)
Yadav et al. 2013	2013	+

@: -/+ = different results in different endpoints, Symbols in parantheses = questionable results

In summary, two questions arise:

1. Is there further distorted or false reporting in the RAR in addition to the examples presented here which were not detected or were tolerated by the BfR? Due to time constraints a complete re-examination of the GTF-assessment was not possible within the scope of the present analysis. Therefore, an answer to this question cannot be given. To live up to the term "Assessment Report" it would have been the task of the BfR to detect and expose the inconsistencies and inaccuracies provided by industry.

2. How will the "weight of evidence", which, according to the GTF and the BfR speaks against genotoxic effects of glyphosate, change if the 21 neglected publications are thrown into the balance tray and, if distorted information such as in case of the paper by Koller et al. (2012) is corrected?

Carcinogenicity

Carcinogenicity is a term that describes the development of tumors – in the current context due to the action of pesticides To assess whether a pesticide poses a carcinogenic hazard, long term (two years) tests in rats and mice are required by EU legislation (Commission Regulation [EU] No 283/2013). The incidence and type of tumors is compared between a control group (not treated with the pesticide) and a low-, mid- and high-dose group of animals treated with the pesticide, which e.g. is mixed into the food of the animals.

Because such two-year studies with hundreds of animals are very expensive they are almost invariably run by industry. However, as discussed below additional information (genotoxicity and specific biochemical effects) can be an indication that carcinogenicity is more likely to be expected than in situations where such additional effects are absent.

In case of glyphosate the BfR concluded that “classification and labelling for carcinogenicity is not considered appropriate” (RAR Volume 1, 31 March 2015 revision, p. 65). On 20 March 2015 the IARC published a two-page-summary in the journal Lancet of their upcoming monograph on five different pesticides, including glyphosate. The summary disclosed that glyphosate had been classified as “probably carcinogenic in humans”, a classification which was based on “limited evidence” in humans, “sufficient evidence” in animals as well as mechanistic evidence, i.e. genotoxicity and oxidative stress. This was in strong contrast to the BfR-Assessment. The BfR responded with a press release (23 March 2015), insisting on its own assessment that glyphosate is not a carcinogen. In its response the BfR repeated its view that they see neither evidence in humans, nor in animals and nor do they see any genotoxicity. Notably, the BfR remained mute about oxidative stress as mechanistic evidence in its response to the IARC announcement. Oxidative stress is the “flooding” of cells and tissues with highly reactive oxygen-containing molecules associated with potential cellular damage, in particular to the DNA. This triggered a search by Testbiotech and PAN Germany whether oxidative stress had been considered at all in the draft RAR of 18 December 2013 in the context of carcinogenicity. It turned out that this had not been the case. Testbiotech and PAN identified at least eight scientific papers on oxidative stress elicited by glyphosate which had not been mentioned in this version of the RAR. In addition the two studies mentioned in the RAR were not discussed in the context of carcinogenicity. In the 31 March 2015 version of the RAR a number of publications on oxidative stress caused by glyphosate are now mentioned or listed (many of them added by public comments as the BfR admitted in a letter to PAN and Testbiotech), but again none of this evidence was discussed in relation to carcinogenicity.

The BfR was asked by Testbiotech and PAN Germany to comment on this omission and responded that on the one hand it is “without doubt” that oxidative stress is a possible mechanism of carcinogenicity, and pointed out that it can also be the reason for various organ damages (BfR-letter to dated 13 May 2015). In the same letter the BfR concluded that oxidative stress cannot be a carcinogenic mechanism for glyphosate, because no reproducible organ damage has been observed in animal studies – a statement that conceals the fact that oxidative stress could cause damage to the DNA without causing microscopically visible organ damage, subsequently leading to carcinogenesis via genotoxic mechanisms (see above). The BfR went on to state that it is not logical to take oxidative stress into consideration, because no carcinogenicity was seen in animal studies.

A closer look at the RAR may help to understand why it was so important to separate the mechanistic evidence (genotoxicity, oxidative stress) from the results of the animal studies, to avoid that the compilation of evidence becomes too strong.

The contention about these regulatory studies circles around the incidence of malignant lymphoma in male mice in four different studies (see Table 2) which were subjected to particular consideration by the BfR. Three of them were carried out in a mouse strain called CD-1, and one in Swiss albino mice. The problem was that a significantly higher incidence of malignant lymphoma was observed in the study using Swiss albino mice (Study Code ASB2012-11492, Table 2). The BfR delivered the following three arguments to explain why this observation was irrelevant:

a) Swiss-albino mice have a high spontaneous incidence of malignant lymphoma, implying that this strain is not suitable;

b) The dose of 10.000 ppm was very high, implying that it is irrelevant;

c) No significant increase of malignant lymphoma was seen in the 3 studies with CD-1 mice. The same “weight of evidence” approach as already used in the genotoxicity part had been applied here, i.e. dismiss significant findings in a valid study by referring to other studies that – allegedly – did not show an effect.

Table 2: Incidence of malignant lymphoma in male mice of 4 regulatory studies. Doses are given in the top row of each cell of the table as ppm (mg glyphosate per kg diet); incidences (number of animals with lymphoma/number of animals in the group) are given in the bottom row of each cell of the table.

Strain/*Study-Code*		Control	Low Dose	Mid Dose	High Dose
CD-1/ *ASB2012-11 492*	Dose	0 ppm	500 ppm	1.500 ppm	5.000 ppm
	Incidence	0/51	1/51	2/51	5/51@
Swiss-Albino/ *ASB2012-11 491*	Dose	0 ppm	100 ppm	1.000 ppm	10.000 ppm
	Incidence	10/50	15/50	16/50	19/50*
CD-1/ *ASB2012-11 493*	Dose	0 ppm	1.600 ppm	8.000 ppm	40.000 ppm
	Incidence	2/50	2/50	0/50	6/50
CD-1/ *TOX9552382*	Dose	0 ppm	100 ppm	300 ppm	1.000 ppm
	Incidence	4/50	2/50	1/50	6/50

* statistically significant according to the RAR
@ statistically significant dose-dependent increase according to Cochran-Armitage trend test.

Concerning a), although Swiss albino mice have a higher incidence of this tumor type[7] than other mouse strains, it needs to be emphasized that the tumor incidence of the top dose group was not only significantly higher as compared to the concurrent control group, but

- it was also above the mean incidence of the historical control;
- it was even outside the range of the historical control, and
- the effect was dose dependent with a visible increase, though non-significant in the other dose groups.

It should be kept in mind that in other situations the demonstration that data were **within** the historical control and were **not dose-dependent** is frequently used by industry to dismiss observed statistically significant differences. Here, the fact that the higher incidence in

[7] The BfR (at the request of an EFSA expert committee, the PRAS 125) put quite some efforts into proving that this significantly higher tumor rate is irrelevant. What is remarkable about these efforts, is that it the majority of the publications used for strengthening BfR’s argument have not been subjected to the usual relevance and reliability check of papers from the scientific literature. If so, they would probably have to be rejected.

malignant lymphoma is **outside** the historical control and that there is **indeed dose-dependency** does not seem to be important for industry and the authorities.

GTF and BfR should answer the question, what else would be needed to accept this is a true effect?

Concerning b), it should be noted that there is a dose dependent trend for an increase starting at the low dose of 100 ppm. In addition, a statistically significant increase of malignant lymphoma exists in a CD-1-mouse study (study code *ASB2012-11492*) with a top dose which is only half the top dose of the one we discuss here (see also the item c).

Concerning c): First of all we face an interesting phenomenon: It is well-known that not all statistically significant differences are biologically relevant. This however is only half of the truth, i.e. this rule applies also the other way round, i.e. not all differences lacking statistical significance are biologically irrelevant. As stated in the applicable OECD-guidance, "similarly, declaring a result non-significant (often designated as P>0.05 or NS, again a nomenclature not favoured by statisticians) should not be interpreted as meaning the effect is not biologically important" (OECD 2012, p. 118). But this part of the rule is virtually never applied by the industry. The BfR too forgot to mention this in its reply to PAN Germany dated 02 September 2015. According to the EFSA-publication referred to in this BfR-letter, "The concept of *biological relevance* implies a biological effect of interest that is considered important based on expert judgement" (EFSA 2011, 2372). This opens up experimental findings to the interpretation by experts.

Here we have a paradoxical situation: While GLP pretty much ensures that the data generated by industry are reliable, the interpretation of these data is subjected to "expert judgement". When experts who make this judgement belong to the industry, this presents a serious conflict of interest. Therefore, it would be of utmost importance, that authorities like the BfR apply another principle which is laid down in item (8) of Regulation (EC) No 1107/2009 – the precautionary principle. However, as we can learn from BfR's handling of the regulatory mouse studies, this authority is very far from applying this principle.

As mentioned above, the increase in malignant lymphoma in Swiss albino mice was dismissed, because the three studies performed in CD-1 mice supposedly did not show a significant increase in the incidence of this tumor type. However, if the Cochran-Armitage-Trend Test is applied, the statistical method explicitly recommended by the OECD (OECD 2012, p. 123), a dose-dependent, statistically significant increase of these lymphoma are identified in Study *ASB2012-11492*. Again: dose-dependence adds to the statistical significance *per se*, and strengthens the argument of biological relevance. In addition, it should be noted that all four studies mentioned above had a – though in other cases only slightly - higher tumor incidence in the highest dose group as compared to the concurrent control group. The BfR with its claim of a detailed, quality-assured examination should have stumbled over this and should have applied the valid statistical method for its assessment. Instead, the BfR dismissed this observation, i.e. "there was no evidence for carcinogenicity up to this dose level" (i.e. 5.000 ppm), only contradict itself in the same paragraph by saying "Even in males, the difference was not statistically significant but a possible effect might be suspected and should be clarified because of the increase in malignant lymphoma in the study by … ASB2012-11491 … and because of a weakly higher incidence in the study … ASB2012-11493."

The first step of this clarification should have been the application of the appropriate statistical method (i.e. Cochran-Armitage-Trend Test, see above). This was not done. Instead, the BfR looked for historical control data. As with the tests of statistical significance

the use of historical control data is a tool which needs to be applied with responsibility. It can help to dissociate accidental from true findings. In case of the male mice of study *ASB2012-11492* (using the CD-1 strain) historical control data were requested from the laboratory which performed this study with the intention to see whether the "possible effect (that) might be suspected" needs to be taken more seriously. Besides that it should be remembered that the "possible" effect, was not only to be "suspected", but statistically significant (see above), it turned out that "**the quality and regulatory value of the historical data is very much compromised**" (RAR, Volume 3, p. 509). After this assessment it was very surprising to read in the important Volume 1 (page 65) of the RAR where the general assessment by the BfR is made, that the observation of "slightly higher incidences in top dose males" was dismissed, because they were "… **fully covered by historical control data**." The only conclusion that can be drawn from this distortion of facts is that this was done by intention. Accepting a carcinogenic effect in this study with CD-1 mice would have invalidated the above mentioned "argument a)", i.e. that Swiss mice are not suitable, because this other strain showed a statistically significant increase in malignant lymphoma too. In addition, accepting a carcinogenic effect in this study would have weakened "argument b)", because in the CD-1 mouse study the high dose was only 5.000 ppm and not 10.000 ppm as in study *ASB2012-11491*.

In summary, the RAR itself contains two mouse carcinogenicity studies with a significant increase of malignant lymphoma in male mice, both with a dose-dependent increase. In one of the studies the findings were supported by historical control data, in the other study it was not possible (although attempted) to question the results by historical control data, because their quality and regulatory value was very much compromised. In addition to these two regulatory studies the IARC identified two further studies with significant carcinogenic effects in mice (EPA 1985a, 1985b, 1986, 1991a; JMPR 2006[8]). In one of these studies a statistically significant, dose-dependent trend was observed for renal tubule adenoma in male mice (CD-1 strain), a rare tumor in this strain of mice. In the other study a significantly higher incidence of haemangiosarcoma was observed. Both studies were statistically assessed using the Cochran-Armitage-Trend test. In a thorough analysis the BfR should have taken these studies into account.

The denial of genotoxicity, the neglect of oxidative stress as a mechanism and the strange way of looking at the data from carcinogenicity studies in the RAR gives the **impression of a purposeful separation and, thereby, weakening of evidence**. If oxidative stress and genotoxicity were recognized as glyphosate effects by the BfR, the carcinogenic effects in the two mouse studies which the BfR tries to dismiss would weigh much heavier than isolated consideration. In contrast, the IARC put together the results of carcinogenicity studies, evidence of genotoxicity and oxidative stress and came to its now well-known conclusion. It seems that carcinogenic effects in mice had to be denied at the same time as oxidative stress and evidence of genotoxicity had to be dismissed! This dismissal was reinforced in a recent BfR press release (FAQs on glyphosate dated 28 August 2015), reasoning that in "some" of the publications describing oxidative stress certain pieces of information were lacking. The question is, if "some" of the publications were lacking information - what about the others? Is it justified to dismiss the accumulating evidence of oxidative stress altogether because "some" publications were lacking details? Shouldn't it give reason to look for additional evidence to close the gap?

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8 For references see the IARC monograph (IARC 2015b)

Reproductive toxicology

Reproductive toxicology investigates adverse effects of chemicals on embryos/fetuses and the offspring or on the reproductive organ system of adults. For risk assessment the Commission Regulation [EU] No 283/2013 requires testing of developmental toxicity (i.e. administration of the test substance to pregnant females and the assessment of the effects on embryos/fetuses/offspring) and a so-called multi-generation test (exposure of rats to the test substance during pre- and postnatal development including their own reproduction).

Others documented numerous toxicological studies, including reproductive toxicology publications missing in the draft RAR of 18 December 2013 (PANE 2014). Here we concentrate on just one study, because of the unique opportunity of analyzing how public comments were taken into consideration by comparing the draft of December 2013 with the updated version of 31 March 2015. The example relates to a study by Beuret et al. (2005) who identified oxidative stress in pregnant rats as well as in their fetuses. Oxidative stress is not assessed in the routine studies required by EU legislation, however – as described above – it is an important toxicological mechanism which should be taken seriously. The example illustrates the distortion of scientific facts and the disregard of the contents of public commenting.

In the draft RAR of 18 December 2013 (Volume 3, Annnex B6.1, p. 659) the following is stated with regard to the paper by Beuret et al (2005):

"Relatively few *in vivo* publications on glyphosate[9] … exist … Some lack appropriate interpretation of basic toxicology; e.g. Daruich et al. (2001, …) and Beuret et al. (2005, …) noted rats treated with a glyphosate based formulation showed reduced food intake, reduced water intake and reduced body weight gains. However the authors did not consider attributing the effects of altered enzyme concentrations to dehydration or restricted diets. Both studies are reviewed by Williams et al. (2012)".

The arguments why the study of Beuret et al. (2005), supposedly lacked "appropriate interpretation of basic toxicology" were derived without scrutiny from the industry-sponsored review by Williams et al. (2012). These authors pretended to provide a thorough scientific assessment by referring to three papers that dealt with reduced food consumption and the "altered enzyme concentrations" mentioned above. However they distorted the facts by concealing that these papers actually describe the opposite of what Williams et al. (2012) claim to prove.

Therefore the following public comment was submitted on 08 May 2014:

"The important findings by Beuret et al (2005) – increased lipid peroxidation10 - are not taken into consideration. Instead the reduced food consumption "that could have affected the altered enzyme concentrations" is criticized. This is a **wrong, misleading assessment**, because studies with restricted diets … show that reduced food consumption actually **decreases** lipid peroxidation whereas here an **increase** of lipid peroxidation and of the corresponding enzyme activity is shown."

Looking at the draft RAR of 31 March 2015, this paragraph now reads as follows:

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9 Concerning reproductive toxicology

10 A measure of oxidative stress

“Some lack appropriate interpretation of basic toxicology; e.g. Daruich et al. (2001). Beuret et al. (2005) investigated the effects of 1 % Glyphosate oral exposure (a trade product from Argentina described as “Herbicygon” was used which is a commercial herbicide formulation) on lipid-peroxidation and antioxidant enzyme systems in pregnant rats and in fetuses. Lipid-peroxidation was higher in both maternal and fetal livers in the glyphosate treated groups. Catalase and Superoxide dismutase activity were not altered. Both studies are reviewed in Williams et al. (2012).”

In other words, the findings of Beuret et al. (2005) are briefly described, but the significance of their findings is not assessed. The “lack appropriate interpretation of basic toxicology” now seems to be restricted to the paper by Daruich et al. (2001), but reference to Williams et al. (2012) and their distortion of facts continues, enabling the use of their review for discrediting Beuret et al. (2005) in the future.

Conclusions

The current analysis demonstrated omissions and distortion of facts in the toxicology part of the draft RAR that was submitted to the EFSA. The BfR which was in charge of this part of the RAR has full responsibility for these gaps and deficiencies. The identified neglect of scientific publications, failure to apply state-of-the-art statistical methods to regulatory studies and distortion of facts in Volume 1 of the RAR (as compared to Volume 3, Annex B.6) fall altogether into areas related to the assessment of carcinogenicity. This weakening of evidence by three different processes, i.e. neglect of studies, failure of analysis and distortion of facts, nourishes the suspicion that this was done on purpose.

In addition examples are provided that the opportunity for public comments of the RAR merely represents an alibi event, because despite of the facts provided during the commenting phase no change of assessment at all could be identified in the analyzed parts of the RAR.

This Renewal Assessment Report needs to be subjected to a complete and thorough re-analysis by a group of truly impartial experts before a decision can be made about the approval or non-approval of the glyphosate in the European Union.

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