Memo n° 24: Peter CLAUSING

Toxicologist, Germany

The Hague, October 15th-16th, 2016

CONTACTS :

emilie@monsanto-tribunal.org

witnesses@monsanto-tribunal.org

Dr. Peter Clausing, born in 1950, graduated as an agronomist at the University of Leipzig and earned his PhD in 1974. After post-graduate studies in toxicology he became a board-certified toxicologist in 1988 (recognized by the German Society for Pharmacology and Toxicology) and held positions at two research institutes of former East Germany. As a postdoctoral scientist he worked at the U.S. FDA's National Center for Toxicological Research from 1994-1996. Thereafter he worked as a toxicologist in a Danish contract research organization (1997-2001) and in a German pharmaceutical company (2001 until retirement in 2010). He published 54 papers and 4 book chapters in the area of toxicology.

In 2014 he became a member of PAN Germany. Since October 2015 he is a member of PAN Gemany's executive board. He contributed to several of PAN Gemany's publications. Since April 2015 he is involved in the debate on the re-approval of glyphosate in the European Union and wrote six PAN Germany position papers concerning aspects of the carcinogenicity of glyphosate.

PAN Germany
Pestizid Aktions-Netzwerk e.V.

Regulatory agencies (BfR, EFSA) used biased arguments to deny the carcinogenicity of glyphosate

Memorandum by Dr. Peter Clausing, PAN Germany,
as a witness to the Monsanto Tribunal

The Hague, Netherlands, 15/16 October 2016

Hamburg – 06 October 2016

Executive summary

The German Federal Institute for Risk Assessment (BfR) and the European Food Safety Authority (EFSA) committed scientific fraud by not rejecting the position of the Glyphosate Task Force (GTF) and stating "that glyphosate is unlikely to pose a carcinogenic hazard to humans and the evidence does not support classification with regard to its carcinogenic potential according to the CLP Regulation" (EFSA 2015, p.10) and that "no hazard classification for carcinogenicity is warranted for glyphosate according to the CLP criteria" (RMS Germany 2015a, p. iii). These statements are contrary to the evidence contained in BfR's own Renewal Assessment Report (RAR), its Addendum, and the draft CLH Report, which is largely based on the RAR and its Addendum and was submitted to the European Chemicals Agency (ECHA) by the German Federal Institute for Occupational Safety and Health.

Most importantly, males of all five mouse carcinogenicity studies considered by the authorities of acceptable quality showed a statistically significant increase in the incidence of one or several tumour types. Notably, three of these five mouse studies exhibited a significant increase in the same type of cancer (malignant lymphoma), underscoring the reproducibility of this finding in studies performed in different laboratories and at different times. This clearly exceeds the criteria for the classification as a carcinogen as given in CLP Regulation (Regulation [EC] 1272/2008). In contrast to these three studies, the fourth study for which the incidence of malignant lymphoma lacked statistical significance has to be regarded invalid, because of severe deficiencies in the histopathological assessment of this type of tumour. The finding of an increased incidence of malignant lymphoma is further supported by epidemiological studies that indicate an association between glyphosate use and Non-Hodgkin lymphoma, and by mechanistic evidence, i.e. genotoxicity and oxidative stress. The authorities used five false arguments in an attempt to invalidate the significant findings of the mouse carcinogenicity studies.

Proper evaluation of the evidence provided in the CLH Report, the RAR and its Addendum inevitably leads to the conclusion that glyphosate is carcinogenic in experimental animals, warranting a Category 1B carcinogenicity labelling of glyphosate.

The five arguments used by the authorities are false or distorted

Five arguments were used by the authorities to dismiss the significant finding of glyphosate-induced malignant lymphoma in mouse carcinogenicity bioassays. They claimed that "no evidence of carcinogenicity was observed in rats and mice" (EFSA 2015, p.10) or that the evidence for glyphosate-induced malignant lymphoma was "equivocal" (ECHA 2016, p. 73), because:

1. of "lack of statistical significance in pair-wise comparison tests (EFSA 2015, p. 11) or "partly contradictory study outcomes, depending on the statistical method applied" (ECHA 2016, p. 73);
2. of "lack of consistency in multiple animal studies" (EFSA 2015, p. 11) or "inconsistent dose response in the individual studies" (ECHA 2016, p. 73);
3. "neoplastic lesions … being within historical control range" (EFSA 2015, p. 11);
4. one "study was re-considered … as not acceptable due to viral infections that could influence survival as well as tumour incidence – especially lymphomas" (EFSA 2015, p. 10) or "a possible role of oncogenic viruses that should not be ignored" (ECHA 2016, p. 73);
5. "the increased incidence of malignant lymphomas occurred at a dose level exceeding the limit dose of 1000 mg/kg bw per day recommended" (EFSA 2015, p. 10).

For convenience, the results of the five mouse studies as derived from the CLH Report (ECHA 2016) are summarized in the table below.

Tabelle 1: Incidence of malignant lymphoma in males of the five mouse studies; number of animals per group and sex: 50, except for the study of 2009 (51) and 1983 (48-50); p-values<0.05 are considered as significant, however it should be noted that the error probability (p-value) is cut in half, if a one-sided test is used (i.e. testing only the assumption of an increase of the Incidence); for pair-wise comparisons p-values are given for the high-dose group, trend test p-values relate to the entire study. The Cochran-Armitage-trend test was used.

Year of Study	Mouse Strain	Doses (mg/kg bw*) Tumour incidence	Statistical method and p-values, all non-trend-tests were pair-wise 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 data, but in the narrative described as non-significant.

*bw = body weight

** according tot he CLP-Report incidences refer only to the assessment of lymph nodes with macroscopic changes

*** Sum of lymphoreticular neoplass, no specific designation of malignant lymphoma

ARGUMENT 1: Lack of, or insufficient statistical significance

This argument is not true.

The incidence of malignant lymphoma was higher in males of all glyphosate treated groups of all five mouse studies. In addition, a statistically significant increase occurred in three of the studies, with a clear dose-dependence in two of them. Of the two studies without statistical significance **the study of 1993 is non-compliant with applicable guidelines as far as malignant lymphoma are concerned** (because only lymph nodes with macroscopic changes were assessed histopathologically). Therefore, it is wrong to refer to this study at all and it is futile to claim: "In the study by Atkinson et al. (1993, TOX9552382), in contrast, there was no dose response and the incidence in the control group was similar to that at the top dose level" (ECHA 2016, p. 68). In the other study with no significant increase of malignant lymphoma, the study of 1983, a deviating histopathological terminology was used. It is not clear whether "lymphoblastic lymphosarcoma" with or without leukemia (ECHA 2016, p. 68, Table 32) are equivalent to "malignant lymphoma". In summary, three valid studies are remaining which all show a statistically significant increase of malignant lymphoma.

In addition the claim of "partly contradictory study outcomes, depending on the statistical method applied" (ECHA 2016, p. 73), i.e. pair-wise comparisons or trend tests, has to be considered as a "constructed" contradiction. According to OECD Guidance "Significance in either kind of test is sufficient to reject the hypothesis that chance accounts for the result. A statistically significant response may or may not be biologically significant and vice versa" (OECD 2012, p. 116). Therefore, the malignant lymphomas that were found statistically significant by the trend tests in the three studies must not be considered as "chance events". In addition, trend tests are explicitly recommended in a flow diagram (OECD 2012, p. 123). Furthermore, according to OECD Guidance No. 116 "In a carcinogenicity study, … a one-sided test may be considered more appropriate, ..."

(OECD 2012, p. 133). Taking this into consideration, the studies of 2001 and 2009 yielded statistical significance even with pair-wise comparisons. Moreover, when discussing statistical significance *vs.* biological relevance, it is often forgotten that this applies in both directions: "Similarly, declaring a result non-significant … should not be interpreted as meaning the effect is not biologically important ..." (OECD 2012, p. 118). With increases of malignant lymphoma in glyphosate-treated males of all five mouse studies, statistically significant increases in three of them and indications of an association between glyphosate use and the incidence of non-Hodgkin lymphoma in humans, this effect certainly should be considered biologically relevant.

ARGUMENT 2: Inconsistent dose-response

This argument is scientifically unjustified.

Strain-specific differences in tumor incidences in general and in malignant lymphoma in particular are a well-known phenomenon. This is even acknowledged in the RAR (RMS Germany 2015b). Therefore it is scientifically unjustified to draw a conclusion of "inconsistency" from a direct comparison of the tumor incidence of studies using different strains, even when the top doses are comparable as for the 2009 and the 2001 study (ECHA 2016, p.71). The authors of the CLP report seem to have missed that the incidences of malignant lymphoma in the control groups of these studies were different too. The important and consistent outcome of these two studies is a dose-dependent, statistically significant increase in malignant lymphoma.

ARGUMENT 3: The observed neoplastic lesions were within historical control range

This argument is false, because it is contrary to the facts.

First of all "it should be stressed that the concurrent control group is always the most important consideration in the testing for increased tumor rates" (OECD 2012, p. 135). Keeping this in mind, the main purpose of using historical control data (HCD) is to facilitate an evaluation in the light of variable results. Therefore, this argument is not only false as shown below, but largely overemphasized in the RAR, its Addendum and the CLH Report.

Variation of tumor incidences between different laboratories, different strains and the known possibility of a background drift over time are the reasons why it is recommended to use data collected over the last five years, from the same strain and the same laboratory (OECD 2012, ECHA 2015). Therefore it appears to be an attempted fraud when comparing the high-dose incidences of the 2009 study (where no acceptable HCD were available) with the Charles River HCD derived from studies in 11 different laboratories, using animals of four different breeding facilities, and performed over a period of 13 years (ECHA 2016, p. 73), and at the same time questioning the relevance of the valid HCD of the 2001 study which are compliant with OECD criteria (!) "since it was based on observations in only five studies employing in total 250 untreated control animals per sex" (ECHA 2016, p. 71). For the two studies with valid historical control data, the following picture arises: The findings of the 2001 study are strongly supported by the HCD, because the control group was within the HCD range (6-30%), whereas the incidences in the mid dose (32%) and the high dose (38%) did not only exceed the average (18.4%), but even the upper limit of the range of the HCD. For the study of 1997 the mean and the range of the HCD were 6.33% and 3.85-19.23%, respectively. The high dose incidence in the actual study was 12%, i.e. about twice the HCD mean.

ARGUMENT 4: Animals of the 2001 study were infected with oncogenic viruses

There is no evidence of a viral infection in the studies reviewed

This is an interesting issue of twisting the facts. In the EFSA conclusion this important study was dismissed "as not acceptable due to viral infections that could influence survival as well as tumor incidence – especially lymphoma" (EFSA 2015, p.10), a statement suggestive of sound evidence. Later, the CLH Report (ECHA 2016) explains: "During a teleconference (TC 117) … , it was mentioned by an U.S. EPA observer that the Kumar (2001, ASB2012-11491) study had been excluded from U.S. EPA evaluation due to the occurrence of viral infection that could influence survival as well as tumor incidences, especially those of lymphomas. However, in the study report itself, there was no evidence of health deterioration due to suspected viral infection and, thus, **the actual basis of EPA's decision is not known**." (ECHA 2016, p. 72, emphasis added). After admitting that such an infection was not proven, the CLH Report in turn twisted the wording of a paper by Tadesse-Heath et al. (2000) to claim that these authors had "emphasized the contribution of **widespread** infections with murine oncogenic viruses to the high but remarkably variable incidence of tumors of the lymphoreticular system in this species" (ECHA 2016, p. 72,emphasis added). However the word "widespread" does not occur in this publication and at the end of their paper the authors state: "It should be noted that the several strains of outbred and inbred Swiss Webster mice designated as CFW in use in the United States and in Europe should not be considered to be identical. We have examined only one population for the highlymphoma–high-MuLV-expression phenotype" (Tadesse-Heath et al. 2000, p. 6836).

In summary, the handling of the issue of oncogenic viruses to dismiss the important study of 2001 is an outstanding example of twisting the facts, in an attempt to justify the dismissal of this study.

ARGUMENT 5: An increased incidence of malignant lymphomas occurred only at excessive doses

This argument is wrong. Malignant lymphoma was not a high-dose-only phenomenon.

In its Addendum the BfR uses the argument of a "high-dose phenomenon" (RMS Germany 2015a, p.35) to dismiss significant findings of the carcinogenicity bioassays in rats and mice. Before comparing these arguments with the recommendations of applicable Guidance and Guidelines it needs be emphasized that two mouse studies (2001 and 2009) with exhibited a statistically significant, dose-dependent increase of malignat lymphoma across all dose-groups. Moreover, the top-doses were 1460 mg/kg bw and 810 mg/kg bw for the studies of 2001 and 2009, respectively. In other words, the top doses were below or only slightly above the 1.000 mg/kg which allegedly represents a "limit dose".

The BfR referred to a top dose of 1.000 mg/kg bw as a limit dose that should not be exceeded in animal studies. It should be noted that a top dose of 1.000 mg/kg is mentioned in the OECD Guideline No. 453 for Combined Chronic Toxicity/Carcinogenicity Studies (OECD 2009a), but not in the OECD Guideline No. 451 for Carcinogenicity Studies (OECD 2009b). In other words, it can be assumed that the "limit dose" refers only to the chronic toxicity part of this Guideline.

The BfR also referred to a recommendation that a 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, the BfR argues that "excessive toxicity" has had a confounding effect, because of the observation that "the body weight gain was decreased by more than 15% compared to controls" at the same time admitting that "survival/mortality was not affected" (RMS Germany 2005a, p. ii).

First, it should be emphasized 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" (OECD 2012, p. 53, emphasis added). But "necrosis" or "metabolic saturation" has not been mentioned in the RAR for the mouse studies discussed here (RMS Germany 2015b). 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%".

However, more importantly, for the study from 1997 it is documented in the RAR 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 (OECD 2012, p. 133-134). In other words, if the body weight is reduced due to lower food consumption the concern is 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.

Taken together, the claim that malignant lymphoma were "occurring only as a high-dose phenomenon" (ECHA, p. 73) is false and not supported by evidence.

Mechanistic evidence / Oxidative stress

The International Agency for Research on Cancer (IARC) assessed that the carcinogenicity found in animal studies on glyphosate is supported by strong mechanistic evidence, i.e. genotoxic effects and oxidative stress. In the following, the oxidative stress part is discussed. It needs to be mentioned that the BfR did not even consider in its final draft of the RAR dated 31 March 2015 any publication on oxidative stress induced by glyphosate as related to carcinogenicity while at least 8 papers were published between 2005 and 2013 showing this effect in fish, tadpoles, mice and rats. Only after the IARC's

monograph was published on 30 July 2015 the BfR added its evaluation in an Addendum (RMS Germany 2015b). The CLH Report (ECHA 2016, p. 93) refers to the Addendum of the RAR and states that "it was concluded in the addendum that from the sole observation of oxidative stress and the existence of a plausible mechanism for induction of oxidative stress through uncoupling of mitochondrial oxidative phosphorylation alone, genotoxic or carcinogenic activity in humans cannot be deduced for the active substance glyphosate and glyphosate based formulations."

However, as explained above, the "deduction" of carcinogenicity is not based on the "sole observation" of oxidative stress. Rather this observation is considered as supportive evidence of the demonstrated increase of tumor incidences, in particular of malignant lymphoma, in animal experiments. Similar to the evidence derived from epidemiology, findings of oxidative stress caused by glyphosate should be part of an overarching assessment and of an appropriate weight of evidence approach. Such publications help to fill the knowledge-gap that exists, because the measurement of oxidative stress parameters is not part of carcinogenicity bioassays or any other guideline-driven study designs.

Overall conclusion

Ample evidence has been provided above showing that European authorities twisted or ignored scientific facts and distorted the truth to enable the conclusion that glyphosate is not to be considered a carcinogen, thereby accepting und reinforcing the false conclusion proposed by the Monsanto-led GTF. The German Federal Institute for Risk Assessment (BfR) and the European Food Safety Authority (EFSA) committed scientific fraud.

References

ECHA (2015): Guidance on the Application of the CLP Criteria. Guidance to Regulation (EC) No 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures Version 4.1. https://www.echa.europa.eu/documents/10162/13562/clp_en.pdf

ECHA (2016) Proposal for Harmonised Classification and Labelling. Based on Regulation (EC) No 1272/2008 (CLP Regulation), Annex VI, Part 2. Substance Name: N-(phosphonomethyl)glycine; Glyphosate (ISO). European Chemicals Agency. http://echa.europa.eu/documents/10162/13626/clh_report_glyphosate_en.pdf

EFSA (2015): Conclusion on the peer review of the pesticide risk assessment of the active substance glyphosate. EFSA Journal 2015;13(11):4302

OECD (2009a): *Test No. 452: Chronic Toxicity Studies*, OECD Publishing, Paris. http://www.oecd-ilibrary.org/environment/test-no-452-chronic-toxicity-studies_9789264071209-en;jsessionid=16odl65wcetn2.x-oecd-live-03

OECD (2009b): *Test No. 451: Carcinogenicity Studies*, OECD Publishing, Paris. http://www.oecd-ilibrary.org/environment/test-no-451-carcinogenicity-studies_9789264071186-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. ENV/JM/MONO(2011)47, Paris. http://www.oecd.org/officialdocuments/displaydocument/?cote=ENV/JM/MONO(2011)47&doclanguage=en

RMS Germany (2015a): Renewal Assessment Report Glyphosate. Addendum 1 to RAR, Assessment of

IARC Monographs Volume 112 (2015): Glyphosate, 31 August 2015. http://registerofquestions.efsa.europa.eu/roqFrontend/wicket/page?0-1.ILinkListener-outputForm-outputDocumentsContainer-documents-1-fileNameLnk

RMS Germany (2015b): Renewal Assessment Report Glyphosate Volume 3; Annex B.6 Toxicology and metabolism. 18 December 2013, revised 29 January 2015, 31 March 2015.
http://registerofquestions.efsa.europa.eu/roqFrontend/wicket/page?0-1.ILinkListener-outputForm-outputDocumentsContainer-documents-1-fileNameLnk

Taddesse-Heath L, Chattopadhyay SK, Dillehay, DL et al. (2000): Lymphomas and high-level expression of murine leukemia viruses in CFW mice. J Virol 74 (15) , 6832-6837

Imprint

Pestizid Aktions-Netzwerk (PAN) e.V.

Nernstweg 32

D-22765 Hamburg

Phone: +49 (0)40-399 19 10-0

Email: info@pan-germany.org

Hamburg, 06 October 2016

Dr. Peter Clausing, board-certified toxicologist (DGPT).

Phone: +49-176 7801 2705, Email: peter.clausing@pan-germany.org,