Author’s response to reviews

Title: A Comprehensive Analysis of the Animal Carcinogenicity Data for Glyphosate from Chronic Exposure Rodent Carcinogenicity Studies

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Author’s response to reviews:

Editor's comments:

Thank you for revising your manuscript. One reviewer suggests that some additional revision would be beneficial. Also, I understand from a recent publication by Kenny Crump in Risk Analysis that he has authored an additional paper on multiple comparison concerns in the bioassay data on glyphosate, to appear in Toxicological Sciences. By more clearly addressing Crump's critique, an EH article on glyphosate would be much more useful.

I have gone to Toxicological Sciences’ website and looked through their accepted for publication manuscripts and it is not there. I cannot review this article until it is available. Crump simply lists it as submitted.

Reviewer #3: Overall I found the author was very responsive to the reviews and made valuable improvements to the paper, which have strengthened its contribution. Clearly some of my requests involved more effort than I anticipated; the additional documentation included is very valuable so I appreciate they were addressed. In a few places the revision falls short of my hopes for improvement and I defer to the editor to address these remaining details. (In particular, I find it difficult to ensure I understand the contributions made by the authors (vs. other researchers) when their paper is written in passive voice. I won't belabor this further.)

I have gone back through the paper and removed all passive voice for the analyses that I have personally conducted for this review. The difficulty for me is that these studies are in the past so, even though my analysis is new, the actual findings are in the past. However, hopefully this corrects your concern. All discussion of literature results are passive.

Here are additional comments that I suggest be addressed in the final version.

1. The author should correct the errors I found in the current draft and look carefully to ensure no others remain.
   a. The number of significant findings in the abstract differs from the main text

There are two different numbers to consider here. First, number of significant tumor sites. There are 37 as stated in the abstract and in the comparison to EPA’s evaluation. However, in the analyses done, there are 41 significant findings. The extra 4 findings are as follows: For kidney adenomas in male mice from Study C, the p-value is 0.009 against historical controls. Since there are no carcinomas, the same number appears for adenomas and carcinomas combined. But the finding is still only the one
increased tumor, adenomas. However, since my evaluation scheme involved doing both adenomas and carcinomas, I counted both of these for the overall false-positive rate. The same holds true for Harderian gland adenomas in females in Study C and for hepatocellular adenomas in males in Study L. Finally, for C-cell carcinomas in females from Study C, both the historical control evaluation and the comparison against the concurrent control are positive, two positive findings, but one tissue. Hence 37 significant tumor/tissue responses but 41 total positive findings in the evaluations. Minor change made to false positives section.

b. Equation (2) is missing an i subscript.
Corrected

c. Takahashi is Study E not Study L
Corrected.

2. Add a concluding paragraph to the discussion. Right now the paper falls flat at the end. I think a few key points are worth restating at the end of the paper, for instance
   a. The regulatory bodies aren't doing their own analyses and this is leading them to miss important evidence.
   Done
   b. Summary statements highlighting the organs/systems where there is clear evidence that glyphosate is causing cancer, with some mention about those that are particularly compelling given the other evidence reviewed in this paper.
   That is done just before the end of the paper and throughout the text. Since there are 13 tumors/sites with clear evidence, this would take a great deal of space and reiterate what has already been said. No change.
   c. The tumors with "clear evidence" where regulatory bodies reached different conclusions or did not consider them at all.

I hesitate to be too specific about the failures of the regulatory authorities since that is not the intent of the paper. Basically, they dismiss all positive findings as due to chance. Some wording in new last paragraph.

3. Consider revising the abstract to incorporate more of the distilled findings summarized in Table 6 and some of the most important points made in the discussion. Also in the abstract please consider these suggestions:
   a. Glyphosate is the most widely used agricultural chemical of any kind worldwide and also the most widely used pesticide (of which a subset are herbicides).

I changed herbicide to pesticide, but did not use agricultural chemical because I have no idea about the magnitude of use of fertilizers of different types.
b. I think the abstract can be reworded to not repeat the idea of consistency across sex, species, strain in two consecutive sentences.

Done

4. Consider a bit more elaboration on the NTP criteria to bring along readers unfamiliar with them. While the cancer evaluation criteria are clearly referenced, the paper does not give good insight into any of the terms. (For example, I don't consider a statement like "Clear evidence (CE) indicates it is clear that glyphosate caused these tumors" a particularly informative definition.)

The reference to NTP has been dropped since, technically, their definitions refer to the entire study and not a single tumor. I have used their criteria as a guide and now provided my definitions for these categories. The differences have to do with causation and chance. For CE, I do not believe chance explains the results. For SE, chance, although unlikely, cannot be ruled out. For EE, chance is as likely an explanation as glyphosate for the associations. For NE, chance is the most reasonable explanation.

5. Table 6 (e.g. in a footnote) or the text describing it does not say anything about Table 6's blank cells. What do they mean?

Done.

6. As a reviewer not familiar with animal carcinogenicity tests, I don't know how to put into context when there is evidence of a certain type of cancer in a single gender, species or strain, but not in other subgroups. For instance, there is clear evidence for adrenal cortical carcinoma, but only for one gender, species and strain subgroup. How is a reader unfamiliar with animal carcinogenicity testing supposed to think about this evidence when it isn't replicated in any of the other subgroups? Are there some cancers where we expect specific strains to be more likely to show clear evidence? Some comment about these points would be helpful. This was the gist of my general comment number 4.c.iii. in my first review that Dr. Portier did not understand and thus did not appear to respond to.

It is generally true that any tumor can show dose-related changes in one sex but not another or in one strain or species but not another. Many examples of this appear in the literature and this is well known. I have added a sentence and a reference.

7. Is it worth making a comment in the introduction about the unusually large number of animal carcinogenicity studies available for glyphosate, at least compared to other compounds? (This is my understanding; please correct me if I am wrong.)

I had thought about adding a comment to that effect, but I may be wrong since I don’t really know how many pesticides have this many studies available and most of that literature is very difficult to research. It is a minor point so I left it out.
8. P 3: There are some generic statements that could be made more specific in the last paragraph of the introduction. I think it is important to say that all known studies were reviewed for this review (add number), and that those with the raw data available in the public domain were included in the reanalyses done in this review.

Done.

a. line 10: Is it fair to say this study reviewed all available studies of pure glyphosate? The wording is currently so generic as to not help readers understand the magnitude and context of this contribution.

I have added “glyphosate” but not used pure glyphosate since no study used 100% pure product.

b. Line 11: "data is available" is very generic. The point is that there are studies where the data are not in the public domain in any way, and these are omitted. The number of these studies could be given.

Changed wording to “sufficient quality and detail”; the actual criteria are in the methods section.

9. Methods
a. P 3 line 30: Many readers may not understand that the full laboratory reports are often not available to the public.

Done.

b. P 3 Line 39: I suggest adding a sentence that gives the most important reasons for exclusion.

Done.

c. There are two Takahashi 1999 studies; they should be distinguished, e.g. 1999a, 1999b. The study L reference on p 6 line 2 appears to be incorrect. I'm unclear if there are other details for discussion of references [15] and [33] that need to be corrected in the text and tables. For instance, Table 1 has no mention of JMPR, but Table 2 does, but the text seems to be making a different point.

Done

d. P 7 line 10: Add Ha

Done

10. Discussion
a. P 20: I had trouble following the paragraph starting on line 35 so please revise for greater clarity. Tables 3-5 don't address preneoplastic lesions.

Done. Tables 3-5 summarize the tumor findings. The results from evaluation of the non-neoplastic toxicity are only summarized in the text. These cannot be easily placed in a Table since not all studies looked for the same types of toxicity.
b. P 21: EPA also excluded findings for doses above the limit dose. The discussion implies that EFSA did this but not EPA. This should be clarified.

EPA’s original review used being above the limit dose for exclusion. But they were criticized for this by their Science Advisory Panel and excluded it from their “reasoning” in the final document.

c. P 22 1 21-3: This is an important comment that should be brought into the summary at the end and the probably also into the abstract.

I hesitate to highlight this issue. The purpose of the paper is to get all of the information out there for everyone to see. While I had to deal with the differences between my review and that of the agencies, I still want the findings of the analysis to be the main point of the manuscript. Some modifications were made.

11. It would be really helpful to provide websites for some of the references, e.g. to regulatory guidance documents such as the NTP Cancer Evaluation Criteria [100].

The NTP reference has been removed. The remaining references are, in my opinion, clear. Many have websites, but they sometimes place revisions in the same place, so the website may not remain correct. For the memos, etc. from EPA, they no longer have these online so I have to use the document number to identify them.