Reviewer’s report

Title: THE RAMAZZINI INSTITUTE 13-WEEK PILOT STUDY ON GLYPHOSATE-BASED HERBICIDES ADMINISTERED AT HUMAN-EQUIVALENT DOSE TO SPRAGUE DAWLEY RATS: EFFECTS ON DEVELOPMENT AND ENDOCRINE SYSTEM

Version: 0 Date: 14 Dec 2018

Reviewer: Robin Mesnage

Reviewer's report:

This manuscript describes the results of a pilot study investigating endocrine disrupting properties of glyphosate, and of a Roundup formulation. This pilot study is well conducted and well interpreted. It is a very interesting study because it is an unexplored topic. This study provides an answer to a very important gap. Authors have performed a large range of analysis. The main gap of this study is that only one dose has been tested. Most statistically significant differences are scattered and it is difficult to find a clear toxicity pattern. It is thus very difficult to understand if the health effects which are detected in this study are dose dependent and if they would be replicable in other studies.

Addressing the few comments below would improve the clarity of the study and could provide more insights into the consistency of the statistical differences.

L 60 - In the conclusions of the abstract, I would specify that it is a pilot study

L 77 and further - Which Roundup formulation? It is an important detail since formulations are likely to have different effects. This remark is valid for other citations in the manuscript. In the case where several formulations have been tested, better stay general and mention GBH. The name 'Roundup' is a trade name referring to the formulation 'Roundup' also called MON 2139 (glyphosate 360g/l and ethoxylated tallowamine). The Roundup herbicide tested in this study, Roundup Bioflow, is the representative EU formulation 52276 (https://www.sdslibrary.monsanto.com/Lists/MSDS%20Library/DispForm.aspx?ID=151). This is important to take into account as it is likely that this formulation does not contain an ethoxylated tallowamine surfactant because they were banned from the European market.
L 109 - 'No evidence of interaction of glyphosate and GBHs'. Are you sure that the EDSP program included tests on GBHs?

L 111 - 'EPA dismissed statistically significant differences consistent with estrogenic activity'. Was there a consistent profile indicating estrogenic effects across different assays? It would be more accurate to indicate which assays were found to be positive.

L 143 - 'exposure to GBHs' -> exposure to a GBH

Was the analysis of estrous cycle characterization and sperm analysis, and other aspects of the experiment, performed in a blinded manner?

L 355 - Which statistical software have you used?

L 380 - If your statistical software can do it, it is always better to indicate the exact p-values instead of thresholds. A p-value is a probability and it's value is a very important piece of information.

L 402 - 'borderline significant (p = 0.056)', I would avoid calling this borderline 'significant' since it is not significant
Table 2, and other tables. It will improve the clarity of the article if the authors could provide dot plots showing the spread of the data for some important statistical differences. I understand that it will not be reasonable to do this for every variable, but the spread of the data is a very important information to evaluate the quality of a dataset and the accuracy of interpretations. It can be provided as a supplementary material.

The addition of these dot plots may provide important information for some statistical differences. For instance, I am wondering if the statistical difference for the age at first oestrous is not due to potential outliers since the variance for this point is larger in comparison to the variance for glyphosate or for the control. In another case, I would like to see to which extent the spread for the male AGD values are overlapping of if the effect size is very clear. The most important is perhaps the effect on DHT levels in males for the 13-week cohort. It seems that it is the most pronounced effect in this study. The exposure to Roundup Bioflow decreased the DHT serum levels by a factor of 10.

Were there toxic effects in other tissues that could explain these endocrine disrupting effects?

**Level of interest**

Please indicate how interesting you found the manuscript:

An article of importance in its field

**Quality of written English**

Please indicate the quality of language in the manuscript:

Acceptable
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I collaborated with FM, FB and DM to conduct an investigation of glyphosate toxic effects. I also served as a consultant on glyphosate risk assessment issues as part of litigation in the U.S. over glyphosate health effects.

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