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: Michael Antoniou

Reviewer's report:

This manuscript describes one of the few studies that has compared the effects of glyphosate alone with a glyphosate-based herbicide that is representative of formulations in use within Europe. The authors test a single but regulatory relevant dose of glyphosate/Roundup (US chronic reference dose). An important component of the experimental design is that exposure to the test substances was initiated pre-natally, representing a more realistic scenario with respect to human exposures. The authors have conducted a very thorough analysis to evaluate effects on hormone systems and developmental/reproductive consequences. All analyses are conducted to a very high technical standard and thus the results obtained are compelling as far go for a single dose experiment. Given the controversies surrounding the toxicology of glyphosate based herbicides this is a very timely article that will be found of interest to many in the field and by the general public.

The authors find statistically significant outcomes in a number of measurements suggesting disruption of hormone systems (eg TSH, DHT, BDNF). Associated developmental defects observed were an increase in anogenital distance (males and females) and delay in first estrous. Interestingly, more frequent and pronounced disturbances were found in the Roundup compared to the glyphosate alone treatment groups. This suggest that the co-formulants/adjuvants in the Roundup are a significant contributing factor to the observed physiological/biochemical disturbances. Again this is of importance with respect to human health as populations are exposed to the commercial herbicide formulation and not just glyphosate alone.

Nevertheless, the following points should be addressed before publication should be considered.

1. Anogenital distance (AGD) was measured at post-natal day 4 and found to be increased in Roundup treated males and females and in glyphosate treated males. Was this increase in AGD maintained or did it resolve and return to normal as the animals aged to adulthood?
2. Between lines 85 to 98 the authors summarise findings from other studies of Roundup formulations that found alterations in hormone systems in vivo. However, the authors fail to refer to study by Seralini and colleagues (Seralini R et al. Environ Sci Eur. 2014;26(1):14), which also found hormone (testosterone, estrogen) changes in response to even a very low dose of Roundup over a 2 year period of treatment.

3. Line 144: "Admissible" should be "Acceptable".

4. Lines 400-401: it is stated that "for females only a few samples were available for these further analyses". Why?

5. Lines 445-446: it is stated that "Our findings suggest that both Roundup and glyphosate result in an increased in utero exposure to androgens". It is not clear to this reviewer how the authors arrive at this conclusion since androgen measurements were not taken during pregnancy. Androgen measurements are only shown in the offspring. This point needs to be clarified.

6. There are a number of measurements that show significant differences at 6 weeks but not at 13 weeks; increase in TSH levels in glyphosate-treated males; Roundup increases BDNF levels in males; increase in E2/SHBG ratio in Roundup treated males; decrease in fT/TT ration in glyphosate treated males. Can the authors offer any suggestions as why this is the case? That is, why were the 6 week differences not maintained at 13 weeks?

7. Lines 503-510: it is difficult to follow the arguments of the authors in this section and thus not possible to understand the point they are trying to make. Clarification is required.

8. Line 586: "30.000" should be "30,000".

9. Sentence structure and grammar is poor in many places. The manuscript should be reviewed by a native English speaker to raise to the required standard so that the risk of confusion is avoided.
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:

Needs some language corrections before being published

Declaration of competing interests
Please complete a declaration of competing interests, considering the following questions:

1. Have you in the past five years received reimbursements, fees, funding, or salary from an organisation that may in any way gain or lose financially from the publication of this manuscript, either now or in the future?

2. Do you hold any stocks or shares in an organisation that may in any way gain or lose financially from the publication of this manuscript, either now or in the future?

3. Do you hold or are you currently applying for any patents relating to the content of the manuscript?

4. Have you received reimbursements, fees, funding, or salary from an organization that holds or has applied for patents relating to the content of the manuscript?

5. Do you have any other financial competing interests?

6. Do you have any non-financial competing interests in relation to this paper?
If you can answer no to all of the above, write 'I declare that I have no competing interests' below. If your reply is yes to any, please give details below.

I currently have an ongoing collaboration with the group, which has submitted this manuscript.

I agree to the open peer review policy of the journal. I understand that my name will be included on my report to the authors and, if the manuscript is accepted for publication, my named report including any attachments I upload will be posted on the website along with the authors' responses. I agree for my report to be made available under an Open Access Creative Commons CC-BY license (http://creativecommons.org/licenses/by/4.0/). I understand that any comments which I do not wish to be included in my named report can be included as confidential comments to the editors, which will not be published.

I agree to the open peer review policy of the journal.