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: 13 Dec 2018

Reviewer: Andrea Gore

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The goal of this study was to determine how human-relevant exposures to glyphosate or glyphosate-based herbicides (GBH) in Sprague-Dawley rats affected developmental and endocrine outcomes at differing life stages. Comparisons of the commercial product and the active ingredient is a strength of the study. Several significant effects were found: for AGD (larger, especially in Roundup groups), hormones in males: DHT (13-week Roundup), TSH (6-week glyphosate, 13-week Roundup), BDNF (6-wk Roundup), fT/TT ratio (6-wk glyphosate, 13-wk Roundup), E2/SHBG (6-wk Roundup); and in females, the DHT/TT ratio and TT/SHBG ratios (13-wk Roundup). The study is sound and the data suggest that endocrine systems are disrupted by glyphosate and/or Roundup, with more effects of the commercial product. The authors are commended for excellent statistical data analysis.

Critique:

1. The Discussion needs to be reframed. Authors focus on several endpoints that were affected (TSH, BDNF, steroid hormones, etc.) and they do a lot of speculation based on single-point hormone measures. For example, increased TSH in the absence of measuring other thyroid hormones is not terribly informative about "subclinical reduced thyroid function" proposed by authors (line 439). The discussion on both thyroid function (beginning line 488) and BDNF (line 500) can be shortened, as the biological meaning is unknown. The fact that animals were killed across a large range of times introduces the possibility that diurnal rhythms contributed to differences; and females were not killed on the same day of the estrous cycle, making hormones in females difficult to interpret. Rather, it would be more interesting for authors to discuss important issues such as: why do the 6- and 13 week endpoints differ? Why do results of glyphosate and Roundup differ? The Discussion needs some rewriting to focus on key findings.

2. Sample size information is unclear. Specify the number of dams per group, the numbers of offspring, and how litter was considered as a variable in analyses if multiple littermates were included in the same endpoint group.
3. The weakness that females were not euthanized on the same day of the cycle, and that rats were killed sometime between 9 am and 3 pm, is acknowledged by the authors. They do not state, though, whether they plan to correct these experimental design weaknesses in future work - please do that. The wording describing blood collection [Line 269 ("Blood collection was continuous from 9 a.m. to 3 p.m….")]] is confusing and should be changed. Blood collection was not continuous - that implies multiple sampling within animals, which was not the case.

4. Hormone assays: Details on each hormone assay coefficient of variation need to be provided.

5. The Introduction is far too long and is a review of the entire literature. Please condense.

Minor points:

6. Line 85 ("In vivo, sexual development…"), cite the original literature on sexual development, not Dallegrave et al. 2007.

7. Line 292, sperm analysis: What do authors mean by single male animals? N=1 per group? This is not a meaningful sample size and this section should be excluded if this is the case.

8. Results: The use of sub-headers would be helpful in delineating separate outcomes.

9. Table 8a: In the table legend, what do the lettered footnotes mean (e.g., a refers to 7 out of 8, etc.)? 7 out of 8 what? Weren't the hormones measured in all animals?

10. Title. Why is it important to refer to the Ramazzini Institute? This is extraneous information.
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