Title: A Repeated Measures Study of Phenol, Paraben and Triclocarban Urinary Biomarkers and Circulating Maternal Hormones during Gestation in the Puerto Rico PROTECT Cohort

Version: 0 Date: 13 Nov 2018

Reviewer: Medina Jackson-Browne

Reviewer's report:


This manuscript examines gestational phenol and paraben concentrations in relation to reproductive and thyroid hormones in 602 pregnant women in the PROTECT cohort. The authors observed both positive and negative associations between many phenols and paraben with hormone levels measured between the 2nd and 3rd trimesters. The authors implemented a linear-mixed model to account for intra-individual correlation of hormone measurements, and also used a linear-mixed model with biomarker concentrations as a time-varying variable to assess critical periods of paraben and phenol exposures. Although there is evidence of large day-to-day variability, methods implemented to reduce exposure misclassification bias make this a strong addition to the literature on the potential effects of paraben and phenol exposure on maternal hormone levels during pregnancy. I have a mostly minor comments below that I hope will help improve further iterations of the manuscript.

Methods

2.1 Study participants

- Which samples were used for analyses should be clearer; i.e. although there were 3 study visits, you only used information from the 1st and 3rd (the assumption is because these were the visits that blood was collected as well)? Or were urinary biomarkers from the 2nd visit used also? Page 5: lines 44-53

- the number of participants from each visit should be stated. Page 4: line 49

- what is the total number of participants in the cohort? (not just the total included in analyses). Page 4: line 56

- where the differences due to loss to follow-up or missing measurements/covariates?
2.4 Statistical Analyses

- demographic characteristics should be listed and how they were included in analyses (continuous vs. categorical). Page 6: line 41

- Although the authors explained why they used a different socio-economic index for certain models, did they perform sensitivity analyses using both? It is hard to justify using different covariates in regression models and then comparing the results. Page 7: lines 36-48.

Tables

Table 1:

- table should be comparing the demographic characteristics of those included and excluded (full cohort) in analyses.

- demographics should better reflect regression analyses by having the characteristics for each visit in addition to the full analyses cohort (see above comment).

- title should be reworded; it is not the demographics of the study population but of population included in analyses.

- what are the concentrations of the biomarkers/hormones based on these demographics? Seems they should be included in this table as well.

Table 2:

- this table could also be reworded to include the term "distribution" and "difference" since the distribution is shown and the p-values for differences between visits are reported.

- Again, if urinary biomarkers were used from visit 2, they should be included in this table as well.

- Total n should be included for each biomarker/hormone instead of the total n for each visit.
Tables 4 and 5:

- aren't these just tables of results shown in figure 2? Seems that these tables should be moved to supplemental instead being in main manuscript.

- It would be helpful to include unadjusted models, so that the reader can understand the overall direction of confounding. This would aid the reader in deciding whether further adjustment would tend to strengthen or attenuate the results.

Figures

- Are the results based on LOD substituted concentrations as well? Seems worth adding this to footnote.

- Figure 1: are these correlations of log- or un-log-transformed concentrations? Should be stated in the figure and in results section.

- Figure 2 is incorrectly labeled as Figure 1.

Results

- The term "suggestive" was inconsistently used throughout the results section. In some areas "suggestive" is used and in others, "no association" or "not statistically significant" was used. I assume all of the above is referencing "no statistical significance"; if so, it would be best to just report the direction of the effect estimate and 95% CI and state that it the null is included or that the effect estimate did not reach statistical significance. I would make sure all statements are parallel.

- "data not shown" was stated on Page 10: line 27, it would be best to either include the data in the tables/figures or not include these results in the manuscript.

Discussion

- Could you say something about the overall sizes of the estimates? How large or small are they?

- Discussion of different findings across studies: to what extent can exposure differences explain these findings?
Please clarify the term "vulnerable" on page 12: line 36.

Please include citations for this statement: "...due to the unique role CRH plays in human pregnancies..." Page 14: lines 36-37.

There is a human study that examined triclosan during pregnancy as a potential vulnerable period as well. Page 15: lines 32-36. Please discuss this human study in addition to the rat study. Jackson-Browne et al 2018 published in Environmental Health Perspectives: "Identifying vulnerable periods of neurotoxicity to triclosan in children."

There is also an additional study examining triclosan and thyroid hormones by Braun et al. 2017 published in Hormones and Behavior: "Associations of early life urinary triclosan concentrations with maternal, neonatal, and child thyroid hormone levels." Please discuss your studies results compared to theirs. Page 15: lines 39-53

The Wang et al paper cited [80] also looked at triclosan and fetal testosterone from cord blood. Please include results in discussion. Page 16: line 4

In your limitations/strengths sections, you did not discuss whether your results generalize to other populations. You discussed how your associations differed or agreed with results of similar studies but the addition of how the concentrations of biomarker and hormones in this cohort relate to other cohorts would help address the issue of external validity. In addition, adding a column to Table 1 to help identify how the 602 participants in this study are different from the overall cohort help address internal validity. i.e. are those included in the analyses cohort more educated, higher income, etc.

**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
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 declare that I have no competing interests.

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.