Author's response to reviews

Title: Exposure to Phthalates among Premenstrual Girls from Rural and Urban Gharbiah, Egypt

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Author's response to reviews: see over
Philippe Grandjean, University of Southern Denmark  
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Environmental Health, Editors-in-Chief

Dear Editors-in-Chief and Environmental Health Editorial Team,

Thank you for the opportunity to provide a revision to our manuscript entitled “Exposure to Phthalates among Premenstrual Girls from Rural and Urban Gharbiah, Egypt: a pilot exposure assessment study” which my co-authors and I are pleased to resubmit for publication in Environmental Health (MS: 1658845535136815). The revised article describes our original work, and it has not been submitted for publication elsewhere.

We thank the Reviewers for the helpful comments. In order to expedite your decision, we have incorporated the suggestions into our revised manuscript and have highlighted (with “tracked changes”) our revised content. Below please find our detailed responses to the reviewer and editorial comments.

Reviewer 1:
1. To compare the results between rural and urban, how about the log transformed data use? So, I think you can use parametric statistics.

• Response: Log transformation of the data did not yield a normal distribution for five of the phthalate metabolite compounds, as measured by the Shapiro-Wilk test for normality, thus we used non-parametric statistics for comparisons between groups. This change was reflected in the text: “Comparisons of concentrations of urinary phthalate metabolites between urban and rural Egyptians were conducted via Wilcoxon rank sum test, as log transformation of the phthalate metabolite data did not yield a normal distribution.”

Reviewer 2:
1. The PC approach should be saved for the later study. It adds nothing to this analysis, and the interpretation is unclear - the PC-plots look identical for the 2 groups in both figures. It would be more helpful to show GM or Means adjusted for some of the covariates. The crude differences are precisely what is repeated in the PC results.

• Response: The PC approach has been deleted and replaced by comparisons between covariate adjusted mean urinary phthalate metabolite concentrations. Changes were made in the Methods (see below), the Results:

   “Least squares adjusted means and standard errors of urinary phthalate metabolites
were calculated, adjusting for specific gravity, age, and BMI (Table 4). Adjusted mean phthalate metabolite concentrations were compared between urban and rural Egyptians as well as between individuals who reported storing food in plastic containers against those who did not. There were no statistically significant differences in covariate adjusted mean concentrations between urban and rural Egyptians. Individuals who reported storing food in plastic containers had significantly higher urinary concentrations of MiBP when comparing covariate adjusted means. Additionally, while only MiBP concentrations were found to be statistically significantly higher in individuals reporting food storage in plastic (p = 0.04), adjusted mean concentrations were found to be higher for every phthalate metabolite measured.”

And in the Discussion:
“Food storage in plastics is becoming ubiquitous in many areas that once utilized ceramic and clay containers, since plastics are inexpensive and widely manufactured. When covariate adjusted means were compared for all measured metabolites, individuals who reported storing food in plastic containers had significantly higher urinary concentrations of MiBP.”

2. Add this explanation to the Methods

- Response: The statistical methods for comparison of covariate adjusted means was added to the Methods: “Covariate adjusted least squares means and standard errors of phthalate metabolite concentrations in urine were calculated using PROC GLM and the LSMEANS statement in SAS 9.2. Means were adjusted for age, BMI, and urinary specific gravity. Adjusted means between urban and rural individuals, as well as individuals who reported storing food in plastic containers or not, were compared by T-test, with a p<0.05 considered statistically significant.”

3. p 7: contamination often arises from handling, regardless of the quality of the vials, because of pipets, tubing, gloves, etc. Further, while MECPP - the most reliable DEHP metabolite - is similar between urban/rural (unadjusted), the phase-I metabolites are different. Thus, there may be a chance that contamination could have occurred in the urban not the rural situation, or is it that urban setting had plastic seat covers in the collection site. This information might also be useful for the "later study”.

- Response: All subjects (Rural and Urban) were provided bus transportation to the Tanta Cancer Center for sample collection and interviews. This has now been clarified in the Methods: “Urban girls were chosen by systematic random sample of girls from the census records of Tanta city as the urban location. Rural girls were chosen by a systematic random sample from two villages from two districts in the province. Study subjects were approached to participate in the study and no refusals were encountered. All study subjects were provided bus transportation to the Tanta Cancer Center.” Since sample collection for both the rural and urban girls occurred in the same facility and under identical circumstances, it is not likely that differences in phase-I DEHP metabolites are due to contamination in Urban participants.
4. p 10, li 221 and final response note about bivariates: were differences observed in multivariable adjusted means?

- Response: This information was added to the Results section following the reanalysis of the data: “There were no statistically significant differences in covariate adjusted mean concentrations between urban and rural Egyptians. Individuals who reported storing food in plastic containers had significantly higher urinary concentrations of MiBP when comparing covariate adjusted means. Additionally, while only MiBP concentrations were found to be statistically significantly higher in individuals reporting food storage in plastic, adjusted mean concentrations were found to be higher for every phthalate metabolite measured.”

We again thank the reviewers for their helpful comments and for the kind consideration of our revised manuscript for publication in *Environmental Health*. Please contact me if you need any additional information.

Sincerely,

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