Author's response to reviews

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

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Version: 2 Date: 22 March 2011

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

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

Please find enclosed a revised version of our article entitled “Exposure to Phthalates among Premenstrual Girls from Rural and Urban Gharbiah, Egypt”, 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 you and the Reviewers for your constructive comments. In order to expedite your decision, we have incorporated these 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:

Introduction

1. line 63-67: Phthalates can be categorized into higher molecular weight and lower molecular weight. As DEHP is categorized into the higher molecular weight group, further explanation/knowledge is required on specific lower molecular weight phthalates.

• Response: Thank you for the comment. To clarify for readers, we added specific low molecular weight phthalates, which are typically used in different industrial applications than high molecular weight phthalates: “Higher molecular weight phthalates, such as di-(2-ethylhexyl) phthalate (DEHP), are most often used as plasticizers in polyvinyl chloride (PVC), while lower molecular weight phthalates, such as diethyl phthalate (DEP) and dimethyl phthalate (DMP), are used in cosmetics, insecticides, and pharmaceutical applications [2].”

Method

1. line 131: The phthalate metabolites concentration in urine of workers who are use the phthalates in their workplaces showed higher at post-shift than pre-shift (Ref: Ann Occup Hyg 2009;53(1):1–17). Also, adult male who do not use the phthalates in their workplaces showed higher concentration at evening than those of next morning (Ref: J Prev Med Public Health 2010;43(4):301-308). It means that the urine should be collected after evening for assessing the phthalate exposure. Do you think it is appropriate to collect urine samples at 12:00 – 4:00?
• Response: We agree with the reviewer that due to the non-persistent nature of phthalates, researchers must take temporal variability into account when collecting urine for analysis of phthalate metabolites. In our study, we standardized our collection times to the afternoon (12-4 PM) to ensure that concentrations that we measured in our population would be internally comparable. We also compared our data to that collected as part of the National Health and Nutrition Examination Survey (NHANES). NHANES collects urine for analysis of phthalate metabolites at three different periods during the day (morning, afternoon, and evening). We are not currently aware of a standard established for time of urine collection for analysis of phthalate metabolites. The studies cited by the reviewer suggest that collection of urine in the evening, subsequent to a day’s exposure to phthalates, show the highest concentrations. However, measurement of phthalates at this point may overestimate daily phthalate exposure as phthalate metabolite levels may be measured at their daily peak and may not reflect true average daily values. We chose an afternoon collection time because we felt it would accurately convey an average phthalate metabolite concentration in urine and allow for comparisons to data collected in NHANES. In addition, we took the reviewer’s comment below (iii. Information about sampling time) into account and restricted our analysis of NHANES data to only those samples collected during the afternoon to minimize temporal variability.

Results
1. In my opinion/view point, comparison of study results and NHANES unavailable without considering the following (Table 2) i. Without adjusting the specific gravity ii. Differences in lifestyles between countries iii. Information of sampling time

• Response: We thank the reviewer for the comment. Regarding point (i), unfortunately specific gravity was not measured in NHANES and is not available as a covariate. Urinary output in NHANES was estimated via urinary creatinine concentration. We discussed potential differences in routes of exposure between US and Egyptian girls in the discussion and due to the reviewer’s suggestion in point (ii) added the following two sentences: “Elevated MiBP concentrations in Egyptians compared to US individuals suggest differential routes of exposure to DiBP, a phthalate commonly used as a plasticizer as well as in inks and paints. Since individuals who reported consuming canned foods as well as storing food in plastic had significantly higher concentrations of MiBP in their urine, food storage and consumption could potentially explain this difference between the Egyptian and US populations.” To address the reviewer’s point (iii), we restricted our analysis of NHANES data to only urine samples collected in the afternoon, matching the time of day that we collected our samples. These updated values can be observed in Table 3, with a modification in the methods: “Additionally, we restricted our NHANES analysis to individuals who participated in the afternoon session, to most closely match the time of day when we collected urine from the Egyptian study subjects. Concentrations of urinary phthalate metabolites were available for 97 comparison subjects, except for MCNP and MCOP, where concentrations were available for 44 subjects because MCNP and MCOP were not measured in NHANES 2003-2004.”
Reviewer 2:

The exposures and sources are a contribution, but the small dataset seems overanalyzed. …Not clear that there is power to undertake principal components, and the data would be more easily understood if simple bivariate comparisons were provided in tabular fashion, with appropriate statistics.

- Response: While there is no hard rule for a necessary sample size or subject to item ratio in principle component analysis, our n=57 and subject to item ratio >5 fits within previously published minimum recommendations for principle component analysis (reviewed in Osborne and Costello, 2004, Practical Assessment, Research & Evaluation, 9(11)). Larger sample sizes are always advantageous, however, we believe our analysis provides sufficient framework for this methodology to be adapted in a larger environmental epidemiology study.

In Table 1, there is no reason to include anthropometry as it is not involved in the study; rather include more SES variables and perhaps data about the exposure sources, such as plastics, personal products, and construction materials. Were any of these sources correlated with SES other than urban/rural?

- Response: We thank the reviewer for the comment. We included anthropometric measures for readers who may be interested in the body composition of the study population. As the reviewer suggested, we added summary statistics for the food storage/consumption behaviors into Table 1.

In the figures, it would be more informative to delete the PCs and plot the food sources vs urban/rural.

- Response: As food sources and urban/rural status are categorical variables, we thought it would be more appropriate to include these data in a table. Table 1 now includes food storage and dietary habits by urban/rural status as appropriate for categorical variables.

“toxic” on page 3 seems misused.

- Response: Toxic has been removed from this sentence: “Exposure of rats to high doses of phthalates orally (0.5 – 2 g/kg/day) has been associated with a range of health outcomes including prolonged estrous cycles and mid-pregnancy abortion [11-12]”

Effects” on the last line 82 of page 3 should be changed to associations; and “carcinogenic” is a stretch.

- Response: Per the reviewer’s suggestion, we changed “effects” to “associations” and removed a reference to carcinogenicity: “Epidemiological studies of exposure to phthalates also suggest potential developmental and reproductive associations.”
On page 7, were the collection materials phthalate-free?

- Response: We collected the samples in polypropylene tubes provided by the CDC, which do not require the use of phthalates as plasticizers.

Provide a reference for lines 168-170.

- A reference is now provided, and the text has been updated to reflect this change: “Three samples had specific gravity values less than 1.005. These samples were excluded from the analyses because of the urinary sample being too dilute, adapting previously established criteria for male workers to this young female population [20]” (p 9, li 199-200, provide references; however, it is strongly suggested not to include PCs in an underpowered study.

- Response: A reference on the use of principle component analysis was added, as suggested.

Li 215 “95th ..”, clarify.

- Response: The meaning of the sentence was clarified: “Girls in both the urban and rural groups had MEP urinary concentrations above the 95th percentile of the sample population, signifying that the highest exposed individuals in the population were distributed in both urban and rural areas.”

P 10, li 221, clarify.

- Response: The sentence has been clarified: “Urinary specific gravity levels were significantly higher in rural compared to urban Egyptians by the Wilcoxon rank-sum test (p=0.043) and specific gravity adjusted urinary concentrations of three phthalate metabolites, MBzP, MCPP, and MiBP, were found to be significantly higher in urban individuals (Table 3).”

P 12, a recent study reported levels in Peru, which might be included.

- Response: We thank the reviewer for pointing out this interesting study of phthalate exposure in another vulnerable population, pregnant women in Peru. We have now included this study in our discussion and modified the following text: “A study of
phthalate metabolites measured in urine collected from pregnant women in Peru found significantly lower creatinine adjusted concentrations of MBzP, MCPP, MEP, and MiBP compared to pregnant US women [25]. A recent study of a group of pregnant women from Jerusalem, Israel, however, observed a different pattern of exposure when comparing to US women, with MBzP urinary concentrations lower and MiBP levels higher in Israeli women [26]. The findings of the Israel study are similar to the present study, where median MBzP levels were lower but MiBP levels were higher in Egyptian girls compared to US girls.”

Add specific gravity to Tables 2 and 3.

• Response: Specific gravity levels were added to Table 3, as suggested by the reviewer. Unfortunately, specific gravity is not available as a covariate in NHANES, so we were unable to adjust the NHANES levels for specific gravity and thus cannot report them in Table 2.

Consider deleting tables 4-5 and the figures, and including bivariate comparisons of foods/urbanity/SES/other sources and phthalates that are of interest.

• Response: Results of the bivariate comparisons for food storage and consumption were added in the text: “Individuals who reported storing food in plastic bags or containers were found to have significantly higher specific gravity adjusted concentrations of miBP by the Wilcoxon rank-sum test (p=0.01). Individuals who reported consuming canned food had significantly higher levels of miBP (p-value = 0.02) and mEHP (p-value = 0.04). There were no significant differences in urinary phthalate metabolite concentrations observed in individuals who reported using plastic utensils or plastic plates.”

Formatting Concerns:
We now ensure that the revised manuscript conforms to the Environmental Health journal style, as detailed here:

• The figure files are now formatted correctly.

• The title on the Title Page now includes the study design.

• Author superscript numbers begin on a left-justified line.

• The institutional addresses now include Department, School, and Institution.

• The line for correspondence reads Corresponding author with the * after the corresponding author's superscript number with all the other information removed.
• The spacing is double-spaced throughout.

• Names in the e-mail addresses section as initials e.g. JAC, etc.

• Line numbering has been removed throughout.

• Italicized text has been changed to normal except where indicated in the reference section.

• The headings in the Abstract section now appear above the text; the colons have been removed.

• The first section after the Abstract is titled Background.

• General numbers 0-9 are now written out, including on p.5 (e.g. two villages from two districts).

• Before the List of abbreviations, we inserted the heading Conclusions. In this section we now briefly state the main conclusions of the research and give a clear explanation of their importance and relevance.

• The list of abbreviations section is now in sentence format as abbreviations. The term and the pairs are separated with semi-colons.

• The following section is now titled Competing Interests.

• All headings now appear above their text, and the colons after the headings have been removed.

• The acknowledgement section is now titled Acknowledgements and Funding.

• The references are formatted as number, period, space and reference.

• All authors are listed (no et al.).

• All lines in the tables are visible.

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

[Signature]

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