Author's response to reviews

Title: Urinary bisphenol A and pubertal development in Chinese school-aged girls: a cross-sectional study

Authors:

Maohua Miao (miaomaohua@163.com)
Ziliang Wang (wangziliang1986@126.com)
Xiaoqin Liu (xq_liu8008@163.com)
Hong Liang (lucylhcn@163.com)
Zhijun Zhou (zjzhou@fudan.edu.cn)
Hui Tan (htan@fudan.edu.cn)
Wei Yuan (yuanwei11@yahoo.com)
De-Kun Li (De-Kun.Li@kp.org)

Version: 1 Date: 09 May 2017

Author's response to reviews:

Dear Editor,

Thank you and the reviewers very much for the comments and suggestions. We have revised the manuscript based on the reviewers’ comments and our responses are outlined below:

Reviewer #1: Summary: The authors conducted a cross-sectional study on the association between bisphenol A exposure and pubertal development. Data for the analyses came from three schools in Shanghai and the final sample consisted of 655 girls aged 9-18. The age of onset of pubertal development is continuing to decline, and environmental toxicants are thought to play a role. Thus, the paper addresses an important public health concern. The comments below will help to improve the clarity of the manuscript:

ABSTRACT

The authors may wish to explain in the abstract what PH2 and PH5 represent in your study.
Response: We thank the reviewer for the suggestion. We have added explanation on PH2 and PH5 in the abstract.

Lines 37-40: I would suggest removing the "But the associations were not statistically significant after potential confounders were controlled" sentence.

Response: The sentence has been deleted as suggested.

INTRODUCTION

Page 3, line 52: Please include a reference for the "one of the environmental stressors to developing children is bisphenol A (BPA)" statement.

Response: A reference has been added (line 80).

Page 4, line 84: The authors should clarify that they're using Tanner stages to assess onset of pubarche and thelarche in girls.

Response: We have made the revision according to the suggestion (line 112).

METHODS

P6, line 118: Please clarify how creatinine was adjusted for.

Response: Creatinine was adjusted as urine BPA level divided by creatinine; we have added this in the methods session (line 146).

P6, line 120: Please clarify whether the same trained physician conducted all Tanner staging assessments.

Response: Yes, the Tanner staging assessments were conducted by the same trained physician. We have added this in the methods session (line 150).

Unclear why the authors chose to categorize BPA exposure as exposed (>LOD) and unexposed (<LOD). If the rationale was because so many girls were <LOD, then the authors should clarify this. Studies will often replace levels below the LOD with (LOD/square root of two). Did the authors consider this approach? Alternatively, the "<LOD" category could be kept as is, and then the ">LOD" group could be categorized at the median, for instance. This way the readers would be able to see the distribution of the BPA exposure. Overall, the manuscript could be improved by providing further rationale for the categorization of BPA exposure.

Response: Given that 40% of the study population had non-detectable BPA, the actual median level is somewhere slightly above LOD. As suggested by the reviewer, we used “Median among detectable BPA” to further categorize BPA exposure level, similar results were observed using
the new categorization, although there was no further stronger association among girls with high BPA exposure (>median) compared with those with moderate exposure (LOD-median), which is consistent with the report of the nonmonotonic dose-response effect of BPA.

Please provide a more detailed rationale for the restriction by age for each of the outcomes. The current research question is difficult to investigate using a cross-sectional study, thus the authors attempt to address this issue by restricting the outcomes to certain age groups. However, this greatly limits the sample size. Further, the rationale for the chosen cut-offs is unclear. It would be helpful if the authors indicated (perhaps in table format) a) how many girls were B2+/PH2+ and b) how many had reached menarche. It would also be helpful if the authors conducted sensitivity analyses to assess if the results for each of the outcomes change with different age cut-offs.

Response: We chose the cutoff according to the distribution of Tanner stages in the study population. At age 9, 76.0% of the girls had evidence of breast development, the proportion increased to 90.4, 97.5 at age 10 and 11. At age 12, all girls reached breast development stage 2 (theelarche). With regard to pubic hair development, the percentage of girls who had reached pubic hair stage 2 was 5.1%, 12.2%, 43.8%, 92.5%, and 96.9% for girls at age 9, 10, 11, 12 and 13. At age 14, all girls reached pubic hair development stage 2 (pubarche). We chose the age of 12 as the cutoff point for the puberty onset analysis, since all girls at ages over 12 had reached theelarche and the effect of BPA is no longer observable, thus irrelevant in the context of this study’s objectives. Similarly, we conducted analysis on BPA and menarche at age less than 14 according to the distribution of menarche. (lines 197-206)

We have added a table presenting the distribution in the result session as suggested (table 2). We also conducted sensitivity analysis using other cutoffs including 11 and 13, the results were consistent.

The authors may wish to clarify that Tanner staging is used to assess stages of breast and pubic hair development and B2+ and PH2+ represent onset of puberty.

Response: We thank the reviewer for the suggestion and the revision has been made accordingly.

How were the covariates chosen? Did the authors consider previous literature or create a directed acyclic graph?

Please be more specific here.

Response: We identified potential confounders according to previous literature. We have added this in the methods session (line 180).

Please describe the covariates in more detail. For example, how was an unbalanced diet defined? Why was a cut-off of 10 chosen for the depression score? How was sleeping quality evaluated? This additional information would greatly improve the clarity of the manuscript.
Response: We thank the reviewer for the suggestion. The median of the depression score was 10. We used 10 as the cut-off since few girls meet the clinical criteria of depression. The information on unbalanced diet and sleep quality were both self-reported. We have added this in the methods session (lines 181-183).

Did the authors consider a formal assessment of mediation for BMI? The discussion of mediation by BMI is a bit sparse. Either the mediation by BMI should be omitted from the paper, or discussed further.

Response: We tried to evaluate the effect of BMI since it has been reported as a modifier of hormonal exposures in studies examining EDCs in relation to female puberty (Wolff et al., 2008). However, we did not observe the mediation effect as expected (the risk estimate has no significant change after BMI was adjusted), largely due to the small sample size and the cross-sectional design (we collected current BMI instead of BMI at pubertal onset, which might be the relevant stage). In such cases, our ability to assess the mediation effect of BMI is quite limited and thus we deleted the analysis on mediation of BMI (lines 184-185).

RESULTS

Table 1: Please describe how urban/rural status was defined.

Response: The urban/rural status was defined according to residential registration, which is widely accepted in China, in which areas mostly consisting of farmers are considered as rural and those mostly consisting of workers are considered urban.

The number of missing observations for each variable is included in a footnote under the table. This information would be better represented as a row under each variable to help with the clarity of the results.

Response: We have revised the table according to the suggestion.

Table 1: It would be helpful to include the age distribution in the table. For instance, the authors could categorize age as "9-12" and "13-18" as that's how it's commonly presented in the other tables.

Response: We have added the distribution of age in table 1, but we used a narrower categorization in order to present the full picture of the distribution of BPA across age groups.

Table 2: The exposure distribution by age is a bit unnecessary, particularly because the authors do not assess stratum specific estimates by age. Instead this information could be presented in a separate table, or in Table 1. Unclear how the percentages in Table 2 are calculated. For instance, what does the 0.0% represent? It's worth clarifying that the total sample size for adjusted analyses is 573, and indicating that 82 girls had missing data.
Table 3: Similarly, the distribution by age is a bit distracting, particularly when the authors do not stratify by age. Further, unclear why age 12 is omitted from the table?

Response: We have deleted the distribution of menarche, PH2+ and PH5 across BPA exposure by each age. We can present this as a supplemental table if needed.

The percentage of 0.0% means that no girls reported having experienced menarche at the examined age. And we have added information on the total sample size for adjusted analyses.

Age 12 was not included in the table since the analysis was conducted among those aged <12. We have corrected the age range to 9-11 in the revised manuscript. We apologize for the error.

DISCUSSION

Page 9, line 189: This is a bold statement given your study design. There's no way of assessing causality with cross-sectional data. This sort of statement may be appropriate for a longitudinal study design where the outcomes are assessed in the same group of girls, but may be a bit misleading with cross sectional data.

Response: We accept the suggestion and the sentence has been deleted.

Page 10, line 219: Would be worth clarifying that girls could be exposed to BPA through other routes of exposure, not just diet.

Response: We thank the reviewer for the suggestion and we had made revisions accordingly (discussion session, paragraph 7).

The limitations section is a bit sparse. The authors may wish to expand on the limitations of using cross-sectional data, i.e. it's tricky to assess associations due to difficulties with temporality. The authors may also want to address the limitations of the way BPA exposure was categorized, or the fact that 40% of participants were below the LOD.

Response: We thank the reviewer for the suggestion and we had made revisions accordingly (discussion session, paragraph 7).

Page 11, line 228: What about the menarche results? This is worth commenting on.

Response: We thank the reviewer for the suggestion and we have added a comment on the menarche results (discussion session, paragraph 2).

Reviewer #2: The authors reference the appropriate scholarly context. They don't cite enough relevant publications in the field: it should be further added the importance of prenatal exposure (es. The effect of maternal exposure to endocrine disrupting chemicals on fetal and neonatal

Response: We thank the reviewer for the suggestion and the literature recommended. We have added discussion on the importance of prenatal exposure (discussion session, paragraph 5).

We hope that the manuscript has been revised in a manner that will make it suitable for publication.

Thank you!

Yours sincerely,

Wei Yuan