Author's response to reviews

Title: Association between bisphenol A exposure and body mass index in Chinese school children: A cross-sectional study

Authors:

He-xing Wang (wanghexing@tom.com)
Ying Zhou (yingchou@fudan.edu.cn)
Chuan-xi Tang (tchx8012@126.com)
Jin-gui Wu (shcnwu@163.com)
Yue Chen (Yue.Chen@uottawa.ca)
Qing-wu Jiang (jiangqw@fudan.edu.cn)

Version: 4 Date: 4 September 2012

Author's response to reviews: see over
September 4, 2012

Dear Editors:

We must thank you and all other reviewers for the critical feedback. We feel lucky that our manuscript went to these reviewers as the valuable comments from them helped us not only with the improvement of our manuscript, but obtain some helpful perceptions in this research field. Please do forward our heartfelt thanks to these experts. Based on the comments we received, careful modifications have been made to the manuscript. All changes were marked using the track changes mode. We hope the new manuscript will meet your magazine’s standard. At the bottom of this letter, you will find our point-by-point responses to the reviewers’ comments.

Thanks and Best regards!

Yours sincerely,

Ying Zhou.
yingchou@fudan.edu.cn.

**Major Revisions**

1. New Table 1, Table S1, and Table S2 have been generated for better presenting our results and answering the questions by the reviewers.

2. In ‘Statistical analyses’ of the ‘Methods’ section, we have provided more detail information. (Page 7~9)

3. The calculation and discussion of outcome variation by using adjusted $R^2$ in multiple linear regression has been removed from this article.
4. In ‘Discussion’ section, a full paragraph has been added to explain the association of BPA exposure with BMI (Paragraph 3 of the ‘Discussion’ section, page 12).

5. In ‘Discussion’ section, one paragraph has been added to explain the differences in the effect of BPA exposure between our study of human population and other studies of cells and animals (Paragraph 8 of the ‘Discussion’ section, page 14).

Editors’ Revisions
1. The study design has been included in the title.

2. All the information about the corresponding author has been removed, and replaced by the phrase * Corresponding author. The * has been placed after the corresponding author’s superscript number(s).

3. In the title page, all the author names and email addresses have been listed as name initials:email address, and inserted below the heading.

4. In the final section, the ‘Conclusion’ has been changed to ‘Conclusions’.

5. All the author names of each reference in this paper have been listed because the author numbers of each reference are below 30, and the ‘et al’ was not used.

6. For the tables, all horizontal lines have been made visible.

Reviewer: Justin Teeguarden

Major Compulsory Revisions

Answer 1: The effect of first morning void on BPA exposure level has been discussed in the ‘Paragraph 10 of the ‘Discussion’ section, and two references by Ye X et al. and Teeguarden et al. have been added for this [1,2]. (Page 15)

Answer 2: The use of urine outputs of 600 and 1200 ml/day has been justified in the ‘Paragraph 1’ of the ‘Statistical analyses’ section (Page 7). In this article, urine output was estimated by age (older subjects were conferred by higher urine output). Body weight or BMI increases with age in children. Therefore, daily urine outputs are
expected to be larger in children with higher body weight or in older subjects. Two variables related to body weight, age and sex, have been included in multiple linear regression analysis and analysis of variance.

**Answer 3:** The study conducted by Eijsvogels et al. (2011) showed that obese subjects had reduced urine outputs than normal weight ones during prolonged excise status, but it was not clear whether this was applicable to subjects during routine life status. Since our participants were expected to be during routine life status, this applicability during routine life status was important for the interpretation of our results. Siener et al. (2004) reported that BMI was not associated with urine output. Gundersen et al. (1966) reported an inverse relationship between total body water and degree of obesity except in grossly obese subjects. Therefore, there is a lack of strong evidence that obese subjects have reduced urine outputs. A reduced urine output may result in high urine specific gravity. The specific gravity correction alleviated the potential impact weight related urine outputs.

**Answer 4:** We totally agree that total daily exposure is the best metric of exposure for the associations with obesity when blood concentrations are not available. But daily intake estimates adjusted by individual body weights after BPA exposure, were not appropriate to be used to explore the association of BPA exposure with BMI in cross-sectional study because body weights used here have been influenced by BPA exposure. If higher BPA exposure causes heavier body weight, it would result in a decreased BPA exposure estimate if daily intake estimates adjusted by body weights. Therefore, daily intake estimates adjusted by body weights after BPA exposure tends to underestimated true daily intakes in the subjects with high BPA exposure, and therefore weakens the association between BPA exposure and BMI. For this reason, daily intake estimates not adjusted by body weight have replaced those adjusted by body weight (Table 2) for analysis of the association of BPA exposure with BMI. The results showed that the daily intake estimates were significantly associated with BMI (Table 2 and S2). Second, as shown in Table 3 and S2, multiple linear regression
analysis showed that high urine BPA and daily intake estimate were significantly associated with an increase in BMI value in all subjects whether those values were corrected by specific gravity or not. The non-significant association after stratified by sex and age might also be attributed to the reduction of sample size (see the ‘Paragraph 3’ of the ‘Discussion’ section, page 12).

**Answer 5:** In the ‘Paragraph 2’ of the ‘Background’ section (Page 4), the exposure doses tested in the cited studies by Rubin et al and Sargis et al were pointed out, and another literature reported that $10^{-4}$-$10^{-12}$M level of BPA induced lipid accumulation in HepG2 cells replaced the one from Masuno et al. In ‘Discussion’ section, one paragraph was added to give some plausible explanations for the exposure dose differences between our study and other lab researches (Paragraph 8 of the ‘Discussion’ section, page 14).

**Answer 6:** A full paragraph in ‘Discussion’ section was added to explain the reason why urine BPA was significantly associated with BMI (Paragraph 3 of the ‘Discussion’ section, page 12).

**Answer 7:** In the ‘Discussion’ section, one paragraph was added to compare the exposure doses in this study to those in cell and animal studies, and give some plausible explanations for the dose differences between our study and these lab studies (Paragraph 8 of the ‘Discussion’ section, page14). A reference about the activation of membrane estrogen receptor-$\alpha$ at as low as pM level (Wozniak et al. 2005) was added into the ‘Paragraph 5’ of the ‘Discussion’ section (Page 13).

**Reviewer: Elizabeth Hatch**

**Major Compulsory Revisions**

**Answer 1:** Daily intake estimates were used to further or in another aspect explore the association of BPA exposure with BMI. Because daily intake estimates individually
corrected by body weights weakened the association (specific reason has been provided in Major Compulsory Revisions 4 by the reviewer of Justin Teeguarden), daily intakes not corrected by body weights have replaced those corrected by body weights in Table 2. The method for daily intake estimates by Lakind and Naiman has been described in more detail. Why urine outputs were fixed in two categories (600ml/day and 1200 ml/day) has been explained in the ‘Paragraph 1’ of the ‘Statistical analyses’ section (Page 7).

Answer 2: The statistical analysis has been further described in ‘Statistical analyses’ section. Analysis of variance was mainly used in descriptive statistics to examine the association of BPA exposure with age subgroups, sex, and BMI subgroups. Multiple linear regression was mainly used to investigate the association of urine BPA or daily intake estimate with BMI in various conditions, such as whether urine BPA or daily intake estimate corrected by specific gravity or not, whether stratified by age subgroups or sex or not, and whether included of age and sex as covariates or not.

Answer 3: The calculation of adjusted $R^2$ in linear regression model has been removed from Table 2.

Answer 4: The study design of this article has been pointed out in the title and the ‘Methods’ of ‘Abstract’ section. (Page 2).

Answer 5: In the ‘Paragraph 2’ of the ‘Results’ section, significant mean differences (95% CI) of naturally log-transformed urine BPA concentrations and daily intake estimates among BMI subgroups (normal weight, overweight, and obesity) have been added in addition to p-values. (Page 9~10)

Answer 6: In the title of Table 2, the results have been pointed out to be from multiple linear regression analyses, and the regression coefficient ($\beta$) has been interpreted in the ‘Paragraph 3’ of the ‘Results’ section (Page 10). The mean differences of BMI
with quartiles of urine BPA were displayed in Figure 1.

**Answer 7**: The calculation of outcome variation explained by urine BPA, based on the difference of adjusted $R^2$ between with and without urine BPA in linear regression model, has been removed from Table 2.

**Answer 8**: The specific gravity correction was important for urine dilution, so Figure 1 has changed from only including the significant associations of urine BPA with BMI to including all the associations in adjusted model 2 (corrected by specific gravity).

**Answer 9**: In paragraph 3, page 7 of the paper published by Lang et al, the authors pointed out that logged BPA concentrations showed no significant differences between the BMI categories after adjustment for age, sex, and urinary creatinine concentrations by formal testing. Therefore there might be a discrepancy of the associations of BPA exposure with obesity between the studies conducted by Lang et al. and Carwile et al. because Carwile et al. found the significant positive associations of BPA exposure with obesity.

**Answer 10**: In the ‘Paragraphs 9 and 10’ of the ‘Discussion’ section, the ‘second’ limitation has been changed to the first limitation.(Page 14~15)

**Answer 11**: The ‘Discussion’ section of this article has been refined and reorganized.

**Reviewer: George Zhang**

**Major Compulsory Revisions**

**Answer 1**: In the ‘Method’ section of ‘Abstract’, how 6 schools and school children were chosen has been further explained, and the study design has been specified. (Page 2)

**Answer 2**: A new table (Table 1) has been generated to summarize the distribution of
the numbers of participants, weights, heights, and BMI among age subgroups (8-9 years, 10-11 years, 12-13 years, and 14-15 years), sex, and BMI subgroups (normal weight, overweight, and obesity). (Page 7~8)

**Answer 3:** The specific gravity-corrected BPA concentrations and daily intake estimates have been described in geometric mean and 95% confidence interval in Table 2. The influence of specific gravity correction on the significance level of BPA-BMI association has been pointed out in ‘Result’ section of ‘Abstract’. (Page 2~3)

**Minor Essential Revisions**

**Answer 1:** The exclusion / inclusion criteria for participants, the numbers of primary or middle candidate schools, the total student counts in each of 6 selected schools, and the frequency distribution of 360 eligible among these 6 schools have been pointed out in the ‘Study population’ of ‘Methods’ section (Page 5~6). The time gap (about 3 months) between physical examination (October 2011) and urine collection (January 2012) was pointed out in the ‘Measurement of urine BPA’ of ‘Methods’. (Page 7)

There were some possible variations of participant characteristics that impacted the BPA exposure-BMI associations. Urine output was estimated by age (older subjects were conferred by higher urine output) in this article. Body weight or BMI increases with age in children. Therefore, daily intakes might be expected to be larger in high body weight subjects. This may result in a false association of daily intake estimates with BMI. For solving this potential confounding, two variables related to body weight, age and sex, have been included in multiple linear analysis and analysis of variance as covariables. The different dietary patterns between normal weight and obese participants possibly influenced the associations. Because food is thought as the main exposure route of BPA in human population, it is possible that increased overall energy intake or different diet composition of obese individuals might increase the
risk of BPA exposure, compared to normal weight ones. This possible confounding was pointed out in the limitations of this article (in the ‘Paragraph 9’ of ‘Discussion’ section, page 14).

**Answer 2**: The cut-point values for the identification of normal weight, overweight, and obesity in school children 7-18 years of age proposed by Working Group on Obesity in China (WGOC) have been listed in supplementary information Table S1.

**Answer 3**: How to construct and calculate the 95% CI for geometric mean of urine BPA concentration and daily intake estimates has been explained in the ‘Paragraph 2’ of the ‘Statistical analyses’ section (Page 8). A mistake was made for the minimum urine BPA concentration, which should be 0.05 ng/mL (i.e. the LOD of 0.07 ng/mL divided by the square root of 2), and the minimum BPA concentration of 0.03 ng/mL has been corrected to 0.05 ng/mL in Table 2.

**Answer 4**: First, urine BPA concentrations corrected by specific gravity were categorized into the quartiles, and then mean difference analyses across the urine BPA concentration quartiles (quartile 1 was reference) were carried out in all subjects or stratified by age (8-11 and 12-15 year age groups) or sex after adjustment for age in years and sex (see the ‘Paragraph 5’ of the ‘Statistical analyses’ section, page 9). This analysis indicated the mean difference of BMI corresponding to quartiles 2, 3, and 4 of urine BPA concentrations with quartile 1. Because quartile 1 was reference and set to zero, no error bar was with it. The quartiles were represented by numerical values of 1, 2, 3, or 4 according to from low to high urine BPA concentration.

**Answer 5**: “shot time span (one month)” has been corrected to “short time span (one month)” in the ‘Paragraph 1’ of the ‘Discussion’ section. (Page 11)

**Discretionary Revisions**

**Answer 1**: In view that some studies also used the method that assigned a default
value of LOD divided by the square root of 2 for the measurements below the limit of detection [3, 4], we still used it here.

References: