Author’s response to reviews

Title: Prenatal and postnatal bisphenol A exposure and social impairment in 4-year-old children

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Author’s response to reviews:

Reviewer #1: Reviewer Comments

This well-written manuscript examines the association of prenatal and concurrent urinary BPA concentrations with 4-year old children's social behaviors in 304 mother-child pairs from Korea. The authors report null findings when examining linear associations between prenatal or concurrent BPA concentrations among all children, but report that: 1) concurrent BPA is associated with poorer social behaviors in girls, but not boys, 2) prenatal BPA is associated with poorer social behaviors at concentrations >3.0 ug/g, and 3) associations between BPA > 3 ug/g and social behaviors appear to be stronger in girls than boys. The strengths of this study include the prospective design, pre- and postnatal BPA measurements, and reasonable sample size. The major limitations include examination of individual-item level data from the K-SCQ, over-interpretation of the non-linear associations, no formal testing of whether these non-linear effects are significant, and absence of specific methodological details.

Major Comments

1. It is not typical to examine individual level data from psychometric tests like the K-SCQ (Table 2). Conceptually, these individual items were chosen for the K-SCQ because as a whole they represent the construct of social behaviors; however, when examined by
themselves they do not. Pragmatically, I believe that their inclusion detracts from what could be a succinct manuscript. I strongly recommend dropping these individual items and focusing on the three summary scales.

=> Thank you for your insightful comment on individual items. As the reviewer suggested, we dropped the individual item analysis.

2. Did the authors formally test the non-linear association between prenatal BPA and social behaviors? Based on the presented results, I do not believe that there is sufficient evidence to declare a non-linear association. It appears that I could fit a straight line within the 95% CI of the smoothed regression line (Figure 1a) with a great deal of imprecision at the lowest and highest concentrations relative to the median BPA concentration. In addition, there appears to be a positive association between prenatal BPA and SCQ scores at the lowest levels of BPA. What do the authors make of this?

=> As the reviewer commented, Figure 1a is not sufficient to declare a non-linear association. Therefore, in addition to visualization of the relationship between BPA and total SCQ, we compared AIC and deviance values of linear and spline models. Description for the comparison was added in the Methods and Results.

=> In the Methods,

“Generalized additive models (GAMs) were constructed to investigate the relationship between prenatal and postnatal BPA concentrations and social impairments at age 4. BPA has non-monotonic effects [42, 44]; therefore, we constructed 2 models, including 1 with a linear BPA term and another spline model for BPA exposure (4 degrees of freedom). When the shape of the association in GAMs looked nonlinear, we compared the AIC of linear and spline models to select a better-fit model for a given set of data. We also calculated the difference of deviances for the fitted models and tested statistical significance of the difference, which followed a chi-square distribution.”

=> In the Results,

“Figures 1(a) and 1(b) show the associations between prenatal and postnatal BPA and K-SCQ scores, respectively. The regression curve deviates from a straight line and has a flexion point at around 3.0 μg/g creatinine of prenatal BPA at the mid-term of pregnancy (Figure 1(a)). AIC in the spline model with 4 degrees of freedom was slightly smaller compared to the linear model (1495 for the spline model vs. 1497 for the linear model), suggesting that the spline model is a better fit to assess prenatal BPA and social impairments. The difference in deviance was statistically significant (P = 0.0284). Furthermore, 18% of mothers (55 of 304) had values above this level of 3.0 μg/g creatinine, which is 1.5 times greater than the mean prenatal BPA
concentration in our sample. The maximum BPA level was 13.0 μg/g creatinine. The assumption of linearity was met for the association between social impairments and postnatal BPA levels at 4 years of age (Figure 1(b)). AIC values were 1498 for the spline model with 4 degrees of freedom and 1495 for the linear model, suggesting that the linear model is a better fit for our data regarding the association between postnatal BPA and social impairment. Difference of deviance was not statistically significant (P = 0.2391).”

3. If the authors can verify that the association is truly non-linear, then some caveats around the interpretation on pages 12 and 13 is necessary. The authors should discuss that the range of BPA exposures in this study was quite narrow and this makes it surprising that there is a non-linear association given the narrow range of exposure. If anything, Moreover, the exposures experienced by these mothers and children was not likely to overlap with the doses typically employed in animal neurotoxicity studies. Specifically, low-level human exposure is estimated to be on the range of <1 ug/kg/d (see work by LaKind, Stacy, and others). Animal studies typically employ doses of 5 to 50 ug/kg/d.

=> Thank you for your helpful comment. As the reviewer suggested, we explained more about the non-linear relationship citing references which also showed non-linear relationship in the low-level human exposure as follows:

“While toxicology studies frequently assume the presence of linear relationships, this assumption may not be valid for receptor-mediated mechanisms [54]. We found that the linear association between social impairment and prenatal BPA was not statistically significant at the α = 0.05 level. However, considering the flexion point in the non-linear relationship, total K-SCQ was strongly associated with above-threshold prenatal BPA concentrations (16.9% [95% CI, 2.3% to 33.5%]). This association was particularly strong for social communication in girls (58.4% [95% CI, 6.5% to 135.8%]). The range of BPA exposures in this study was quite narrow compared to the doses typically employed in animal neurotoxicity studies [55, 56]. Therefore, we cannot conclude that the non-linear relationship indicated by our study is consistent with non-linear relationships in animal neurotoxicity studies. However, the non-linear relationship is similar to the findings of other human observational studies with low-level exposure to BPA, which are comparable to the exposure level in the present study [25, 42].”

4. If the authors do indeed verify a non-linear association, I request that they formally test the difference in association between boys and girls.

=> As the reviewer suggested, we tested the difference in associations between boys and girls as follows:
“The magnitude of the increase in social communication scores associated with prenatal BPA concentrations at or above the threshold (3.0 μg/g creatinine of BPA) was greater in girls than boys (4.7% [95% CI, -22.4% to 41.3%] for boys vs. 58.4% [95% CI, 6.5% to 135.8%] for girls) (Table 4). This difference was marginally significant (P-value < 0.1) (Supplementary Material, Figure S2). Social interaction and behavior patterns were not associated with prenatal or postnatal BPA concentrations in the total sample or for boys and girls separately.”

5. The authors need to acknowledge that there is the potential for reverse causality of the postnatal BPA-SCQ association. For instance, children with more behavior problems may have different dietary or mouthing behaviors that increase their BPA exposure.

=> As the reviewer suggested, we added a statement regarding reverse causality as follows in the Discussion:

“Finally, we did not investigate the potential for reverse causality of the association between postnatal BPA and social impairments. Children with more behavior problems may have different dietary or mouthing behavior [61, 62] that may increase their BPA exposure.”

Minor Comments


=> As the reviewer suggested, we included the work of Braun et al. as a reference in the following sentence: “However, cohort studies examining prenatal exposure to BPA have not found significant associations with social impairments in children 4-9 years of age [19, 20].”

7. Introduction, line 9: The detection of BPA in serum does not necessarily indicate exposure and could be indicative of contamination (see the work of Calafat).

=> As the reviewer suggested, we corrected the sentence, “In particular, BPA has been detected in maternal serum, placenta, and umbilical cord serum collected during pregnancy or at delivery [5-8].” to “In particular, BPA has been detected in the urine of pregnant women and children [5-11].”

8. Introduction, Line 34: Please refer to the official diagnosis of this disorder: autism spectrum disorders.
=> As the reviewer suggested, we corrected ‘autistic-like trait disorders’ to ‘autism spectrum disorders’.

9. Introduction: Line 36: The European Union did not conduct this study, but it was an extrapolation of the effect and cost of EDCs on neurodevelopmental disorders in the European Union.

=> As the reviewer suggested, we corrected the sentence as follows:

“The European Union has expressed concern regarding data [21] indicating that exposure to endocrine disruptors may contribute to neurobehavioral deficits and disease, which cost more than €150 billion per year in Europe. In an effort to better control endocrine-disrupting chemicals, the European Food Safety Authority reduced the safety level of BPA from a combination of sources (e.g., diet, dust, cosmetics, and thermal paper) from 50 µg/kg of body weight/day to 4 µg/kg in 2015 [22].”

10. Methods, page 6: Please clarify why only 2,085 children were selected for follow. Moreover, the high rate of loss to follow-up is troubling (~70%) and should be formally evaluated and discussed. Specifically, could you please compare the baseline characteristics of those not followed-up with those who were?

=> As the reviewer suggested, we clarified the sampling strategies as follows:

“The present study, the Environment and Development of Children (EDC) Study, is a prospective cohort study of the growth and development of children. The participants are children whose mothers participated in another study of birth outcomes, the Congenital Anomaly Study (CAS). The CAS cohort consisted of pregnant women who received prenatal care at 1 of 8 hospitals in the metropolitan areas of Seoul and Incheon, the Republic of Korea. The study enrolled 13,484 women during the second trimester of pregnancy and 11,085 of these women remained in the study until they gave birth between August 2008 and July 2011. At the time of enrollment, blood and urine samples were collected after more than 8 hours of fasting and a questionnaire regarding demographics and lifestyle was administered by trained nurses. The CAS cohort included 115 children with congenital anomalies. After excluding mothers having children with congenital anomalies (n = 115) and those with invalid addresses (n = 218), 10,752 mothers were target participants for a new birth cohort comprising the EDC study (Supplementary Material, Figure S1(a)).

We determined that a sample size of 610 (effect size 0.017 [33], alpha 0.05, power 0.90) would sufficiently examine the association between BPA exposure and children’s growth variables such as body mass index (BMI); we inflated the sample size to 645 children in order to allow for an
~5% drop-out rate. Between 2012 and 2015, we contacted 2,085 mothers chosen randomly from the 10,752 target participants, until enrolling 645 mother-child pairs (615 mothers, including 30 multiple births) in the EDC study (response rate, 31%). We conducted follow-ups when the children were approximately 4 years of age, between March 2013 and December 2015 (Supplementary Material, Figure S1(b)). The children underwent health examinations at the Seoul National University Hospital located in Jongno-gu, Seoul, Republic of Korea. The mothers’ depressive symptoms and children’s dietary habits were assessed using the Center for Epidemiologic Studies Depression (CES-D) [34] and food frequency questionnaires (FFQs) [35], respectively.”

=> In the results, we compared the baseline characteristics of those not followed-up with those who were as follows:

“Characteristics of mothers (n = 615) included in the present EDC study were different from excluded mothers (n = 10,137) in the CAS cohort; differences in the EDC cohort included that the mothers were older (31.2 years vs. 30.6 years for included and excluded mothers, respectively), children were born at an earlier gestational age (39.2 weeks vs. 39.3 weeks), there were more twin or triplets (3.9% vs. 1.7%), and there were more current or past smokers (45.6 vs. 41.3) (Supplementary Material, Table S1). Characteristics including maternal age, prenatal BPA levels, and K-SCQ scores of the children in the present study (N = 304) were similar to those of the excluded children (N = 341). However, the included children were slightly younger (47.7 vs. 48.0 months; P = 0.0270) and had lower creatinine-adjusted BPA levels at 4 years of age (4.9 vs. 5.7 μg/g creatinine; P = 0.0006) compared to the excluded children (Supplementary Material, Table S1).”

6. Methods, page 7: Was free and conjugated BPA measured? If so, how was the deconjugation done?

=> To answer the reviewer’s question, we modified the Methods as follows:

“We measured the total concentrations (free and conjugated species) of urinary BPA. Urine samples were treated with β-glucuronidase/sulfatase to hydrolyze conjugated BPA species [13].”

7. Methods, page 8: What log base was used? Was the creatinine-standardized BPA concentration log-transformed?

=> Yes, the creatinine-standardized BPA concentration was natural log-transformed. ‘Natural’ was added in the sentence.
8. Methods, covariates: How were these covariates selected? Did the authors have data on maternal smoking during pregnancy? If so, I suggest adjusting for it.

=> As the reviewer suggested, we clarified how to select covariates and compared models with or without covariates including maternal smoking that the reviewer suggested.

=> In the Methods,

“Potential covariates for inclusion in the statistical models were selected a priori, following a literature review [12, 13, 42]. Prenatal information was obtained using questionnaires at the time of recruitment; variables of interest included maternal age (years), gestational age (weeks), smoking (yes or no), drinking alcohol during pregnancy (yes or no), educational attainment (≤ or > than high school), parity (first vs. second or later child), and CES-D scores (0–60 points). Children’s characteristics such as age (months), gender, BMI (kg/m2), birth weight (kg), childcare (home, daycare, or other), exposure to second hand tobacco smoke (yes or no), and infant feeding type (breast feeding, bottle feeding, or mixed) were obtained at the follow-up visit. From various dietary habits queried by the FFQs, we selected those that were likely to be associated with BPA levels (P-value < 0.1), including canned food or drinks (< or ≥ 1 per week), instant rice (< or ≥ 1 per week), and use of plastic dishes in the microwave oven (yes or no).

Covariates were first determined by searching for variables that reduced the Akaike information criterion (AIC) [43] in the model by >10%, compared to the base model (prenatal and postnatal BPAs were independent variables in the base model). Second, we selected variables that were significantly associated with total SCQ scores (P-value < 0.05) after controlling for other covariates. In the final model, covariates included gender, parity, maternal education, birth weight, and use of plastic dishes in the microwave oven. We also controlled for prenatal and postnatal levels of urinary BPA.”

=> In a sensitivity analysis,

“Finally, we compared percentage changes in SCQ total scores associated with prenatal and postnatal BPA, with or without adjusting for covariates that were excluded in the final model; the covariates included maternal age, gestational age, smoking during pregnancy, drinking alcohol during pregnancy, mother’s depression, child’s age, infant feeding type, second hand smoke, place of childcare, canned food or drink, and instant rice.”

=> In the Result:

“Additional adjustment for covariates did not change the main findings (Supplementary Material, Table S4).”
9. Conclusion: I think the policy level statement should be dropped and a summary of the strengths and limitations should be provided.

=> As the reviewer suggested, we dropped the policy statement and included statements regarding strengths and limitations as follows:

“The prospective cohort study design is a strength of this study investigating the relationships between prenatal and postnatal BPA concentrations and social impairments at 4 years of age. Although the study has several limitations, including parent-reported questionnaires to evaluate social impairments and no participants with severe social impairments, the study makes a significant contribution to research on endocrine disruptors' impact on children health because the relationship between BPA exposure and neurodevelopmental effects has not been fully elucidated in humans, and our results elucidate BPA exposure effects related to social impairments. Specifically, prenatal BPA exposure was significantly associated with impairments at or above the flexion point of 3.0 μg/g creatinine, whereas there was a linear association for postnatal BPA exposure. Further studies to evaluate the health implications and underlying mechanisms of these findings are warranted.”

Reviewer #2: The paper by Lim et al. investigates possible effects of maternal exposure to the estrogenic chemical bisphenol A, on neurobehavioral development of children at 4 years of age, by evaluation of social functions through psychometric methods. The paper is of high interest in the light of the emerging evidence of long term, sex-specific, neurobehavioral effects of developmental exposure to BPA in animal models. Albeit the intrinsic limitations of correlational studies, that should be clearly outlined in the discussion, the study is well designed and carefully conducted and may contribute to the current research on endocrine disruptors' impact on children health. For this reviewer, however, a problem is the difficulty to understand the data analysis and the consequent discussion of results.

The authors should add some more explanation on data analysis and on the rationale of linear regression (see also below).

As the authors themselves recognize, an additional limitation is the lack of a direct, observational assessment of neurobehavioral functions. However, the present study offers interesting and promising inputs for future research.

I have some additional issues that I think should be addressed in the paper:
1. Breast feeding vs bottle feeding (BPA releasing bottles??) vs soy-based milk were not mentioned as factors considered in the analysis. This is a potentially highly relevant exposure factor that need to be addressed.

=> As the reviewer suggested, we included breast vs. bottle feeding in the analysis. However, we did not obtain information on soy-based milk. Although feeding type was not significantly associated with social impairment in this study, therefore we did not include it in the main analysis. However, we conducted an additional analysis to examine a possible confounding effect of feeding type on the association between prenatal or postnatal BPAs and social impairment.

- In the Methods,

“Potential covariates for inclusion in the statistical models were selected a priori, following a literature review [12, 13, 42]. Prenatal information was obtained using questionnaires at the time of recruitment; variables of interest included maternal age (years), gestational age (weeks), smoking (yes or no), drinking alcohol during pregnancy (yes or no), educational attainment (≤ or > than high school), parity (first vs. second or later child), and CES-D scores (0–60 points). Children’s characteristics such as age (months), gender, BMI (kg/m2), birth weight (kg), childcare (home, daycare, or other), exposure to second hand tobacco smoke (yes or no), and infant feeding type (breast feeding, bottle feeding, or mixed) were obtained at the follow-up visit. From various dietary habits queried by the FFQs, we selected those that were likely to be associated with BPA levels (P-value < 0.1), including canned food or drinks (< or ≥ 1 per week), instant rice (< or ≥ 1 per week), and use of plastic dishes in the microwave oven (yes or no).

Covariates were first determined by searching for variables that reduced the Akaike information criterion (AIC) [43] in the model by >10%, compared to the base model (prenatal and postnatal BPAs were independent variables in the base model). Second, we selected variables that were significantly associated with total SCQ scores (P-value < 0.05) after controlling for other covariates. In the final model, covariates included gender, parity, maternal education, birth weight, and use of plastic dishes in the microwave oven. We also controlled for prenatal and postnatal levels of urinary BPA.”

=> In a sensitivity analysis,

“Finally, we compared percentage changes in SCQ total scores associated with prenatal and postnatal BPA, with or without adjusting for covariates that were excluded in the final model; the covariates included maternal age, gestational age, smoking during pregnancy, drinking alcohol during pregnancy, mother’s depression, child’s age, infant feeding type, second hand smoke, place of childcare, canned food or drink, and instant rice.”
In the Result:

“Additional adjustment for covariates did not change the main findings (Supplementary Material, Table S4).”

2. Did the authors consider whether children were in day-care or home - maternal, relatives, baby-sitter's care - as an environmental factor potentially affecting social behavior?

=> As the reviewer suggested, we included place of childcare in the analysis. Although place of childcare was not significantly associated with social impairment, therefore we did not include it in the main analysis. However, we conducted an additional analysis to examine a possible confounding effect of place of childcare on the association between prenatal or postnatal BPAs and social impairment.

=> Please see the response to comment #1.

3. Nothing is mentioned about possible sources of BPA - do you have any information about mothers' and/or children's dietary habits, for instance?

=> As the reviewer suggested, we included dietary habits (e.g. canned food or drink, instant rice, use of plastic dishes for microwave) in the analysis. Because dietary habits except use of plastic dishes for microwave were not significantly associated with social impairment, therefore we did not include them in the main analysis. Instead, we conducted an additional analysis to examine possible confounding effect of dietary habits on the association between prenatal or postnatal BPAs and social impairment.

=> Please see the response to comment #1.

=> In addition, we stated how the dietary habits were measured as follows:

“The mothers’ depressive symptoms and children’s dietary habits were assessed using the Center for Epidemiologic Studies Depression (CES-D) [34] and food frequency questionnaires (FFQs) [35], respectively.”

4. Personality traits have high hereditability - was this controlled for by evaluating parents' social functions?

=> We did not specifically evaluate parent’s social functions in the study. However, we collected information of mothers’ depressive symptoms. Although the score on mothers’ depressive symptoms was not significantly associated with social impairment, therefore we did not include
it in the main analysis. Instead, we conducted an additional analysis to examine confounding effect of mothers’ depressive symptoms on the association between prenatal or postnatal BPAs and social impairment.

=> Please see the response to comment #1.

=> In addition, we stated how mother’s depression was measured as follows:

“The mothers’ depressive symptoms and children’s dietary habits were assessed using the Center for Epidemiologic Studies Depression (CES-D) [34] and food frequency questionnaires (FFQs) [35], respectively.”

5. Do the authors look for any correlation between pre- and postnatal BPA level?

=> As the reviewer suggested, we included the result of correlation as follows:

“Creatinine adjusted prenatal and postnatal BPA concentrations were not significantly correlated (Pearson’s correlation = 0.01136).”

6. Body weight could be an additional target of prenatal, sex-biased BPA effects. Was an association between maternal BPA and bw at birth and at 4yrs of age analysed?

=> As the reviewer suggested, we included birth weight in the analysis. Weight at 4 years of age has been already used to compute BMI in the previous version of manuscript. We conducted an additional analysis to examine a confounding effect of children’s’ BMI on the association between prenatal or postnatal BPAs and social impairment.

=> Please see the response to comment #1.

exposure to bisphenol A. Proc Natl Acad Sci U S A. 2011; 108:11715-11720) and Palanza et al 2016 for a recent review (already in the ref list)

=> As the reviewer suggested, we modified the sentence by including the references:

“In addition, sex- and time-specific effects on neurodevelopment or behavioral outcomes following BPA exposure have been reported in animal studies [28-32]; however, the traits that are most sensitive to BPA in humans have not been fully elucidated [12, 14-16].”

SPECIFIC COMMENTS

8. pag. 4, 2nd period:

It is not correct to say that EU has suggested that EDCs do have contributed to children neurobehavioral deficit. A more conservative approach is suggested here, such as "The European Union has EXPRESSED CONCERN on DATA INDICATING that exposure to endocrine disruptors MAY HAVE contributed to neurobehavioral deficits and disease, costing more than €150 billion per year in Europe [17]."

=> As the reviewer suggested, we corrected the sentence as follows:

“The European Union has expressed concern regarding data [21] indicating that exposure to endocrine disruptors may contribute to neurobehavioral deficits and disease, which cost more than €150 billion per year in Europe. In an effort to better control endocrine-disrupting chemicals, the European Food Safety Authority reduced the safety level of BPA from a combination of sources (e.g., diet, dust, cosmetics, and thermal paper) from 50 µg/kg of body weight/day to 4 µg/kg in 2015 [22].”


=> As the reviewer suggested, we quoted the references.
The sentence: "We also estimated prenatal and postnatal BPA effects at concentrations less than, or equal to or greater than, threshold BPA concentrations using piecewise linear regression models and the thresht function in the HEAT package." is unclear to me. Please explain the rationale for estimating BPA concentrations for acclared non monotonic effects of BPA.

=> As the reviewer suggested, we modified the paragraph as follows:

“Generalized additive models (GAMs) were constructed to investigate the relationship between prenatal and postnatal BPA concentrations and social impairments at age 4. BPA has non-monotonic effects [42, 44]; therefore, we constructed 2 models, including 1 with a linear BPA term and another spline model for BPA exposure (4 degrees of freedom). When the shape of the association in GAMs looked nonlinear, we compared the AIC of linear and spline models to select a better-fit model for a given set of data. We also calculated the difference of deviances for the fitted models and tested statistical significance of the difference, which followed a chi-square distribution.

After visualizing the relationship between BPA exposure and social impairments, we estimated the contributions of BPA to the linear and piecewise linear regression models. First, to estimate the overall linear effects of BPA on social impairments, we constructed regression models for BPA exposure and social impairments. Second, we estimated prenatal and postnatal BPA effects at concentrations that were either less than, or equal to/ greater than, threshold BPA concentrations, using piecewise linear regression models and the thresht function in the HEAT package [45] of R software (R Development Core Team, https://cran.r-project.org/). Piecewise linear regression analysis has been used to determine flexion points in non-linear relationships [42, 46, 47] using AIC as a measure of the relative quality of a statistical model for a given set of data. We modeled scores on the K-SCQ as a Poisson distribution and estimated the effects of BPA on the total and subcategory scores for social impairments (social interaction, social communication, and behavior patterns). All models were controlled for gender, parity, maternal education, birth weight, use of plastic dishes in the microwave oven, and prenatal or postnatal levels of urinary BPA. To examine gender differences in our analyses, we stratified our samples by children’s gender.”