

Author's response to reviews

Title: A cohort study of the association between secondary sex ratio and parental exposure to polybrominated biphenyl (PBB) and polychlorinated biphenyl (PCB)

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Dear Editors and Reviewers:

I am pleased to submit our revised manuscript “A cohort study of the association between secondary sex ratio and parental exposure to polybrominated biphenyl (PBB) and polychlorinated biphenyl (PCB)” by Metrecia L. Terrell, Alissa K. Berzen, Chanley M. Small, Lorraine L. Cameron, Julie J. Wirth, and Michele Marcus. We would like to thank the Reviewers for their comments, which have added to the depth of this manuscript. We have incorporated the Reviewers’ comments into this final and complete version of the manuscript. Our specific responses to the Reviewers’ comments are as follows:

Editor’s Comments:

Reviewer 1: No revisions (major, minor or discretionary)

Reviewer 2:

Major compulsory revisions

1) Tables 2 & 3

a. The number of fathers in the lowest paternal exposure group seems too small to maintain that as a separate category. Instead I think it would be preferable to divide the fathers in half at the median.

As the reviewer suggested we re-examined our results dividing exposure at the median for fathers and for mothers as well as continuously (see your Comment #9). We found that the odds of a male birth were consistent with results presented in the adjusted models in Table 2 although they were somewhat attenuated (Model 1 becomes: maternal PBB ≥ 3 $\mu\text{g/L}$: AOR=0.93, 95% CI: 0.72–1.20; referent group: maternal PBB < 3 $\mu\text{g/L}$); Model 2 becomes: paternal PBB ≥ 5 $\mu\text{g/L}$: AOR=1.12, 95% CI: 0.72–1.74; referent group: paternal PBB < 5 $\mu\text{g/L}$; Model 3 becomes: maternal PBB ≥ 3 $\mu\text{g/L}$ and paternal PBB ≥ 5 $\mu\text{g/L}$: AOR=1.35, 95% CI: 0.84, 2.15; referent group: maternal PBB < 3 $\mu\text{g/L}$ and paternal PBB < 5 $\mu\text{g/L}$). While we agree that the number of fathers in the lowest paternal exposure group is small, we do not think it is appropriate to combine fathers with no detectable PBB exposure with fathers who have PBB levels up to 5 $\mu\text{g/L}$. The fathers in this cohort generally have higher PBB concentrations than the mothers so that separation of exposure levels is both more important and more difficult because of small numbers of unexposed. Therefore, we have retained the three categories of exposure in Tables 2–4 but have reported results of ancillary analyses in the text (page 15).

b. The numbers suggest to me, not an increase in SSR with increasing paternal exposure (seen marginally), but a possible decrease in SSR that is confounded by maternal levels. It does not seem that models were run that simultaneously adjust for maternal and paternal exposure levels (among those children where both parents' levels are known). (An interaction between them could be tested, but it doesn't look to me like it's there.)

Because maternal and paternal exposures were correlated ($r_s=0.64$, $p<0.001$), in the paper we did not include the analyses where they were modeled simultaneously. However, for

illustrative purposes, when both maternal and paternal PBB were in the model at the same time (not as an interaction), the results were consistent with Table 2 adjusted Model 1 (maternal PBB >1–<4 µg/L: AOR=1.14, 95% CI: 0.68, 1.91; maternal PBB ≥4 µg/L: AOR=1.47, 95% CI: 0.82–2.65; referent group: maternal PBB ≤1 µg/L) and Model 2 (paternal PBB >1–<6 µg/L: AOR=1.28, 95% CI: 0.56, 2.91; paternal PBB ≥6 µg/L: AOR=1.38, 95% CI: 0.54–3.55; referent group paternal PBB ≤1 µg/L). We attempted to run an interaction term between maternal and paternal PBB exposure (9 possible categories), but this resulted in unstable models because of small numbers in some of the cells. Thus, for combined maternal and paternal exposure we considered only where both mothers and fathers had low exposure compared to where both mothers and fathers had high exposure. Further, the results for paternal exposure do not suggest a decrease in the odds of male birth in any of the performed analyses for PBB exposure.

c. These same comments apply to the PCB analyses.

Same reasons as above for PBB, although PCB concentrations were not as correlated ($r_s=0.19$, $p=0.002$). The results were similar to Table 4 adjusted Model 1 (maternal PCB >5–<8 µg/L: AOR=1.08, 95% CI: 0.74–1.58; maternal PCB ≥8 µg/L: AOR=1.46, 95% CI: 0.86–2.48; referent group: maternal PCB ≤5 µg/L) and Model 2 (paternal PCB >5–<9 µg/L: AOR=0.72, 95% CI: 0.49, 1.05; paternal PCB ≥9 µg/L: OR=0.88, 95% CI: 0.55–1.42; referent group: paternal PCB ≤5 µg/L).

2) Better description of the cohort. Items that are mentioned without any detail

include:

a. How many individual fathers and mothers were there? Overall and in the different subsets that go into the different analyses?

We have added the number of individual fathers and mothers to the Results section (pages 11–16). In summary, all offspring included in the study had a mother in the cohort, but not necessarily a father in the cohort. After exclusions our final sample included 479 cohort mothers (for n=865 offspring). There were 171 pairs of mothers and fathers where both were in the cohort and had a PBB measurement (for n=300 offspring). There were 144 pairs of mothers and fathers where both were in the cohort and had a PCB measurement (for n=253 offspring).

b. What is the distribution of timing between births and blood collection?

The blood collections were mostly done during 1976–1979 when parents were enrolled in the cohort. Their offspring included in the present study were born during 1975–1988. So, there are varied years from the offspring's birth and the parents' blood collection. For the mothers, samples were collected from 3 years before the offspring was born to 12 years after the offspring's birth (average=4 years). For the fathers, samples were collected from 3 years before the offspring's birth up to 11 years after the offspring's birth (average=1 year). We have added this detail to the Exposure Assessment section (bottom of page 8). Since the estimated half-life of PBB is approximately 13.5 years [1], the measurements taken at enrollment are likely to accurately represent the ranking of the parents' serum levels around the time of conception with a small amount of random error. Using a PBB decay model to estimate parents' serum levels around the time of conception yielded similar results.

c. PBB/PCB adjustments are made based on models that include information on breastfeeding, but how/when was this breastfeeding information collected? What is its distribution.

Estimated maternal PBB at the conception date of the offspring was estimated using a previously developed decay model described in Terrell et al. [2]. The breastfeeding variable was a time-dependent covariate included in a mixed-effects model with other factors. We found that breastfeeding was associated with a faster decay of serum PBB levels. We ascertained breastfeeding information for each woman's live births (whether or not they breastfed and how long they breastfed) from telephone interviews. Of the 479 women in our study, 347 women reported breastfeeding histories for their offspring and approximately 65% of these women breastfed at least one of their offspring. We have added sentences to the Exposure Assessment section (pages 8–9) that further explains how the decay model was implemented for use in this study. For the estimated maternal PBB, we calculated a decay estimate (λ) using the parameters specified in the decay model described in Terrell et al. [2] which includes the mother's age at exposure to PBB, body mass index (BMI), smoking history, parity, and breast-feeding history. We then calculated the estimated PBB based on the formula [estimated PBB=enrollment PBB x $\exp(\lambda t)$], where (t) is the time between the offspring's conception date and the date when the mother's serum sample was collected.

d. Need to be clearer in tables what the n's refer to (children I assume).

Yes, all reference to N's in the tables refers to the offspring (not the parents). We have added the word "offspring" to the Tables 1–4 in reference to N.

e. In the results it's stated that 116 children had no maternal PBB measurement and were excluded. Is it just coincidence that also 116 children are reported in the discussion (this should come earlier, at least in results section) to have been born before the mother's blood was drawn for PBB analysis?

Yes, we have verified that these numbers were coincidentally the same with 116 children excluded due to no maternal PBB measurement and 116 children born before the mother's blood was drawn.

3) It seems that even in models with only maternal exposures, only children born 1975-1988 are included. Why not all 1,392 born 1975-2005?

There were no cohort fathers identified for births from 1989–2005. Since the sex of the offspring is determined primarily by the father (except for selective survival *in utero*), we have restricted the study sample of offspring to the earlier births from 1975–1988. We have revised the Methods section (page 7) and the Results section (Population Characteristics pages 11–12) to reflect this change in sample size.

4) It seems odd that father's PBB levels are not extrapolated to the time of birth of the children while the mothers' levels are. While there may not be an exact model, the PBB exposure was close to a point source and presumably at the very least a simple time decay should be considered. If not, I think there should be stronger justification for why not.

Because the offspring could have been born many years before or after parents had their enrollment PBB measurements collected (refer to your Comment #2b), we agree that an estimated paternal exposure was needed as we had done for maternal exposure. Using a similar methodology as that described in Terrell et al. [2], we developed a mixed-effects decay model for the males in the cohort that adjusted for their age at exposure and BMI at enrollment. We used the estimates from this model to calculate the estimated PBB at conception date of the offspring for each father in this study. We have detailed the calculation of the estimated PBB in the Exposure Assessment section (page 9) and have included a new table that has the odds ratios for a male birth by estimated maternal PBB exposure and estimated paternal PBB exposure (Table 3).

Minor essential revisions

5) What was the covariance structure of the GEE model?

We used an exchangeable covariance structure, as we assumed that the correlation would be the same between any two offspring births from the same mother (or father). The covariance structure has been added to the Statistical data analysis section (page 11).

6) What was correlation between PCBs and PBBs?

PBB and PCBs had a weak positive correlation. For the 434 mothers that had both a PBB and a PCB measurement, the Spearman correlation coefficient was 0.14 ($p=0.004$). There were 162 fathers with both a PBB and a PCB measurement and the Spearman correlation coefficient was 0.13 ($p=0.10$). This has been added to the Results section (under PBB and PCB concentrations, page 12).

7) *In 2nd paragraph of “Association with sex ratio” in results, it should be made clear for the reported OR what the reference group was and what the overall categories were.*

As suggested, we have clarified the reference group for each of the ORs given (pages 13–16).

Discretionary revisions

8) *Last sentence of page 11: I don’t see how a continuous PCB exposure necessarily means that it will be lower than a one-year PBB exposure. Isn’t the relevant point how high that PBB exposure was?*

Yes we agree that the relevant point is that the PBB exposure was high for parents in this study. We have removed this sentence from the Results section.

9) *Despite the skewing in the contaminant levels, it would be of interest to know what happens if they are modeled continuously (possibly log transformed).*

We have modeled the exposure variables as continuous variables. This has been added to the Methods section (page 10) and to the Results section (pages 14–15). We did not find any significant association with continuous log-transformed PBB or continuous log-transformed PCB exposure and the odds of a male birth. For the adjusted maternal PBB only model the OR=1.00 (95% CI: 0.91, 1.10). For the paternal PBB only model, there was a 16% increase in the odds of being a male birth for a 10 µg/L increase in the natural log of paternal PBB concentration (AOR=1.16, 95% CI: 0.78, 1.72). In the combined maternal and paternal PBB model, there was a 7% increase in the odds of being a male birth (AOR=1.07, 95% CI: 0.96, 1.19) for a 10 µg/L increase in the natural log of

maternal and paternal PBB concentrations. The odds of a male birth for a 10 µg/L increase in the natural log of serum PCB concentrations were as follows: in the maternal PCB only model, a 28% increase (AOR=1.28, 95% CI: 0.78, 2.09); in the paternal PCB only model, a 37% increase (AOR=1.37, 95% CI: 0.58, 3.25); and in the combined maternal and paternal PCB model, a 14% increase (AOR=1.14, 95% CI: 0.84, 1.54). However, modeling the exposures continuously assumes a linear relationship which may not be a fair depiction of the relationship between PBB or PCB and sex ratio, as seen in our data when exposure variables were modeled categorically.

10) I'm still a little unclear on how the breastfeeding data was handled, but since it and childbirth in general might introduce changes in PBB/PCB levels that are hard to quantify, it would be interesting to run sensitivity analyses restricted to the first child after a mother's PBB/PCB concentrations were determined.

Please refer to your Comment #2c where we have provided additional information about the breastfeeding variable in the decay model. We agree that changes in PBB/PCB levels may be affected by childbirth or breastfeeding. In fact, we did find such an effect in our decay model (see Terrell et al. [2]). We ran additional models including only the first child after the mother's PBB exposure or PCB exposure measurement was collected (Results section, page 16). For the maternal enrollment PBB model (n= 407 offspring), the adjusted odds ratios were comparable to Table 2, Model 1 (maternal PBB >1– 4 µg/L: AOR=1.39, 95% CI: 0.86–2.26; maternal PBB ≥ 4 µg/L: AOR=0.98, 95% CI: 0.61–1.57; referent group: maternal PBB ≤ 1 µg/L). Likewise, for the maternal enrollment PCB model (n=346 offspring), the odds ratios were larger but with imprecise confidence

intervals compared to the results in Table 4, Model 1 (maternal PCB >5– 8 µg/L: AOR=1.18, 95% CI: 0.68–2.02; maternal PCB ≥ 8 µg/L: AOR=1.67, 95% CI: 0.94–2.96; referent group: maternal PCB ≤ 5 µg/L).

Reviewer 3:

Major Compulsory Revisions

1. There is only one brief mention of possible biological effect (suspected endocrine disruptor). The authors do not paint a compelling picture of why it might be important to evaluate PBBs/PCBs' effect on sex ratio. In addition, the Authors do not seem to have a clear biologic hypothesis regarding PBB exposure and sex ratio. For example, on page 11 at the end of the section on Population Characteristics the Authors state that “the overall proportion male among these offspring with potential in utero PBB exposure was 0.542...”. How would in utero PBB exposure affect sex ratio?

Environmental exposures have been suggested as one possible factor in altering the sex ratio. To our knowledge, our study is the first to report on the sex ratio of offspring who were born to parents exposed to PBB. Thus, a priori it was unknown how parents exposed to PBB would influence their offspring sex at birth. However, PBBs belong to a class of chemicals that has been associated with decreases and increases in the sex ratio of offspring born to highly exposed parents in other populations [3-7]. Because of these equivocal results, we began our study with a general hypothesis that parents' exposure to PBB may alter the odds of their offspring being a male birth in highly exposed parents compared to low and unexposed parents. We have highlighted the importance of evaluating PBB/PCBs effect on sex ratio in the Background section (page 6). We have

also added a paragraph to the discussion section on the possible biological effects by which PBB/PCBs may influence sex ratio (pages 17–18). The possibilities we highlight are that on the paternal side, whether exposure to these types of chemical causes the preferential survival of Y sperm over X sperm has been suggested in the literature, although the findings are inconsistent [8, 9]. On the maternal side, whether *in utero* PBB/PCB exposure causes an increase in early loss of XX embryos or differential survival of female fetuses is unknown.

2. The Authors need to be more explicit about their statistical analytic methods. For example, there is no mention of what link function was used in the GEE analysis or what type of regression was performed. Logistic regression and odds ratio may not be appropriate due to the high proportion of male births (i.e., a high prevalence of the outcome).

We have expanded the Statistical data analysis section of the Methods (page 11) to include that we used logistic regression analyses to model the odds of a male birth and that the link function was logit. We recognize the Reviewer’s concerns regarding the appropriateness of using logistic regression and odds ratios due to the high proportion of male births. While the interpretation of an odds ratio as a risk ratio for common outcomes remains debated in the literature [10, 11], we have not made such an interpretation. We were interested in modeling the “odds” of a male birth, not the “risk” of a male birth. There are no instances in the manuscript where we have so interpreted the odds ratio as a risk ratio.

3. The discussion regarding the time period of included study participants is confusing. In the Methods, it sounded like the final sample for this analysis was restricted to births occurring between 1975 and 1988; however, the Authors give results for a final sample size of 1,392 offspring born between 1975-2005. Which is it? It seems like the sample should be restricted to the earlier births. Is the overall sex ratio given (0.542) for the 1,392 or the 865 born between 1975-1988? This section needs to be clarified.

As suggested, we have restricted our sample to the earlier births 1975–1988, where the mother was a cohort participant and the father may have been a cohort participant. There were no cohort fathers identified for births from 1989–2005. Since the sex of the offspring is determined primarily by the father (except for selective survival *in utero*), we have restricted the study sample of offspring to the earlier births from 1975–1988. We have revised the Methods section (page 7) and the Results section (Population Characteristics pages 11–12) to reflect this change in sample size.

4. The Authors never discuss the PCB exposure but mention at the bottom of page 11 that it was continuous rather than a one-time exposure like the PBB exposure. Why is that? The Authors should give more background regarding the PCB exposure if they are going to include it in the paper.

PCBs were used in electrical equipment and were banned in the United States in the late 1970's. Accumulation in the environment has led to widespread exposure in humans.

The Michigan PBB Long–Term study participants have PCB concentrations similar to the general population, with PCB exposure likely from contaminated food (primarily fish).

What “continuous” exposure meant was that participants may have consumed PCB

contaminated food throughout the years of the study, whereas participants' exposure to PBB-contaminated food occurred during one year (1973–1974). We have removed the use of the word “continuous.” We have added a section in the Background that clarifies the PCB exposure sources and specific details about PCB exposure in this population (pages 5–6).

5. Why wasn't gestational age at birth characterized in Table 1? Male birth ratio is much higher among preterm births. Is it possible that the births in this cohort had a lower mean gestational age?

Although it did not reach statistical significance, we did find gestational age to be associated with the odds of a male birth, as expected. For offspring born before 37 weeks gestation the odds ratio was 1.78 (95% CI: 0.86–3.67) compared to the referent group of offspring born 37 to < 42 weeks gestation. We have added this information to Table 1. We have also considered gestational age as a potential confounder. However, we and others previously found no association of PBB or PCB exposure with gestational age in this population [12, 13]. We have added this point to the Discussion section (page 19).

6. Why is there no mention of race/ethnicity of the study cohort in the entire paper?

In the Population characteristics section of the Results, we had previously reported that we excluded offspring born to fathers whose race was missing or listed as non-white. Because the Long-Term study is 98% white, we did not have an adequate sample size to examine race as a covariate. We have now added a sentence in the Statistical data analysis section of the Methods to describe this (page 10).

7. From Table 2 the Authors conclude that there was a significant increase in the proportion of males in two combinations of parental exposures, including where one of the parents had moderate PBB levels and the other parent had high PBB levels, giving a range from 0.54 to 0.62. However, there is no indication of any statistical testing that has been performed to draw these conclusions other than a multivariate analysis, which is not explained well. The proportion male among mothers and fathers with the lowest PBB concentrations (0.53) is nearly the same as that from median mothers and highest fathers (0.54). How can the Authors conclude that these are really different from each other?

We agree that Table 2 may have been confusing as it was previously presented. The male proportions given in the table were not adjusted for offspring born to the same parents, and there were no statistical tests done to compare the proportions. In light of this and as a recommendation from Reviewer #4, we have removed the table in its entirety and have included the combined maternal and paternal exposure models in the tables with the separate maternal and paternal exposure models, so that they can be seen in one place (see Tables 2–4). Also, because of small numbers in some of the combined maternal and paternal exposure cells, we now report the odds ratios using the group where both parents had low exposure as the reference to two groups: (1) both parents had high exposure, and (2) all other combinations of maternal and paternal exposure. This has been described in the Methods as such (page 11).

8. A good deal of the discussion centers around other results regarding PCBs.

Considering that the impetus for doing this study was based on the PBB exposure in this

cohort and that there were no significant results with PCBs in the current study, why do the Authors spend so much time on this in the Discussion?

Yes, we agree and have shortened the Discussion section appropriately (pages 16–19).

9. There is also a lot of focus on paternal BMI as a potential covariate when those findings were barely significant, if at all. In addition, all of the examples the Authors give for an association between sex ratio and BMI deal with maternal BMI.

To our knowledge paternal BMI has not been considered in epidemiological studies on sex ratio. However, we have highlighted paternal BMI in this paper because higher BMI has been shown to be associated with slower elimination of PBB from the body [1, 2] and adjusting for paternal BMI in our models strengthens the associations seen with the odds of a male birth in Tables 2–4 (paternal exposure only models and maternal and paternal combined exposure models). We have highlighted this point in the Discussion section (pages 18–19).

10. There is absolutely no discussion of potential biological mechanisms that might explain the effect of PBBs/PCBs on sex ratio. Given the discrepancy in the Results for maternal and paternal exposures the Discussion is missing some focus on the importance of why examine these relationships in the first place.

As described in your Comment #1, we have added a section in the Discussion of potential biological mechanisms that might explain the effect of PBBs on sex ratio (pages 17–18).

The possibilities we highlight are that on the paternal side, whether exposure to these types of chemical causes the preferential survival of Y sperm over X sperm has been

suggested in the literature, although the findings are inconsistent [8, 9]. On the maternal side, whether *in utero* PBB/PCB exposure causes an increase in early loss of XX embryos or differential survival of female fetuses is unknown.

Minor Essential Revisions

11. Why wasn't gestational age at birth characterized in Table 1? Male birth ratio is much higher among preterm births. Is it possible that the births in this cohort had a lower mean gestational age?

We agree and have addressed (refer to your Comment #5 above).

12. Why do the Authors characterize results differently in the text compared to the tables? Why is paternal BMI characterized as "significant" with a confidence interval of (1.0, 2.1) when the CI in the table is taken out to 2 decimal places and contains one?

We have revised all results given in the text to be consistent with the tables out to 2 decimal places.

Discretionary Revisions

13. It might be helpful to put the information from the 3rd paragraph regarding the categorization of PBB/PCB concentrations in a table

The categorizations of PBB/PCB concentrations are given in Tables 2–4 and described in the Methods (page 10).

14. It seems as if Table 1 should just be a frequency table of characteristics about the cohort. The univariate odds ratios are a bit ingenuous since it is likely that parents were a combination of these characteristics. In addition, see note about using odds ratios in a study with a highly prevalent outcome.

Table 1 does give frequencies of population characteristics. We thought it would also be useful to show bivariate associations with the odds of a male birth. For example, we were able to show the expected association of gestational age with the odds of a male birth. We agree with the reviewer that ultimately characteristics of both parents are important and those results are given in Tables 2–4.

Reviewer 4:

Major Compulsory Revisions

1. The statistical tests should be better described. For the crude analysis on page 11, I get $p=0.039$ rather than $p=0.02$ for a two sided test of 754/1392 vs. $H_0 = 0.514$. Two-sided tests are the appropriate ones here given that the authors are concerned about both higher and lower sex ratios.

We agree that a two–sided test is more appropriate. We have made this change in the Abstract (page 3) and in the Results section (page 12).

2. The authors loosely use the term 'significant' to apply to statistical significance at the $\alpha = 0.05$ level. For example, the second sentence on page 12 implies that a 20% decrease in male births is not important, when they mean that wide confidence limits include one.

We have altered the language in the Results and have described odds ratios in terms of “increases” or “decreases” and confidence intervals in terms of “precise” or “imprecise”.

3. For Table 2, the authors assert that the proportion of males where both mother and father had high PBB (0.60) is statistically significantly increased, but compared to what? To the national population figure quoted earlier (0.514)? Or relative to the 19 births in the category of low PBB for both parents? In either case, when attempting to create the actual numbers under the proportions in Table 2, I cannot reproduce their statistically significant results with a chi-square test.

In our original paper, we were attempting to compare the proportion of male births from two groups of parents: (1) the births from the combination of where both parents’ had high exposure, one parent had moderate/high exposure, and both parents’ had moderate exposure (the lower 4 cells in the original Table 2, n=188 births, proportion male=0.580) and (2) the referent group of births from all other combinations of parents exposure (the remaining cells in the original Table 2, n=112 births, proportion male= 0.482). Manually calculating this would give OR=1.48 (95% CI: 0.93, 2.37; chi-square value=2.70, p=0.10). However, this OR was not adjusted for offspring born to the same parents or covariates. What we presented in the original text was adjusted for offspring born to the same parents and adjusted for year of offspring birth and paternal BMI (OR=1.5 95% CI: 1.1, 2.2). The proportions given in the original table were not adjusted for offspring born to the same parents, and there were no statistical test done to compare the proportions. We agree that Table 2 may have been confusing as it was previously presented and we have removed the table in its entirety.

4. *The authors find an association in a multivariate model when both mother and father have high PBB levels, but not when either parent alone has high levels. These are the main findings of the paper and ought to be emphasized. I would place all three of these results in Table 3, so they can be displayed in one place and compared (both parents high, father only high, mother only high).*

We have revised the tables as suggested. We have included the combined maternal and paternal exposure models in the tables with the separate maternal and paternal exposure models, so that they can be seen in one place in Tables 2–4 for enrollment PBB, estimated PBB and enrollment PCB exposures (maternal only, paternal only, and maternal and paternal combined exposure).

5. *The authors unfairly dismiss the odds ratio of 1.58 for fathers alone because it does not reach the conventional p-value, even though it is consistent with (even slightly larger than) the odds ratio of 1.5 when both parents are high.*

We agree that we may not have emphasized the paternal odds ratios in light of it not reaching statistical significance. We have now altered the language in the Results section. Our combined maternal and paternal exposure models are now comparing births where both parents have low exposure (referent) to births where both parents had high exposure. In the paternal PBB exposure only model (Table 2, Model 2), the AOR=1.69 is no longer larger than the odds ratio from the combined maternal and paternal PBB model comparing births where both parents had high PBB exposure to the referent group of

births where both parents had low PBB exposure (Table 2, Model 3, low/low exposure compared to high/high exposure, AOR=2.56).

6. Also, the results in Table 2 are expressed in terms of proportions, while the results in Table 3 are odds ratios, making it difficult to compare the adjusted and unadjusted analyses. A male proportion of 0.60 for both parents high vs. 0.53 for both parents low works out to an odds ratio of 1.3, so it appears that the multivariate analysis has strengthened the estimate of the effect.

Yes, the multivariate analyses did strengthen the effect estimates. As described above, the male proportions given in the original Table 2 were not adjusted for offspring born to the same parents and we have removed the table in its entirety. For the revised Table 2, the odds ratios comparing births where both parents had low PBB exposure to births where both parents had high PBB exposure adjusts for offspring born to the same parents uses GEE and the AOR=2.56 (Model 3 low/low exposure compared to high/high exposure, 95% CI: 1.32, 4.98).

Minor essential revisions

7. Figure 1 needs to be labeled better. The axes are apparently log base e of PBB and PCB, and a legend should be provided for the symbols.

We have better labeled Figure 1 adding “log” in the axes titles. A legend for the symbols was provided, but may have been unreadable or unattached from the Figure. We have corrected this.

8. *At the top of page 7, the authors mention that they had to exclude births outside of Michigan. How many births are these, and is there any potential bias? This could be included in the discussion.*

There were 84 out of state birth records that were excluded (added to Methods section page 7) and although we did not have the birth record to confirm the offspring sex, we did have the mothers' self-report of their offspring's sex. The proportion male among these 84 offspring was not different than the offspring included in the present study (n=84 excluded offspring, proportion male= 0.548; n=865 included offspring, proportion male=0.542). Thus, we do not believe this to be a source of potential bias. This has been added to the Discussion section (page 18).

Discretionary Revisions

9. *Dates for the feed contamination (1973-74) are given in the abstract, but should be in the Background section as well.*

We have added the dates of the feed contamination to the Background section (page 5).

We have included a manuscript with changes highlighted and a clean, unmarked version of our manuscript. This work represents a collaborative effort of all authors. All authors contributed to the conduct of this research and have agreed to its submission. Please address all correspondence to:

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References

1. Blanck HM, Marcus M, Hertzberg V, Tolbert PE, Rubin C, Henderson AK, Zhang RH: **Determinants of polybrominated biphenyl serum decay among women in the Michigan PBB cohort.** *Environ Health Perspect* 2000, **108**(2):147-152.
2. Terrell ML, Manatunga AK, Small CM, Cameron LL, Wirth J, Blanck HM, Lyles RH, Marcus M: **A Decay Model for Assessing Polybrominated Biphenyl Exposure Among Women in the Michigan Long-Term PBB Study.** *JESEE* 2008, **18**(4):410-420.
3. Hertz-Picciotto I, Jusko TA, Willman EJ, Baker RJ, Keller JA, Teplin SW, Charles MJ: **A cohort study of in utero polychlorinated biphenyl (PCB) exposures in relation to secondary sex ratio.** *Environ Health* 2008, **7**:37.
4. Weisskopf M, Anderson HA, Hanrahan LP, Great Lakes Consortium: **Decreased sex ratio following maternal exposure to polychlorinated biphenyls from contaminated Great Lakes sport-caught fish: a retrospective cohort study.** *Environ Health* 2003, **2**(1).
5. Ryan JJ, Amirova Z, Carrier G: **Sex ratios of children of Russian pesticide producers exposed to dioxin.** *Environ Health Perspect* 2002, **110**(11):A699-701.
6. Taylor KC, Jackson LW, Lynch CD, Kostyniak PJ, Buck Louis GM: **Preconception maternal polychlorinated biphenyl concentrations and the secondary sex ratio.** *Environ Res* 2007, **103**(1):99-105.

7. Karmaus W, Huang S, Cameron L: **Parental concentration of dichlorodiphenyl dichloroethene and polychlorinated biphenyls in Michigan fish eaters and sex ratio in offspring.** *J Occup Environ Med* 2002, **44**(1):8-13.
8. Tiido T, Rignell-Hydbom A, Jonsson B, Giwercman YL, Rylander L, Hagmar L, Giwercman A: **Exposure to persistent organochlorine pollutants associates with human sperm Y:X chromosome ratio.** *Human Reproduction* 2005(online edition):1-7.
9. Tiido T, Rignell-Hydbom A, Jonsson BA, Giwercman YL, Pedersen HS, Wojtyniak B, Ludwicki JK, Lesovoy V, Zvezday V, Spano M, Manicardi GC, Bizzaro D, Bonefeld-Jorgensen EC, Toft G, Bonde JP, Rylander L, Hagmar L, Giwercman A, Inuendo: **Impact of PCB and p,p'-DDE contaminants on human sperm Y:X chromosome ratio: studies in three European populations and the Inuit population in Greenland.** *Environ Health Perspect* 2006, **114**(5):718-724.
10. Nurminen M: **To use or not to use the odds ratio in epidemiologic analyses?** *Eur J Epidemiol* 1995, **11**(4):365-371.
11. Cook TD: **Advanced statistics: up with odds ratios! A case for odds ratios when outcomes are common.** *Academic Emergency Medicine* 2002, **9**(12):1430-1434.
12. Givens ML, Small CM, Terrell ML, Cameron LL, Michels Blanck H, Tolbert PE, Rubin C, Henderson AK, Marcus M: **Maternal exposure to polybrominated and polychlorinated biphenyls: Infant birth weight and gestational age.** *Chemosphere* 2007, **69**(8):1295-1304.

13. Sweeney AM, Symanski E: **The influence of age at exposure to PBBs on birth outcomes.[see comment].** *Environ Res* 2007, **105**(3):370-379.