

Author's response to reviews

Title: Decreased sex ratio following maternal exposure to polychlorinated biphenyls from contaminated Great Lakes sport-caught fish: a retrospective cohort study

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PDF covering letter

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Philippe Grandjean,
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David Ozonoff,
Boston University School of Public Health
Editors-in-Chief
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RE: MS# 4527924681218278

Dear Drs. Grandjean and Ozonoff,

We have revised our manuscript, "Decreased sex ratio following maternal exposure to polychlorinated biphenyls from contaminated Great Lakes sport-caught fish: a retrospective cohort study," according to the comments and suggestions of the reviewers. A point-by-point response follows. In particular, we have greatly expanded the methods regarding PCB and DDE analysis as requested by the reviewers. Following suggestions by the reviewers, we have also reanalyzed the data excluding the dummy variable for captain's cohort or infrequent consumer group, and changed the analysis of paternal exposure to include all children of the fathers in our sample. While this did not greatly affect the results, we have modified the text where appropriate. Other changes to the text are specified in our point-by-point response. We have also corrected our old reference #32 (now reference #33) as per your request.

Thank you for considering this revised manuscript.

Sincerely,

Marc G. Weisskopf

Point-by-point responses to reviewers

Dr. Buck:

Minor points:

1. We would consider this study a retrospective cohort because we defined a cohort and then retrospectively looked at prior births to cohort members. The birth certificates were used only to verify information provided via interview. There was matching based on region and fish consumption criteria, but not PCB exposure per se. We have changed the title to reflect this. As requested, we have added n's to the text in the study sample section (p. 4), and we have added text regarding the comparison of subjects with complete vs. incomplete data (Data Analysis section, p. 8).
2. Self-reported children were searched. We have added text to this effect on p. 4. The unit of analysis is the most recent child of mothers and all children of fathers (this has been changed in response to Dr. Buck's point #4) in our study who donated blood. We are certainly missing some children of the parents in our study because the parents were not asked about children before 1970. Other children were asked about directly to each parent so we are fairly confident that we have all of the most recent children, although we cannot rule out children out-of-wedlock about which the parents didn't tell us. We would expect that if this occurred at all it would be more likely to have been the case for the fathers we spoke with than the mothers.
3. When analyzing the sex ratio with respect to mothers' PCB levels only the single most recent child of each mother is counted. For analyses of fathers' PCB levels all the fathers' children were analyzed (as suggested in Dr. Buck's point #4). The children of the 76 couples for whom we had blood from both parents were included in both the maternal and paternal analyses, but these were separate analyses. We do mention results when including both maternal and paternal PCB levels in the same model, and in this the analysis was restricted to the 76 couples and their most recent child was counted only once. We have added text to make this clearer on p. 7.
4. Unfortunately, we do not always have information on the first pregnancy of each mother. Furthermore, we feel that there is already concern about extrapolating PCB levels back in time, and the added uncertainty with respect to how each childbirth would affect maternal PCB levels would introduce a great deal of variability in our exposure measure that is worth avoiding by analyzing only the most recent child. We have added text explaining this further on p. 7. With regard to paternal exposure, we agree with this point since childbirth should not affect paternal PCB levels. Thus, we have redone our analyses considering all children of the fathers in our study using generalized estimating equations to handle the repeated measures approach. The methods have been updated to reflect this, as has the results section, figure 3, and tables 3 and 5.
5. We consider parity as the number of live births. This has been specified in the text added to p. 7. There is not a lot of data on the validity of this aspect, but one paper in a

Washington state population suggests that birth certificate data for prior births is quite good (>97%; Dobie et al. 1998) and another from North Carolina (Piper et al., 1993) finds even better concordance with medical records (>99%). In addition, in our data every child who we knew was not a first child did have an entry under “last live birth” on their birth certificate. We have added text to this effect on pp. 7-8.

6. Previous studies that have reported associations between PCB exposure and the sex ratio have not considered exposure as a continuous variable, although a linear relation between sport fish consumption and time to pregnancy has been reported (Buck et al., AJE, 1997). We are wary of reducing the number of PCB categories and then reanalyzing because we know how the odds ratios look in the different categories and thus a post-hoc recategorization could be influenced by that. It seems that considering a temporal effect, that is a change in sex ratio over the years, would be attempting to have this be a surrogate for exposure to PCBs (since the levels in fish were declining). But the true exposure would be heavily influenced by consumption habits and so year alone would have a good deal of measurement error as far as actual PCB exposure is concerned. Thus we felt the actual PCB measure would be a better representation of exposure. Furthermore, with the PCB measurements we could attempt to take into account the change in fish levels of PCB using the toxicokinetic model.

Thank you for pointing out our use of the word “incidence” with regard to a study using odds ratios. We have changed the word “incidence” to percentage on p. 14.

Laboratory methodology

Major points:

1. We have added tables 1 and 2 that list the PCB congeners assessed, their limits of detection, and the percentage of nondetects.
2. All values were used to compute the median including nondetects, which were assigned 0 for this purpose. We have rewritten the description of our process (see text p. 6), and in so doing discussed bias issues.

Minor

1. We have added figures 1 and 2 showing PCB levels, by year of child’s birth and gender of child for the unrevised values and then for the revised values with the largest decay constant because this leads to the most dramatic change from the unadjusted levels. We have limited these tables to these situations because we felt that adding such a table for every permutation of our tables 4 & 5 would be overbearing for the reader and not add that much information.
2. Yes, it is likely that the high degree of correlation between adjusted and unadjusted values are high because fasting samples were obtained. The correlation we reported was for total summed PCBs not individual congeners. However, we have removed discussion of these issues as per Dr. Rogan’s point #3.

Drs. Mortenson and Andersson

Major points:

1. We have added n's to the study sample section on p.4 (see also Dr. Buck's point #1). We have also added the numbers of mothers and fathers (and children) to tables 4 & 5.
2. We have summarized the QC data as requested. See added text in methods (p. 5).
3. We have added tables 1 and 2 that list the limits of detection and have added text and a citation on how these are determined, as requested (pp. 5-6).
4. We have added tables 1 and 2, which lists percentages of nondetects, as requested. As the reviewers point out, the imputed values could not be greater than the median. This actually simplifies the imputation logic, and we have rewritten the text to reflect this (p. 6).
5. 102.6 is approximately the specific gravity of serum. But we have removed this part of the text as per Dr. Rogan's point #3.
6. It seems that the confusion may stem from the same issue Dr. Buck rose in her point #3. We have rewritten this paragraph and added one that addresses how the children are handled in the analysis (see added text p. 7).
7. Our results are barely changed when the indicator for captain's cohort is not included in the model. Therefore we have changed the results to reflect the analyses without this indicator, and simply mentioned that adding it does not materially affect the results (p. 10).

Minor comments:

1. Thank you for pointing this out. We have explained the abbreviation here.
2. Good point. We have changed this. Thank you.

Dr. Rogan

1. We have replaced "chemicalization" with "contamination." "Chemicalization" had come straight from our reference 17.
2. We have rewritten and expanded this section in the methods. Note that for samples with <50% detectable, the imputation method ends up assigning a value of 0 to every nondetect, and we have clarified this in the methods (p. 6). The difference between the unadjusted PCB sum (counting all non-detects as 0) and the adjusted PCB sum in our subjects is very small, thus the values are likely not grossly overestimated. We have added to table 3 an indication of PCB concentrations if all nondetects are assigned the value 0.
3. We have taken the lipid adjustment text out as recommended.

4. Thank you for pointing this out. We were incorrect in stating that 2 children prior to the most recent were necessary to determine a parity of >2 . Our procedure (and what we should have said) was to classify as parity >2 any child for whom we could not identify a firstborn, but could identify at least one (non-firstborn) child born before any given child. We have rewritten this text and tried to be clearer about this (see p. 7).
5. The odds ratio for male births compares the male:female ratio in two exposure categories. It is true that it definitely isn't close to a relative risk because a male child is not a rare event, but it has standard OR properties. The logistic regression approach allows us to treat each child as the unit and include individual level covariates as potential confounders, and is the approach used in several other papers on this topic (including the del Rio Gomez et al., 2002 paper). We have, though, included the crude sex ratios by exposure category (see table 3) as suggested in point #10.
6. From other comments regarding this work, including those touched on by Dr. Buck in her comments, we feel that attempting to address the interval between the birth and the blood analysis is important, and therefore would prefer to leave it in.
7. Thank you for pointing out this reference. We have now included it in our citations
8. We have significantly altered our discussion revolving around James' work on p. 15.
9. As we discussed in response to point #6, we prefer to leave the toxicokinetic model in, and so prefer to leave the accompanying discussion.
10. As per our response to point #5, we have kept the logistic regression approach, but we have incorporated the good point of also giving the crude sex ratio by exposure category (see table 3).
11. We have added figures 1 and 2 that show the distribution of Log(PCB) values as suggested here. We have also included additional aspects in the figure that respond to Dr. Buck's minor point #1 on laboratory methodology.