Author's response to reviews

Title: Maternal exposure to polychlorinated biphenyls and the secondary sex ratio: an occupational cohort study

Authors:

Carissa M Rocheleau (CRocheleau@cdc.gov)
Stephen J Bertke (SBerke@cdc.gov)
James A Deddens (JDDeddens@cdc.gov)
Avima M Ruder (ARuder@cdc.gov)
Christina C Lawson (CLawson@cdc.gov)
Martha A Waters (MWaters@cdc.gov)
Nancy B Hopf (Nancy.Hopf@hospvd.ch)
Margaret A Riggs (MRiggs@cdc.gov)
Elizabeth A Whelan (EWhelan@cdc.gov)

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Author's response to reviews: see over
Dear Environmental Health Editorial Board,

Thank you for your consideration of our manuscript, “Maternal occupational exposure to polychlorinated biphenyls and the secondary sex ratio in a retrospective cohort.” We thank the reviewers for their thoughtful suggestions. We have revised the manuscript according to their comments; changes are tracked in the attached manuscript and discussed below. Please note that we did not track the reference renumbering throughout the text so that substantive changes would be more easily identified by the reviewers. We also did not track formatting changes to the tables; we had reformatted these substantially to give them a cleaner look, in addition to adding horizontal borders. We felt the numerous tracked changes made viewing the tables very difficult. Content changes are tracked within the tables.

**Editorial changes:**

- We added a description of the study type (retrospective cohort) to the title.
- We removed colons after e-mail addresses.
- We removed abbreviations (PCB and BMI) from the abstract.
- We altered headings so that only the first word is capitalized.
- Numbers 0-9 are now written out where they appear in the text (with the exception of referring to tables and to numbers with units of measurement, for example ‘8ug/L’).
- We removed the underlining from “Conclusions” and corrected its font.
- We removed the extra space in the first sentence of the conclusion between ‘exposure’ and a period.
- The abbreviations list is now in sentence format, with pairs separated by semi-colons.
- We moved the disclaimer and funding to the acknowledgements section.
- We removed the colons after headings and moved the text to below the headings.
- References now conform with EH style (e.g., no volume numbers).
- All horizontal lines are displayed on the tables.

**Reviewer 1**

**Comment 1:** While the new matrices are an improvement over past proxy approaches, there is still the potential for considerable measurement error inherent in this matrix and given that PCB exposure occurs through other routes not addressed by the authors. The so-called unexposed women seem to include women with background PCB exposures, at least through occupational routes, and women with no known exposure assuming that these women have no background exposure from other sources.

**Response 1:** We have revised our discussion to clearly state that all women in our analysis would have some level of PCB exposure due to non-occupational or previous occupational exposures outside the plants. We now refer to the comparison group as “women with only background levels of exposure to PCBs,” “occupationally unexposed or “non-occupationally exposed” (pages 4, 10, 13, 18) instead of “unexposed”. On page 9, we also state:

\[
\text{For the purposes of our analysis, women with only background levels of occupational PCB exposure at the time of conception were considered unexposed.}
\]
Our inability to measure all sources of PCB exposure is an unavoidable limitation of this study, but there is little direct monitoring data available from the cohorts of workers (and years) who were most highly exposed to PCBs. We feel it is important to evaluate these workers because, when exposed, their exposures to PCBs were extremely high compared to other studies. Additionally:

- We expect the level of background PCB exposure to make up a small fraction of the total difference in PCB exposure between those who are and are not occupationally exposed to PCBs; this is supported by serum PCB measures in workers in the cohort and in the communities around them (cited in manuscript).
- Unmeasured background PCB exposure is expected to be non-differential between those we considered occupationally exposed and occupationally unexposed.

Comment 2: The analytic plan requires further consideration to accommodate the distinct differences in the sociodemographic women by occupation site, the competing direction of sex ratios by maternal characteristics and exposure status (Table 2), and further attention to a possible site effect in the logistic analyses, given the apparent marked differences in sample composition (Table 3). If I understand Table 4 correctly, the analyses are stratified by site lowering statistical power rather than including site in the model. The very distinct prevalence of male births by site requires a more carefully explored analysis.

Response 2: In our primary analysis, we considered adjusting for occupation site (plant) as the reviewer suggests. The variable was dropped during the model selection process because it did not meet criteria for inclusion (inclusion criteria described in the methods). Likewise, several sociodemographic variables were considered for inclusion in the final model but did not meet our criteria. The results of our final model selection are displayed in table 2. In a subsequent analysis, we stratified by plant. These results are presented in table 3. To describe our methods more clearly, we have made revisions to the methods and results sections:

- (page 10): Potential covariates evaluated for inclusion in the final multivariate model included […]
- (page 12-13): Neither adjusting for plant in the main analysis nor conducting a separate analysis within each plant (Indiana, Massachusetts, New York) affected the relationship between maternal occupational PCB exposure and the secondary sex ratio.

Comment 3: The voluminous body of demographic literature on sex ratio reflects that maternal age and also paternal age are the most consistent determinant of secondary sex ratio at the population level. Is paternal age available?

Response 3: Paternal age, unfortunately, was not available. We have added this limitation (page19):

We were also unable to obtain information on paternal age, which has been linked to the secondary sex ratio in previous studies.

Comment 4: It would be helpful to have a more complete description how reproductive histories were obtained, particularly if gravidity (# pregnancies irrespective of outcome) is available to assess potential competing risk of PCB and pregnancy loss. Also, not clear how much proxy reporting of reproductive history was included in the sample.

Comment 4: We have expanded our description of the reproductive history questionnaire (page 9):
In the reproductive history section of the questionnaire, a respondent (or her proxy) was asked if she had ever been pregnant; if so, how many times she had been pregnant (including live births, stillbirths, miscarriages, abortions, and tubal pregnancies). For each reported pregnancy, we solicited the pregnancy end date; how long the pregnancy lasted (1-3 months, 4-6 months, 7-9 months); whether the pregnancy was single or multiple; whether the pregnancy ended in a live birth, stillbirth, miscarriage, induced abortion, or other; and the sex of each live born infant.

A detailed description of the reproductive questionnaire was previously published and is cited in the text (Silver et al, 2009). We do not have any information on the gender of lost pregnancies. Future work may examine pregnancy loss and PCB exposure, however.

We added the variable “reproductive history reported by proxy (n, %)” to table 1 in order to clarify who reported the reproductive history.

Comment 5: Further consideration of the “unexposed” women is needed, particularly the comment that this could include women who did “(page 8)…not work in an area of the plant where PCBs were ever used prior to conceiving… or not have worked in the plant until after the conception.” It would seem that exposure classification is after the event for the latter women.

Response 5: Exposure classification for this analysis is at the time of conception; meeting the criteria for inclusion in the cohort may have occurred after the conception, however. The cohort is defined as women who ever worked in the plants for one year or more. We revised the methods to clarify:

- (page 7): The study population of women was drawn from a subset of the 14,178 women included in prior retrospective cohort mortality studies of workers at three electrical capacitor manufacturing plants located in New York (4,855 women), Massachusetts (8,465 women), and Indiana (857 women). To be included in our study population, these women must have worked for at least one year at any of the three capacitor plants (5,752 women), completed a questionnaire about their reproductive history (3,952 women), and given birth to a live infant after at least seven months gestation (2,595 women).

- (page 9-10): For the purposes of our analysis, women with only background levels of occupational PCB exposure at the time of conception were considered unexposed; these women may have worked in an area of the plant where no PCBs were ever used prior to conceiving the child, or may not have worked in the plant until after the conception.

Comment 6: The rationale for excluding preterm births (for which gender is known) or women with low BMIs is not fully supported.

Response 6: We excluded BMI (<15) because this meets the World Health Organization’s definition of starvation. These extremely low reported BMIs could be due to errors in filling out the questionnaire, or in reading handwriting. Unfortunately, we cannot distinguish between extreme values that are real and those that are due to errors. If the reported BMIs are accurate, this is a potential confounder: women who are starving are rarely able to perform hard physical labor, which would exclude them from certain jobs in the plants (and therefore associate with their exposure level). Starvation produces numerous physiological changes in the body, which could be linked to sex ratio – not least of these are changes in hormone profiles. Because only 6 subjects reported these extremely low BMI values, we cannot adjust for low BMI. We have edited the text to make this issue more clear (page 10):

Women reporting a BMI below 15 at age 20 were also excluded (n = 6), since this is below the threshold for starvation and we were unable to distinguish between extreme values that actually
existed, which could create confounding by nutritional status, and those that were due to reporting errors.

We have gender information for the 36 extremely preterm births (for our purposes, less than 7 months gestation). Including these births in our analysis did not alter our findings (less than 5% change in all odds ratios and confidence intervals, and no change in significance/interpretation), so we decided to exclude them for consistency with other studies (extremely preterm births are often considered etiologically distinct from late preterm or full-term births in reproductive studies, and therefore excluded). The revised text now reads (page 10):

Very preterm births (occurring at less than seven months gestation) were excluded (n = 38) from the main analysis for consistency with other studies; including these subjects in the analysis, however, did not alter our results (data not shown).

**Reviewer 2**

**Comment 1:** The authors of the manuscript may wish to expand at the beginning of the second paragraph in the Background. In place of “Because of their estrogen-mimicking structure”, some [other references, 1-e] may be mentioned.

**Response 1:** We have expanded the second paragraph of the background to show the readers that PCBs may disrupt normal endocrine function in a variety of ways (page 5):

Multiple studies have demonstrated that PCBs can disrupt normal endocrine function in a variety of ways. Various PCB congeners have displayed the potential to alter the production of steroid hormones in human cells, thereby changing levels of estradiol, progesterone, testosterone, and cortisol [1]. PCBs may be estrogen agonists or estrogen antagonists depending on their structure [2, 3]; various congeners can also affect activation of the androgen and glucocorticoid receptors [4, 5]. PCBs may also impact thyroid homeostasis [6, 7]. Because of their potential to disrupt normal endocrine function, it has been hypothesized that parental exposures to PCBs could be associated with a reduction in the ratio of male to female live births, called the secondary sex ratio [8, 9].

**Comment 2:** In the Discussion the authors may wish to add a reference to the work of Steinberg RM and colleagues, in Biol. Reprod. 2008 Jun;78(6):1091-101. Animal studies with Aroclor 1221 produced a different result. It may be useful to discuss the possible reasons for the differences between humans and rats.

**Response 2:** We did not to discuss the rodent studies of PCBs and sex ratio, because we do not think these are good models for human secondary sex ratio. For example, rats give birth to large litters (typically around 11-14 pups per litter). The influence of in-utero hormone exposure from siblings and potential for in-utero selection (maternally mediated litter reduction) makes these large litters incomparable to humans’ typically single gestations.

**Comment 3:** Another consideration is that occupationally–exposed women may have been exposed via different routes of exposure, depending on jobs, (dermal, inhalation), than Yusho, Yu-cheng victims (diet), or the general population (diet or inhalation). Route of exposure may yet prove informative.

**Response 3:** This is an excellent point. We have made revisions to the discussion (pages 14) to point out this difference, and also point out that PCB exposure in the Hertz-Picciotto and Weisskopf studies would also have been primarily through ingestion (page 15):
Finally, the route of PCB exposure has varied across all these studies. In our study, most exposure came from dermal absorption or inhalation of PCBs; in the study by Weisskopf and colleagues, exposure primarily occurred through ingestion; and in the study by Hertz-Picciotto and colleagues, exposure likely occurred through both ingestion and inhalation.

Reviewer 3

Comment 1: There needs to be a more detailed description of the job-exposure-matrix and how exposures were estimated. Although this is provided in other publications, it is critical to the analysis and thus needs further description in this manuscript. It is very difficult to understand/interpret what the figures in the cumulative exposure mean. This is very different from PPM years and the readers need to be provided a better grounding in the methodology? This appears to be a mix of qualitative and quantitative measures. It appears quite sophisticated, but the details are lacking.

Response 1: We have rewritten the methods section to provide additional detail on the job-exposure-matrix and how exposures were estimated, adding this text:

- (page 8): Factors that could affect PCB exposure (exposure determinants, such as plant location or specific tasks) were identified for all unique jobs. Jobs with similar exposure determinants were combined into categories, which were rated qualitatively for intensity and frequency of PCB exposures via both inhalation and dermal exposure routes [10-12]. The final exposure rating combined both dermal and inhalation exposure values.

- (page 9): Job categories were rated qualitatively for intensity (baseline, low, medium, high) and frequency (continuous, intermittent) of PCB exposures. Inhalation and dermal exposure were rated separately for each category. Inhalation intensity scores were quantitatively mapped based on air PCB measurements in the plant. No dermal measurements were available, and thus the dermal ratings are unitless. For both dermal and inhalation exposure, the product of the intensity and frequency ratings were calculated; the dermal and inhalation exposure values were then averaged together as a final value for each plant-specific exposure category (and thereby for each plant-specific job within that category) [10-12].

Comment 2: It would be useful to discuss the different routes of exposure in the "matrix". How much of the exposure was from skin contact and how much inhalation? Skin contact might result in a different mix of congeners being absorbed. Are there differences between the groups or plants? Certainly there would be differences by job categories. Mention is made that the air measurements predicted the blood PCB levels, but no data is presented indicating that this holds for this short employment/young segment of the larger occupational cohorts. Were these women at "equilibrium" or were there levels still accumulating?

Response 2: Our revisions to the methods (see comment 1) clarify how dermal and inhalation exposure ratings were combined in the model, and that each exposure group was plant-specific and based on job task and area. More detailed descriptions of job groups and their exposure ratings are extensive, and available in the cited methods papers.

We have also edited the text to clarify that serum PCB correlated with the total exposure variable from the JEM, not air PCB samples (page 17):

Serum PCB levels correlated fairly well with cumulative exposure estimates (combining both inhalation and dermal exposure) from the JEM in a sample of workers in the Indiana plant [11]
Comment 3: The median duration of employment before conception was around 2 years. And most were under the age of 25 so the exposures are not exactly chronic compared to the other workers in this plant or the duration of employment distribution in the mortality studies.

Response 3: Our outcome of interest in this study is not mortality, but sex ratio of the first live-born infant. Therefore, only exposure occurring prior to conception was relevant. Women having their first successful pregnancy were young, and consequently had short work histories prior to that conception.

Comment 4: Although there is discussion of the body burdens seen in these cohorts, it is unclear what the body burdens would have been for these young women with few years in the plant and how different the exposed were from the controls. It would be helpful describe the data available on this segment of the cohort so early in their employment years and at young ages.

Response 4: We do not know the actual body burden of the study subjects. We looked at estimated PCB exposure of mothers, cumulative to the point of conception, based on our JEM. We assume that exposure will correlate with current body burden. This assumption is supported by a study within the Indiana plant, described and referenced on page 19:

*Serum PCB levels correlated fairly well with cumulative exposure estimates (combining both inhalation and dermal exposure) from the JEM in a sample of workers in the Indiana plant [11]*

Participants in this analysis (whether preconceptionally exposed or unexposed) were drawn from the overall cohort, but were subjected to the same inclusion criteria: female members of the occupational cohort, with a pregnancy ending in a live birth. Only preconceptional exposure was considered for both cases and controls. We have edited the methods to better clarify our (page 7):

*The study population of women was drawn from a subset of the 14,178 women included in prior retrospective cohort mortality studies of workers at three electrical capacitor manufacturing plants located in New York (4,855 women), Massachusetts (8,465 women), and Indiana (857 women). To be included in our study population, these women must have worked for at least one year at any of the three capacitor plants (5,752 women), completed a questionnaire about their reproductive history (3,952 women), and given birth to a live infant after at least seven months gestation (2,595 women).*

Comment 5: A bar graph showing the length of employment and number of births in both the "controls" and the exposed group would be informative.

Response 5: Our outcome of interest in this study is the gender of the first live-born infant. Therefore each participant (regardless of exposure status) contributed exactly one birth to this study.

Working in the plant for at least one year is a criteria for entering the retrospective cohort of workers; this work may have occurred before or after the index conception (in which case, length of employment in the plant prior to conception is 0).

Comment 6: Both groups have lower numbers of males than the general population. This indicates a caution and that births may have been missed.

Response 6: We have expanded our discussion of this deviation from typical population rates to include:

*Unmeasured paternal exposure to PCBs might have contributed to the lower rates of male births we observed among mothers working in the Indiana and Massachusetts plant, which previous studies suggest might be related to the secondary sex ratio [13-15]. Unfortunately, we were unable to account for any paternal PCB exposure.*
We do not believe that missed births account for the small deviations from national averages in the secondary sex ratio. Self- or proxy-respondents rarely forget to report a child that was born live; any missing births are also highly unlikely to be biased towards one gender or another (e.g., a live-born infant that was given up for adoption may be missed, but in the U.S. it is unlikely to be linked to whether the child was a boy or girl).

Comment 7. Given the number of women, what would have been the expected number of first pregnancies (first births)? The numbers seem low for the time, but some mention should be made.

Response 7: We cannot predict how many first births would have been expected, because we do not know how many women were sexually active or using contraception.

In general, until the 1960’s most women in the American workforce were unmarried. The prevailing cultural norm was that married women should be homemakers. Many companies routinely fired women who got married; others fired women who were planning to become pregnant or who became pregnant. These historical trends suggest that (in this time period) nulliparity rates among working women would be far higher than among non-working women.

Impaired fertility is an interesting and valuable outcome, but is beyond the scope of this analysis. We added this text to our conclusion:

*Future work may be needed to examine potential associations between PCBs and other reproductive outcomes, such as infertility, spontaneous abortion, and stillbirth.*

We feel these changes address the concerns raised by our reviewers and enhance the clarity and flow of our paper. Thank you for continued consideration of our manuscript for publication in Environmental Health.

Sincerely,

Carissa M. Rocheleau, Ph.D.
National Institute for Occupational Safety and Health
Centers for Disease Control and Prevention