Author's response to reviews

Title: Prenatal polychlorinated biphenyl exposure is associated with decreased gestational length but not birth weight in a prospective cohort study.

Authors:

Pam Factor-Litvak (prf1@columbia.edu)
Katrina L Kezios (klk2131@columbia.edu)
Xinhua Liu (xl26@columbia.edu)
Piera M Cirillo (pcirillo@chdstudies.org)
Olga I Kalantzi (kalantzi@aegan.gr)
Yunzhu Wang (YWang@dtsc.ca.gov)
Myrto X Petreas (mpetreas@dtsc.ca.gov)
June-Soo Park (jpark@dtsc.ca.gov)
Gary Bradwin (gary.bradwin@childrens.harvard.edu)
Barbara A Cohn (bcohn@chdstudies.org)

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Author's response to reviews: see over
I. In response to the editor’s comments:

1. n-3 fatty acid status

We appreciate the editors queries regarding the n-3 (and likely n-6) fatty acid status of the pregnant women in our cohort, and agree that the addition of fatty acid status would provide guidance to pregnant women regarding the risks and benefits of fish consumption. However, sera were collected between 1960 and 1963 and stored in state of the art freezers for that period; these were at -20°C, a temperature in which fatty acids can break down. Thus, we are not able to add these important variables to the analysis.

We also note that the population of the Bay Area during the early 1960s was not a predominantly fish eating population, so it is unlikely that fish consumption would be differential.

2. Structural equations models

We appreciate the reviewers’ feedback suggesting we examine associations using structural equations modeling to account for the correlations between PCBs. We agree that the high correlations between the congeners suggests that perhaps exposure in our sample is derived from a smaller number of underlying constructs. To address this, we now calculate Cronbach’s alphas and Spearman correlation coefficients within our exposure grouping. Within our PCB groupings, Cronbach’s alpha ranges from 0.6 to >0.90, indicating a high degree of internal consistency. We also note that Cronbach’s alpha for sum of PCBs is also above 0.90, which suggests that PCBs in total reflect a latent variable. We agree with the reviewer that an alternative method for assessing mixtures is via structural equation modeling; however, given the high values of Cronbach’s alpha coefficients, we believe that the results would be similar.

3. Manuscript formatting: EH style

We have updated our manuscript to conform to EH style; we have also changed the title per the editor’s suggestion. The files should be correctly formatted now.
II. Response to Reviewers: Reviewer 1. Wilfried Karmaus

1. Is the question posed by the authors new and well defined?

1a) The reviewer feels it is unclear why the authors distinguish between shortened gestational age and reduced birth weight. Several lines of evidence suggest that reduced birth weight and shortened gestational age, while related, also have independent etiologies and consequences. Indeed, a seminal paper by Wilcox (2001) states that many infants born low-birth weight are not necessarily preterm (and vice versa). Thus, we believe it is appropriate to analyze these as distinct outcomes.

- Van den Berg and Yerushalmy (1966), in an early discussion of the CHDS data, argue that low birth weight infants do not comprise a homogeneous group. Some may be a result of shortened gestation while others may be due to exposures or maternal conditions during pregnancy. Thus, they suggest that length of gestation be analyzed as a complementary criterion to low birth weight. They suggest that it is important to examine and distinguish between birth weight and intrauterine growth (determined by both birth weight and gestation).

- In his discussion of the birth weight paradox, Wilcox et al. (2001) describes birth weight as having both predominant (reflecting normal fetal growth) and residual (reflecting small, preterm infants) distributions. He states that these distributions are independent of each other and that an exposure that affects fetal growth does not necessarily affect the risk of preterm delivery. Conversely, a factor that increases the risk of preterm delivery would not necessarily change the average weight of babies delivered at term. He claims that in order to understand birth weight as an epidemiological endpoint, it is essential to grasp this fundamental independence of the two components of the birth weight distribution.

- In a paper about birthweight and perinatal morality, Wilcox and Skjærvén (1992) state that while gestational age is one determinant of birth weight and that the association between an infant’s birth weight and survival is likely due in part to their maturation in utero, there is still additional variation in birth weight that is unexplained by gestational age. This lends support to the idea that there are, perhaps, multiple causal pathways at play and that gestation and birth weight can be analyzed as separate constructs. There may be separate factors that are related to gestation that do not impact the association between birth weight and, in this paper, mortality. The authors also note that while gestational age is a highly important contributor to birth weight, it is only one of several contributors. In this paper the authors find that, with respect to infant mortality, both gestational age (analyzed alone) and relative birth weight at any given gestational age were both strong and “seperable” factors that affect perinatal survival (p. 382).
Likewise, in a paper examining neonatal mortality, Ananth and Platt (2004) state that “our lack of understanding of the complex relationship among birth weight, gestational age and perinatal mortality stems from mixing etiologically distinct pathways to mortality, namely effects chiefly due to fetal maturity (i.e. gestational age) vs those related to fetal growth” (birth-weight-for-gestational-age) (p.e1-e2). Findings from this study show that birth weight and preterm delivery (independent of birth weight) have a strong impact on neonatal mortality. Interestingly, the authors also note that some see birth weight as a marker for fetal size while gestation is an indicator for fetal maturity.

In conclusion, we do not believe that these outcomes can stand in for one another; they are different. In terms of consequences, gestation has its own meaning and own importance separate of the influence/contribution of birth weight.

1b) As the reviewer also points out, we did take into account infants’ birth weight for their gestational age in our analysis of birth weight percentiles. Results with birth weight percentiles as an outcome were consistent with results of our birth weight analyses. Further, when length of gestation was removed as a covariate in birth weight analyses parameter estimates only slightly decreased and remained non-significant and in the positive direction for all PCB exposures (data not shown).

- For example: In birth weight analysis examining PCB_total (non-transformed) as a predictor or birth weight with DDE included in the model and the covariate length of gestation removed from the model, b= 8 (95% CI: -23, 39). For PCB_mono, b=16 (95% CI: -70, 102).

- However, we want to make clear that our analysis of birth weight (in which gestational age was included as a covariate) is distinct from the analyses where birth weight percentiles were created from birth weight and gestational age information for each participant (using race-stratified birth weight for gestational age tables). We feel that leaving gestational age as a covariate out of the birth weight model results in an incomplete analysis. Further, we feel there is a distinction between controlling for gestational age in a birth weight analysis and conducting an analysis of growth restriction by using the birth weight percentiles outcome, which assigns each participant a percent based on their birth weight for gestational age (see discussion under point 1a).

1c) We understand that the reviewer would like us to explain the etiological chain. However, our data do not allow the testing of the etiological chain. The biological model our data is prepared to explore examines how prenatal exposure to PCBs may result in adverse pregnancy outcomes (as measured by examining birth weight and length of gestation), and that the effects of PCBs on these outcomes may be mediated in whole or in part by perturbed maternal thyroid status.
We do not have a way to test different biological mechanism for each separate birth outcome, though, as seen in our results, it is possible that prenatal exposure to PCBs may influence one outcome but not another due, potentially, to distinct etiological chains despite the fact that birth weight and gestation are not completely independent from one another.

However, per the reviewers comment we have updated all in-text tables to additionally show results with and without control for p,p′-DDE.

1d) Based on our reasons given above (in our response to 1a) we do agree that it is understandable to want to consider intrauterine growth retardation above and beyond shortened gestational age. However, we disagree that shortened gestational age and birth weight are not independent effects. Our method of analyzing birth weight and length of gestation as separate constructs is entirely consistent with previous and current literature. We wanted to examine PCB exposure’s contribution to birth weight and gestational age independent of one another.

2. Are the methods appropriate and well described, and are sufficient details provided to replicate the work?

2a) Reviewer 1 questions our methods of assessing intervening variables. We think there may have been confusion in how we described our method of analyzing mediation, and we reworded this section in the text. To clarify, when examining the effect of thyroid hormone as a mediator we looked at 1) the relationship between PCBs and thyroid hormones, 2) the relationship between thyroid hormones and birth outcomes, 3) the relationship between PCBs and birth outcomes (not including thyroid variables in the model) and 4) the mediation model, which looked at PCBs and birth outcomes, controlling for thyroid variables. In doing so there was no relationship between PCBs and thyroid hormones (which is evidence enough for a lack of mediation effect) as well as no change in parameter estimates when thyroid variables were included in regression models.

Example using PCB_{mono} (non-transformed): PCB_{mono} → FT4: b=0.013; p=0.47; FT4 → gestation: b=-0.48; p=0.15; PCB_{mono} → gestation: b=-0.30; p=0.038; PCB_{mono} → gestation (with FT4): b=-0.29; p=0.043.

2b) Thus, because of the lack of association between thyroid hormones and PCBs (in our sample) we do not believe it is necessary to further evaluate “intervening variables” using structural equations models.

We chose to examine mediation by its epidemiological definition:

- From Rothman and Greenland (2008): “Any factor that represents a step in the causal chain between exposure and disease should not be treated as an extraneous
confounding factor, but instead required special treatment as an intermediate factor”.

- From Susser et al. (2006): The exposure of interest’s relationship with the disease should be decreased after adjustment for the potential mediator
  - Mediators are a part of the causal pathway
  - The exposure causes the third variable which causes the disease
  - For there to be mediation present in the first place there must be an association between exposure and mediator (and between mediator and outcome) even before controlling for the mediator. We do not see these associations in our data.

3. Are the data sound and well controlled?

3a) We believe that the layout of our tables may have caused some confusion. We have updated Table 4 in our text and created two separate tables from it (Table 4 and Table 5). Now, results of regression analyses for birth weight and gestation are included in separate tables. We are unsure what the reviewer means by the “same scale” (linear regression?) but separate models were created for each outcomes and different covariates were controlled in these models. Further, in our sample gestational age is normally distributed (skewness: -0.413); this combined with the fact that linear regression methods are robust leads us to believe that our linear regression analysis for gestational age is entirely appropriate. Though the results of our cox-regression analysis provide essentially the same findings, these were secondary/confirmatory analyses and thus we include them in our supplementary tables rather than presenting as primary findings within the text of our article.

3b) We agree with the reviewer that it is unnecessary to present the exposure data using natural-log transformation. The interpretation of our results is unchanged using non-transformed PCBs vs natural-log transformed PCBs, thus we have updated the new Table 4 and Table 5 to reflect results of analyses using non-transformed PCBs. (Further, supplementary tables of logistic regression and cox-regression analyses have been updated as well to reflect the results of analyses using non-transformed PCBs). We agree that interpretation of these results, i.e. “for each unit increase in PCB exposure” is clearer.

4. Does the manuscript adhere to the relevant standards for reporting and data deposition?
   (Reviewer 1: Yes)

   No response to reviewers necessary

5. Are the discussion and conclusions well balanced and adequately supported by the data?
5a) Factual error in the introduction (reference 11)

The error has been corrected (although the reference the reviewer is referring to is reference 13 not reference 11; reference 11 and 12 had fewer than 30 subjects).

5b) On p. 19-20 may be useful to look at paper by Warner et al. (2012)

We appreciate the reviewer pointing us toward this useful reference. We have incorporated information from this article into our paper and updated the bibliography accordingly.

5c) The critique in the introduction that studies did not measure specific PCB congeners needs to be discussed since in this study all congeners (and total PCBs) had the same effect.

We have discussed this in the text, though we do not feel that we were “critiquing” the use of total PCBs as a measure of PCB exposure – we simply mean that methods of grouping PCBs have developed more recently and we aimed to test how these groupings related to outcomes (as well as the ‘older’ way of analyzing by either sum of PCBs or individual congeners).

- Further, we chose our groupings (Wolff) because they have been used widespread in the literature, their dioxin-like behavior, CYP1A/1B inducing behavior and estrogenicity. We chose to examine groupings by degree of ortho-substitution as this has been suggested to be one of the most useful methods of PCB classification (Moysich et al. 1999).

5d) Worth mentioning that PCB concentrations did not decrease with parity.

We agree that this is an interesting observation and have mentioned it at the beginning of our results section. As we now explain in the text, the reason that PCB levels do not decrease with parity in our sample is that this cohort was established in the 1960’s – a time where women were still actively exposed to PCBs. Further, the women in this cohort were less likely to breastfeed (another mechanism by which PCBs are eliminated from the body).

6. Do the title and abstract accurately convey what has been found?

6a) The reviewer believes that we remove the mention of PCB groupings from our abstract and instead emphasize that all congeners were tested. However, we disagree with this suggestion. We a priori proposed to analyze by groupings and these were our primary analyses. We would prefer to keep these results presented first (in the abstract, in-text and conclusions) and discuss the findings of total PCBs and individual congeners supplementary to the groupings analyses.
III. Response to Reviewers: Reviewer 2. Guiseppe De Palma

A. Major Issues

1. Bibliography is not updated enough.

We have updated our in-text citations and bibliography to include the articles which were mentioned by the reviewer.

2. The abstract and methods are inconsistent in number of PCBs that were analyzed.

Corrections have been made to the methods section; 11 PCB congeners were analyzed (including congener 194). In addition we have added to the methods section that the laboratory analyzed $p,p'$-DDT, $p,p'$-DDE and $o,p'$-DDT.

3. The word “recent” should be cleared since we have known for a long time that CYP1A1 is activated by the AhR receptor.

The adjective ‘recent’ was referring to the time at which the article came out (2011); to decrease confusion we deleted and replaced ‘recent’ with ‘a review article from 2011’.

4. Results should be compared with studies such as Bergonzi et al. 2011.

As mentioned above we have included a citation of this article within the text. We note that the main conclusions from this paper that would be more relevant to our study that revolves around pesticides such as $p,p'$-DDE and $p,p'$-DDT. We have a manuscript focusing on these chemicals currently under review.

B. Minor Issues

1. “From labor and delivery records” is not clear.
We have further explained the meaning of “labor and delivery records” within the text.

2. OC is used as an abbreviation and we do not previously specify what OC stands for.
   We have made this correction (we now specify on this page that OC stands for organochlorine compound).

3. Provide an explanation of “reported PCB values”.
   We have provided an explanation on this page (p. 7, line 22) of “reported PCB values”

4. P. 11 line 15 should be “consideration” instead of “considering”
   We have made this correction.

References