Author's response to reviews

Title: Potential effects of polychlorinated biphenyls (PCBs) and selected organochlorine pesticides (OCPs) on immune cells and blood biochemistry measures (NHANES 2003-2004)

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Philippe Grandjean, MD PhD
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Dear Dr Grandjean,

We would like to thank you and the reviewers for your insightful and thorough review of our manuscript (ID# 7466482121417109) entitled “Potential effects of polychlorinated biphenyls (PCBs) and selected organochlorine pesticides (OCPs) on immune cells and blood biochemistry measures (NHANES 2003-2004)”. Enclosed please find a revised version of the manuscript, figures and different supplementary files. All of the comments made by the reviewers were addressed in this revised version. A point by point review of these and other changes follows.

Reviewer 1 Comments:

1) How correlated were each of the exposures with each other exposure? It was mentioned in the discussion that since the non-dioxin-like PCBs were more strongly associated with changes in CBC or blood chemistries, that the use of toxic equivalency factors may not be appropriate. However, exposures to non-dioxin-like and dioxin-like PCBs are often highly correlated and it is difficult to separate the effects of one from the other. In making this conclusion, it would be helpful to know how strongly correlated dioxin-like PCBs are with the non-dioxin-like PCBs in this population.

We thank the reviewer for this suggestion. We have provided a supplementary Excel file presenting Pearson correlation coefficients between individual dioxin like (DL) and non-dioxin like (NDL) PCBs (Supplementary File 1, CorrelationsPCBs). Since the number of individual PCBs is large, these individual numbers are not given in the revised manuscript. Instead we provided the range of the correlation coefficients (page 8). Overall, our analyses have shown that correlation between DL and NDL PCBs are not always significant and correlation coefficients can vary based on the particular chlorinated compound.

2) How were the exposures that were evaluated in this study chosen? Were these all the chlorinated compounds that were available in NHANES, or are they a select subset?

For DL and NDL PCBs: All compounds with available biomarker levels in NHANES 2003-2004 were evaluated. Those with more than 90% below limit of detection (LOD) values were excluded from analyses.
For organochlorine pesticides: Only a select subset of 5 pesticides were considered to limit number of statistical analyses. These were selected from those with less than 90% below LOD values and included the more abundant pesticides trans-nonachlor, oxychlordane, and p-p'-DDE, and the less abundant compounds such as Mirex and p-p'-DDT.

3) What are the values provided in the parenthesis in the tables beneath the geometric mean? Range? 95% confidence interval? Please indicate in table footnote.

Values in parentheses provide the 95% Confidence Interval. Table titles have been revised to include this information.

4) There is information missing on the some of the labels in the tables. For example, in Table 3, abbreviations are indicated for alanine aminotransferase and aspartate aminotransferase as ALT and AST, respectively, however, the abbreviation GGT is missing for gamma glutamyl transferase. Additionally, for the cell number parameters (neutrophil number, lymphocyte number, etc), please indicate the units.

Labels have been revised to provide the missing information.

5) On page 7, there is a discussion about the relative levels of exposures between males and females. There does not seem to be a figure or table of this data. If this data is not provided, “data not shown” should be indicated.

We have added “data not shown” in this paragraph (now on page 8).

6) Please list in the “Blood count and biochemistry data” subsection of the Methods section each of the parameters from the CBC and blood chemistry reports that were used as dependent variables in the analyses. Since the results tables only contain parameters that were significant, it would be helpful to know all parameters that were evaluated to know which were not significant.

All measurements of routine CBC count as well as Blood Biochemistry profile were screened as dependent variables, except for triglycerides and cholesterol. Since serum levels of PCBs and OCPs are adjusted for blood lipids, changes in levels of cholesterol and triglycerides were not considered. We have added individual names of these variables to the text in the revised draft.

7) On page 8, beginning of last paragraph, it is noted that Tables 3 and 4 present “selected blood biochemistry data of statistically significant changes”. It is not clear from this wording if you are presenting all statistically significant parameters or a selection among the statistically significant parameters.

We thank the reviewer for this careful observation. We have revised the text to clarify that all of the statistically significant changes were presented in Tables 3 and 4. Upon careful evaluation we also noted some errors: Table 4 included some variables that did not have significant change (albumin) or barely reached significance at p=0.05 (glucose). These were removed from the table and were replaced with 3 blood markers that were missed from the earlier draft (sodium, chloride, osmolality).

8) While the results for the “Total” PCB effect (sum NDL PCBs and sum DL PCBs) are presented in the main Tables, it is also mentioned in the text which individual PCBs appeared to have the greatest effects.
Providing the estimates of the effects of the individual PCBs in a supplementary table would allow readers to evaluate the magnitude of the effect of individual PCBs.

We have now provided a new supplementary Excel file (Supplementary File 2, Survey Regression Individual PCBs) that presents results of regression models for individual PCBs.

9) Can you justify why exposures were split into quartiles rather than using them as continuous variables? Using the exposure as a continuous measure is more often used for this type exposure and should provide a more robust analysis compared to quartiles.

The main reason to use 4 separate groups for exposure variables is the large variation of non-detected values (below LOD) across individual PCBs. While some PCBs had a greater percentage of below LOD values, others had only a small number of nondetected values. In order to provide a consistent comparison that would account for this variation, we created four groups. The first group contained all individuals with a below limit of detection measurement. Based on the number of observations the remaining values were separated into 3 equal sized groups. A more detailed description of the exposure groups is provided in the ‘Statistical analyses’ section.

10) There are a number of medical conditions that affect the CBC and blood chemistry parameters that were evaluated this study. Information for many of these conditions are available in NHANES. It is mentioned in the discussion that these conditions were not taken into account for these analyses. Was this because this information was not available for all of the individuals that were included in the analysis? If this information was available but it was decided not to include, can you estimate, based on the frequency of individuals with these various conditions, the extent to which omitting this information from the analysis may affect the results?

As the reviewer notes, many conditions may impact CBC and blood biochemistry data. All of the models in the manuscript were adjusted for two main confounders: age and gender. Additionally, we have considered autoimmune diseases, such as rheumatoid arthritis (RA) and Type 1 Diabetes Mellitus (T1D) as well as Cancer as potential confounders. To understand their impact we have repeated regression models in different data subsets:

1) Individuals with self-reported ‘cancer’ excluded.
2) Individuals with potentially autoimmune diseases, RA and Type 1 DM (defined as those who use insulin and have Diabetes diagnosed before age 20) excluded.
3) Individuals with self-reported anemia treatment excluded.

We provided several supplementary excel files (Supplementary files 3 to 8) to present results of DL, NDL, and OCPs with these subsets of data (e.g., multiple comparisons cancer removed PCBs). We have also added Table 7 to show changes in white blood cell & red blood cell counts by exposure groups of DL and NDL PCBs. Overall, removing individuals with these self-reported conditions did not alter the direction of our results. The text has been modified to reflect these additions.

Reviewer 2 Comments:

This paper is very well written and the conclusions are interesting. The main issue is whether the results were sufficiently adjusted for confounding especially age!
All of the regression analyses are adjusted for age and gender. To evaluate additional effects of selected medical conditions we repeated our analyses in 3 subsets. Please see our response to item 10 above.
Reviewer 3 Comments:

No changes are necessary with the exception of a few typos.

We corrected all typos that we noticed.

We thank our reviewers for their thoughtful and constructive comments. We think that these changes have greatly improved the clarity and contribution of the paper and hope that our revised manuscript might now be appropriate for publication in *Environmental Health*.

Best Regards,

Berrin Serdar