Author's response to reviews

Title: Analysis of the Effects of Exposure to Polychlorinated Biphenyls (PCBs) and Chlorinated Pesticides on Serum Lipid Levels in Residents of Anniston, Alabama

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Version: 3  Date: 25 November 2013

Author's response to reviews: see over
22 November 2013

Dr. Philippe Grandjean
The Environmental Health Editorial Team

Dear Philippe:

Thanks for the additional comments on our manuscript, now entitled “Analysis of the effects of polychlorinated biphenyls and chlorinated pesticides on serum lipid levels in residents of Anniston, Alabama.” As usual the comments were helpful, and we have attempted to address each of the specific issues raised. I’ve copied the comments below and added our response to each of them in capital letters. We have also corrected some of the stylistic issues identified on our previous submission.

Comments of Mary Turyk:
I am suggesting two mandatory, but minor revisions.
For tables 3-7, please add in the footnotes the alpha level that was considered significant for the analyses presented in each table. I am confused by the bolding in the tables. For example the text referring to Table 4 on page 7 of the manuscript suggests that associations in this table were considered to be significant at alpha<0.0018, but p-values that are larger than this figure are bolded in the table. I suggest that a superscript next to the significant betas would be clearer than bolding.
WE HAVE DONE THIS THROUGHOUT. THE BOLDING HAS BEEN REMOVED AND SIGNIFICANT VALUES INDICATED BY ASTERIKS.

Please check for inconsistencies between tables and text. For example, the following sentence is confusing to me because I also see a significant association of tri-tetra ortho congeners with triglycerides (p<0.0001) in model 2 Table 4.
"When adjusted for other POPs (model 2) only tri plus tetra ortho PCBs showed statistically significant positive correlations with total lipids and and total cholesterol".
THE INCONSISTENCIES BETWEEN TEXT AND TABLES HAVE BEEN CORRECTED.

It would be clearer if the association with triglycerides was also discussed. In addition, if the significance level for this table is 0.0018 (as I assume from the text) then the association with total cholesterol would not be significant (p=.0047).

These errors have been corrected.

Comments from Monica Lind:
I appreciate the extensive revision of the manuscript and I agree that the section on multicollinearity may be kept as an appendix. The current version of the
manuscript reads much easier while still providing additional details.

There are some minor essential issues remaining in order for the manuscript to read more clear:

1. For all tables, write explicitly which p-value threshold that was used for statistical significance, e.g. P-value for statistical significance was <0.05
   THIS HAS BEEN DONE FOR EACH TABLE.
2. In the previous version of the manuscript, the results in table 4 read slightly different in the result section than in the current version: the last section on tri-/tetra-ortho PCBs LDL cholesterol and Triglycerides are still statistically significant on p<0.05 level alongside total lipids and total cholesterol but this has been removed from updated version, for unclear reasons.
   THIS WAS ALSO IDENTIFIED BY MARY TURYK AND HAS BEEN CORRECTED.
3. I strongly advise you to consider beginning the discussion with the main findings then followed by the paragraph that is now nr 3 ("The results reported here are consistent...", as was done in the previous manuscript version (although avoiding a numbered list). After that you may discuss paragraphs 1 and 2. This would allow the reader to easier follow your thoughts.
   THIS IS A GOOD SUGGESTION AND HAS BEEN ADOPTED.
4. Regarding the issue on lipid-adjustment or not, I do appreciate the addition to the discussion. However, the sentence “Our results are all based on wet weight measurements of levels of POPs, which is preferable to lipid adjusted levels [48], but still may lead to some level of error in lipid measurement [49].” in the discussion is still not clear. The first half seems to relate to why you did not want to lipid-adjust the exposures (POPs) when studying effects on lipid parameters, and the second half is unclear. Regarding the first half, Schisterman et al did a simulation study investigating POP, lipids and a health outcome, whereas here the lipids are the outcome in this study which makes is slightly different. Would you know of another reference supporting the use of wet weight POPs when investigating lipid levels?
   WE HAVE EXPANDED THIS SECTION IN THE DISCUSSION ON PAGE 17 OF THE TRACK-CHANGES VERSION OF THE MANUSCRIPT. IT IS NOT THAT WE DON’T “WANT” TO USE LIPID ADJUSTMENT, IT IS THAT ONE CANNOT DO THAT WHEN THE OUTCOME VARIABLES ARE FOUND IN THE LIPID COMPARTMENT. WE HAVE COMPARED WET WEIGHT, TREATING LIPIDS AS A CONFOUNDER, AND LIPID ADJUSTED VARIABLES IN PREVIOUS PUBLICATIONS WHEN THE OUTCOME VARIABLE WAS NOT IN THE LIPID LAYER, AND DID NOT FIND SIGNIFICANT DIFFERENCES. THE DISCUSSION OF THIS POINT IS IMPORTANT TO THE UNDERSTANDING OF OUR RESULTS, AND I BELIEVE THAT THIS NEW PARAGRAPH EXPLAINS OUR REASONING MORE CLEARLY.

   a. Regarding the second half: Do you mean that even though you prefer to use wet weight POPs, this may lead to errors in estimating the effect on the lipid levels? I.e. that there might be misquotation of the wet weight POPs? Or incorrect estimation of the lipid parameters?
WE CAN’T OF COURSE RULE OUT SOME MISQUANTIFICATION, BUT THE MAJOR POINT IS THAT YOU CAN’T ADJUST FOR LIPIDS SINCE THE POPS ARE ALL IN THE LIPIDS.

b. When using this short formula for estimating the total lipid levels from total cholesterol and triglycerides, how close to the true lipid value you will come using this estimation? Will you assume underestimation or overestimation of the true lipid levels? Are the populations where the formula was created similar to your study population? Do you think the gravimetric method is “gold standard” in estimating the total lipid contents in blood? Also, as stated by Bernert, “Regardless of the method used to calculate the total lipid concentrations from enzymatic lipid analyses, uncertainties will always result from the unavoidable assumptions that are made”. Relevant to bear in mind is that neither exposure nor outcomes might be completely correctly quantified, and if this may have any implication on the findings.

THESE ARE ALL GOOD COMMENTS, AND ONES WE AGREE WITH. WE HAVE CONSIDERABLE EXPERIENCE WITH GRAVIMETRIC MEASUREMENTS, AND THEY ALSO HAVE PROBLEMS. THE BERNERT FORMULA CERTAINLY IS BASED ON A SET OF ASSUMPTIONS, AND WE RECOGNIZE ITS LIMITATIONS. HOWEVER THE POINT IS STILL THAT ONE CANNOT DO LIPID ADJUSTMENT WHEN YOU ARE MONITORING RELATIONS WITH SOMETHING IN THE LIPID LAYER.

5. You state in the discussion that you “have adjusted results for the important confounders of age, sex, race, BMI, alcohol, smoking and exercise..” but you do not present univariate relationships for each confounder against the outcomes, or coefficients or p-values for any of the multivariate models. I would prefer that either you add some information on univariate analysis, or state (with references) that the confounders were chosen on biological grounds as they are known to be associated with both POP exposure and lipid levels.

WE HAVE ADDED A STATEMENT TO THE METHODS SECTION ON HOW EACH OF THESE CONFOUNDERS WAS DEALT WITH INDIVIDUALLY. THESE ARE WELL KNOWN CONFOUNDERS IN ESSENTIALLY EVERY STUDY OF CARDIOVASCULAR DISEASE, AND ONES WHICH WE HAVE ALWAYS CONSIDERED IN OUR PREVIOUS STUDIES THAT ARE WELL REFERENCED. WE DID NOT FEEL IT NECESSARY TO ADD ADDITIONAL REFERENCES ON THIS ISSUE.

I hope that you will find this revised version of our manuscript acceptable for publication in Environmental Health. Thank you for your consideration.

Yours sincerely,

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