Author's response to reviews

Title: Dietary exposure to methyl mercury and PCB and the associations with semen parameters among Swedish fishermen

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Author's response to reviews: see over
Dear Editor

We are grateful for the constructive comments from the reviewers, and we have thoroughly revised the manuscript according to given comments. Hopefully you will find the revised manuscript acceptable for publication in Environmental Health.

Responses to reviewer Melissa Perry

**Provide more rationale for why focus on CB-153 out of all the other PCB congeners.**

That has been the rationale for using professional fishermen and their wives as a study base for epidemiological evaluations of human health effects of POPs. We use 2,2′,4,4′,5,5′-hexachlorobiphenyl (CB-153) as a biomarker for POP exposure, because it correlates very well with both total concentration in serum of PCBs, with the PCB derived dioxin-like effect as well as with the total POP derived dioxin-like effect. Another relevant exposure biomarker is p,p′-DDE, which is present in relatively high serum concentrations in subjects consuming fatty fish from the Baltic Sea. The sentences have been added to the background section.

**Provide comment on whether the analysis was sufficiently powered to detect a significant difference between the groups.**

We refrained from giving measure of statistical power since it should normally be calculated before the study is performed. The sentences below have been added to the discussion. Although the studied population was relatively large, the categorization of exposure resulted in groups of only some 30 men. Thus, some of the comparisons may have had insufficient statistical power to detect any differences. However, this categorization method was preferred to be able to detect possible trends with increasing exposure. Moreover, analyses were performed comparing the lowest to highest exposure category, thereby maximising differences in exposure levels.

**Reorder discussion to comment on the study's limitations first, then the strengths.**

This has been done.

**Explain abbreviations in tables and abstract including A+B and DFI.**

This has been done.

**Response to reviewer Russ Hauser.**

The authors should consider an additional table to provide the reader with more insights (quantitative) into how the outcome changes (or doesn’t change) with varying levels of exposure. Such a table has been added.
Background: a comment about dose levels used in animal studies relative to those found in the population in this study would be helpful to the reader. The animal studies mentioned in the submitted manuscript concerns several species. It is fair to assume that different species are susceptible to different levels of MeHg, therefore we failed to see the relevance including these exposure levels.

Methods: Did the authors attempt to associate MeHg levels with reported fish consumption? The purpose of the present study was to investigate reproductive outcomes in relation to MeHg exposure. The men in the studied population were selected since they were assumed to have a high such exposure. However, the actual relation between fish consumption and MeHg, although interesting, was not the purpose of this study.

Because response rate was so low, it would be informative if any data were available on non-respondents (such as general demographics). We have already given all available information about the non-respondents in the second paragraph in method section.

Statistical Analysis:
On page 14, line 260. Specifically, how was the model fit determined not to be satisfactory? Please provide details on methods used to determine this. Limiting the analysis to the use of only categorical statistical methods limits the ability to explore more subtle trends in the data. For instance, on page 17, MeHg vs outcomes, only presenting p-values does not provide the reader with a sense of how these variables varied by exposure categories. If exposure was used as a continuous measure, more advanced statistical methods may be applied. Therefore, the decision that the fit was not satisfactory needs to be supported more fully in the paper.

We have added information regarding the test of the model fit. Moreover, analyses have been performed using the Jonckheere-Terpstra test to evaluate possible trend. Also a table has been added providing the reader with outcome information for each exposure category as well as p-values for the tests performed.

Was consideration given to using outcome as a categorical variable? No, we felt that data would be optimally used by not doing this. Therefore, all analyses performed to evaluate a possible relation between MeHg and the different outcomes have been done using original data. However, in the interaction analyses suggested by this reviewer (see also below), the outcomes were dichotomized.

The approach used to test for interaction included categorizing CB-153 and MeHg at their medians, respectively, and then defining 4 groups based on this. The authors define 'Low' and 'High' as values below and above the median, respectively. However, since the exposure ranges are wide and CB-153 and MeHg levels are correlated, it seems important to show that e.g. 'High exposure CB-153 level" in combination with either Low or High MeHg exposure levels are equivalent, and the so called interaction test is not influenced due to the fact that PCB levels in the High/High group are much higher than in those in Low (MeHg)/High (CB-153).
group. The same question one could ask for combinations "the other way around". For instance, in Table 2, there is evidence that High MeHg median differs depending on whether it is paired with Low CB-153 (MeHg median was 3.64) or High CB-153 (MeHg median was 4.0). The same holds true for High CB-153 paired with High MeHg (CB-153 median was 317) versus high CB-153 median of 241 when paired with low MeHg. Please comment on this and how it may impact your results. The point below follows from this observation.

A discussion on this has been added.

Beyond the point raised above is the question of why other tests of interaction were not attempted. For instance, one could calculate a relative excess risk of interaction (Hauser, R EHP 2005; 113:425-430). One could also use regression models in which the categories and their interactions were modeled as predictors of the outcome. Please comment on whether other methods were attempted. I realize that you were limited because of difficulties with model fit as mentioned above. But it seems that additional analysis is warranted.

Calculations of RERI have been included.

Overall, the presentation of the data (relationship between exposure categories for MeHg and CB-153) is too limited given the richness of the dataset. The authors should consider adding another table in which they more fully present the outcomes by exposure levels. Currently, the reader is limited to p-values which do not provide insight into the data. I realize the p-values are above 0.1 but a table presenting the results more fully would make the data more reader-accessible. This has been done.

Discussion, first paragraph on p. 20: Based on the metabolic properties of the two compounds, would it be appropriate to suggest that a high correlation between MeHg and CB-153 represents recent exposure (i.e., recent consumption of fatty fish) among the population?

The half-lives for MeHg and CB-153 differ a lot. As for MeHg the half-life is approximately 44 days and for CB-153 it is several years. Thus, one might speculate that a relatively high correlation between these two compounds indicates stable fish consumption in the sense that the individuals with the highest current consumption also were the ones with the highest consumption earlier. However, it is still possible that the consumption for the whole population has decreased over time.

Figures: Figure 1 may be more appropriately displayed using logarithmic scale on both axes (e.g. tick marks at 0.1, 1, 10, 100, 1000, etc.). We have tried this, but did not find it more informative than the original figure. Moreover, we feel that un-transformed data are more easily understood. Thus, we would prefer to keep the figure as is in the original manuscript.
Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)
1) Results: Table 1 does not appear to be referenced in the text
2) Tables: Table 2 needs a heading/title
3) Abstract: Please define DFI
This have been done