Author's response to reviews

Title: Persistent organic pollutants and non-alcoholic fatty liver disease in morbidly obese patients: a Cohort Study

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Author's response to reviews: see over
Dear Professor Philippe Grandjean,

Please find below specific answers to comments raised by the Reviewer 2 on the revised version of our manuscript entitled “Persistent organic pollutants and non-alcoholic fatty liver disease in morbidly obese patients: a Cohort Study”.

I have numbered the questions raised by the reviewers and made corrections accordingly. In the manuscript changes were made with the track changes function of Microsoft Word. In this Response document all changes made to the actual manuscript are written in italics to help to distinguish these from other text. Also to easily separate reviewer comments and our response to them, all reviewer text is in grayscale background.

Please note, that no changes were made to the Tables as compared to first revision submitted on 13th August 2015. For this reason these same tables were not reloaded.

We hope the answers given may assist in the acceptance of the manuscript for publication in the Environmental Health.

Sincerely yours

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Reviewer: 2

The authors’ effort to reply to my comments and questions is much appreciated. The authors have successfully addressed the issues raised. However, I consider that there is one more issue related to the new data presented that is worthy to be raised. This refers to the conclusions of the comparison between the multivariate-adjusted estimates (reported in the original version) and the estimates adjusted only for age (added in the revised version). The editor and the authors may wish to consider the two minor essential revisions listed below prior to manuscript’s acceptance for publication:

Major comments:

1) Lines 246-247 “Associations for b-HCH and PCB-118 in the models adjusted only for age were similar to those in the fully adjusted model and are shown in the supplementary information (Table S1).”

Effect estimates in the two models are indeed very similar for b-HCH, however, it can be argued that this is not the case for PCB-118. Adjustment for additional covariates in the PCB-
Model 118 seems to increase the precision of the effect estimates (as shown by the narrower 95% CI in the fully adjusted model compared to the model adjusted only for age) increasing the magnitude of most of the associations shown. For example, the associations between PCB118 and diagnosis or grade of steatosis become significant only after the inclusion in the models of additional covariates (Tables 3 and S1). This is somewhat unexpected because the inclusion of additional covariates in the models commonly tends to decrease the precision of effect estimates (i.e. the 95% CI become wider) especially when the sample size is small. My suggestion is to notify these differences for the PCB-118 associations in the text and further, briefly explain, perhaps later in the discussion, what is a possible explanation for the increased precision of estimates in the multivariate-adjusted model. My assumption is that this is due to the inclusion of the BMI covariate in the models; however, if BMI were a confounder, related to both the exposure and the outcome, then I would expect the inclusion of BMI in the models to influence the effect estimates but not to influence that much the precision of effect estimates (or to decrease, but not to increase, the precision).

**Answer:** We appreciate the comments of the Reviewer noting the differences between the models including additional covariates. It is in fact very difficult to make any reliable interpretations comparing models alternatively including BMI, serum lipids and fasting insulin, because these variables correlate highly with each other. However, we try to briefly address these comments in appropriate sections of the text.

In the Results section word “mainly” was added on the sentence on lines 246-247 to clarify that this does not apply to all histology subtypes. Right after this the following sentence was added:

“However, in fully adjusted model association of PCB118 with diagnosis and steatosis grade was significant and the precision of the effect estimates was increased as compared to models adjusted only for age (Table 3 and Table S1).”

As suggested by the reviewer, this was further explained in an additional paragraph of the Discussion section (added on line 316 and forwards) as follows:

“In part of the models the number of covariates included increased the significance of associations and improved the precision of effect estimates. Regarding the association of PCB118 with diagnosis and steatosis grade (Table 3 and Table S1) serum insulin level was the key covariate. In addition to a significant difference in the insulin levels between different diagnosis groups at baseline (Table 1), insulin also correlated with liver inflammation ($R^2=0.207, p=0.008$) and steatosis grade ($R^2=0.217, p=0.006$). Similarly, for PFAAs and inflammation (Table 4 and Table S2), the most important covariate was serum lipids followed by serum insulin, sex and BMI. Here, serum lipids correlated with inflammation ($R^2=0.269, p=0.001$). As insulin, and possibly also lipids may be confounders in the models, related to POPs [32], PFAAs [34] and NAFLD [4], they can be expected to have an impact on the effect estimates but less so on their precision. However, the mechanism by which e.g. impaired insulin sensitivity would change the distribution of POPs in this population of morbidly obese is not known.”

2) Lines 251-253 and Tables 4 and S2: As the authors also clarify in the text, associations of PFASs become significant after inclusion of additional covariates in the models. Similar to my comment above, the precision of effect estimates is largely improved in the multivariate-adjusted model. For example, for PFNA the ORs (95% CI) for the outcome categories change from 0.96 (0.23, 3.97) and 0.15 (0.01, 2.16) into 0.29 (0.05, 1.61) and 0.02 (<0.01; 0.66),
respectively. This is surprising and some explanation to justify the changes in the precision of estimates is required for the readers.

**Answer:** A mention of the change in the precision of effect estimates was made on line 252 (underlined text) of the results section:

“Respective associations in the models adjusted only for age were all non-significant and the precision of the effect estimates was decreased (Table S2).”

A comment on this issue was added in the Discussion in the same paragraph as the respective comment on POPs (see answer to comment 1).