Author’s response to reviews

Title: Prenatal exposure to perfluoroalkyl and polyfluoroalkyl substances and the risk of hypertensive disorders of pregnancy

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Author’s response to reviews:

Dear Editor,

Thank you for your letter and comments on our manuscript titled “Prenatal exposure to perfluoroalkyl and polyfluoroalkyl substances and the risk of hypertensive disorders of pregnancy” (ENHE-D-18-00201R1). These comments helped us improve our manuscript, and provided important guidance for future research.

We have addressed the reviewers’ comments to the best of our abilities, and revised the manuscript accordingly. Revisions were highlighted in red. We hope this meets the requirements for a publication. Thank you and the reviewers for the useful comments. Our responses are as follows:

Reviewer 1

Major Comments:
C1. The authors indicate that international studies (plural) provide a prevalence of HDP ranging from 5-8%, but only give a single citation, which is quite outdated. I suggest reviewing more up to date citations (e.g., Hutcheon JA et al. Best Pract Res Clin Obstet Gynaecol, 2011; 25(4), 391-403, and Duley L. Semin Perinatol, 2009; 33 (3), 130-7), which indicate the prevalence of HDP may be up to 10%. Further, the Chinese report cited by the authors provides a prevalence of 5.2% which, while within the range of other studies, is on the low end.

R1: Thank you for the suggestion. We have now cited these new references, and revised the sentence to “…occurred in 5.2% of pregnancies [1], which is at the lower end of that reported by other international studies…”.

C2. Only a single original article from Canada in 2005 is provided as a reference for the statement of multiple PFAS exposure pathways (page 4, line 51-53). This is inappropriate given the robust literature on this topic. Further, exposure pathways in China are likely to be different than in Canada. At a minimum, a more thorough citation for this statement is needed.

R2: Thank you for your suggestions. We have now done a more thorough literature review on exposure pathways of PFAS in the Chinese population, and added several up-to-date citations.

C3. No mention is made in the introduction (page 5, lines 11-40) of the potential impact of varying exposure levels on inconsistent results of the three previous studies, nor of potential differences due to timing (i.e., given that concentrations are declining in many parts of the world). How does exposure differ between the US-based C8 population, MoBA, and Chinese populations?

R3: Thank you. We have now added discussion on the effects of varying PFAS exposure on the association of interest in the Introduction on Pages 5-6. Also we have added text comparing the exposure level in the Chinese women with that in women from Norwegian and US in general (Page 6 ).

C4. It’s unclear from the methods, but were women recruited at the time of birth? If yes, then this is a cross-sectional study, not a prospective cohort study. Perhaps the parent study was intended as a prospective cohort and these women and their children are currently being followed, but the present analysis is cross-sectional, using only the baseline data.

R4: Thank you. Pregnant women were recruited at the time of birth. In the Methods in Page 6, we have now modified the sentence to “From 2011 and 2012, 687 women who had a singleton pregnancy and came for delivery at two large hospitals in Shanghai were recruited.”

C5. Please provide the LODs for PFOSA, PFHPA, PFOS, PFNA, and PFHxS.

R5: We have now added the description of LODs for these substances at the footnote of Table 2.
C6. What was the justification for the inclusion of age, education, and pre-pregnancy BMI as a priori confounders?

R6: As shown in Figure 1, confounders were selected based on DAG. We have now modified the sentence as follows “For regression models, covariates that were selected based on a directed acyclic graph were considered as potential confounders, including maternal age, educational level, parity and pre-pregnancy BMI (calculated as weight in kilograms divided by height in meters squared).”.

Figure 1. Directed acyclic graph (DAG) of the association between prenatal exposure to PFAS and hypertensive disorders of pregnancy.

C7. Why were other covariates (e.g., smoking, parity, socioeconomic status, occupation, etc.) not explored as potential confounders? Given toxicokinetics, parity in particular has been shown as a very important variable when examining associations of PFAS and pregnancy outcomes.

R7: We have already adjusted for “parity” in the analysis, so we have now corrected the sentence as follows “For regression models, a set of covariates that were selected based on a directed acyclic graph were set as confounders, including maternal age, educational level, parity and pre-pregnancy BMI (calculated as weight in kilograms divided by height in meters squared).”. We used maternal education level to partly reflect the overall socioeconomic status of the family. Because only a small proportion of mothers smoke before pregnancy (N=10, proportion=1.5%) and during pregnancy (N=3, proportion= 0.4%) in our subjects, smoking is unlikely to confound the association assessed in this study. We have now explained why smoking is not included as a confounder in the Methods.

C8. The discussion section of the manuscript seems to indicate that a primary motivation for this analysis was the very limited information on health effects of PFBS; if this is the case, it should have been made clearer in the introduction.

R8: We have now modified the Introduction, which made it clear that PFBS was the emerging substance in the PFAS family members.

C9. There is no discussion in the manuscript regarding the present study's results compared to previous studies, including some comparison of exposure levels.

R9: Thank you. We have now compared the results of our study with previous studies, taking into account the different exposure levels.(Page 14-16)

C10. The sentence on page 13, line 67, starting with 'a direct causal association…' should be omitted. No study will provide "direct causal evidence". This sentence is unnecessary and irrelevant.
R10: Thank you. We have now deleted this sentence.

C11. On page 15, line 109, the word 'causation' should be changed to 'association'.

R11: We have now changed the word “causation” to “association” in this sentence.

C12. On page 15, the authors indicate that studies provide evidence that PFAS are 'by and large consistent across pregnancy'. But, only a single study from the DNBC is cited. Moreover, contrary to what the authors indicate, while Fei et al report high correlation between PFOS and PFOA concentrations in 1st and 2nd trimester samples, they also indicated that on average, concentrations decreased. Given urine as a major route of elimination, it is likely that PFAS concentrations decrease across pregnancy as the GFR increases - this is actually supported by the findings in Fei et al. as well as by Fromme 2010 [pmid: 20722433], Monroy 2008 [pmid 18649879], and recent PBPK modelling (see Loccisano et al, pmid 23151209] of PFOA and PFOS. In addition to revising the statement, they should provide some indication on how the presumed decrease in concentrations across pregnancy may impact their results.

R12: Thank you very much for clarifying the differences of PFAS level across pregnancy. We have now revised the statement and added possible impact on our results.

C13. 'confirm the causal relationship' should be omitted from the conclusion. As the authors indicate, the association they observed may be spurious given preeclampsia may impact the toxicokinetics of PFAS - this possibility should also be further discussed.

R13: We have now deleted it.

Minor comments:

C1: Hypertensive disorders of pregnancy is plural, thus the first sentence in the introduction (page 1, line 3) should be revised accordingly.

R1: We have now changed “is” to “are” in that sentence.

C2. HDP includes several separate diagnoses but there is no background regarding whether these have different or similar etiology.

R2: We have now mentioned in the Introduction that HDP is a group of disorders with heterogeneous etiologies (Page 4).
C3. Importantly, though the two US studies of PFAS and HDP are from populations with high PFOA concentrations, they experience background concentrations of PFOS and other PFAS; this should be noted.

R3: Thank you. We have specifically pointed this out in the Introduction.

C4: When discussing associations (page 5, lines 10-40), it would be helpful to provide some indication of the magnitude of associations, rather than just saying 'weak', 'modest', 'positive' or 'inverse'.

R4: Thank you. We have now added the magnitude of the associations.

C5. Medians, GMs, SDs, and ranges are not used to describe "basic characteristics of subjects" (page 7, lines 38-44).

R5: We have now deleted this sentence.

C6. I believe the authors mean 'a priori' rather than 'a prior' on page 8, line 48.

R6: We have now modified this sentence as “For regression models, a set of covariates that were selected based on a directed acyclic graph were set as confounders, including maternal age, educational level, parity and pre-pregnancy BMI (calculated as weight in kilograms divided by height in meters squared).”.

C7. In the methods, the authors indicate that detailed methods for measuring PFBS were already described…does this imply that these methods differ from those used to measure the other PFAS?

R7: Thank you for asking. All PFAS was measured in the same method. So we have now changed “PFBS” to “PFAS”.

C8. Given this study is among a Chinese cohort, it's unclear of the relevance of the statement (page 12, line 50) regarding the phase out of PFOA and PFOS.

R8: We have indicated that with the phase out of PFOS and PFOA in Europe and North America, they were relocated to China, and PFBS has been increasingly produced as a substitute of PFOS in China.

C9. The first sentence in the discussion is missing some context. The authors state that "our study used elastic net regression models to select a subset of PFAS components"…but it should
indicate that they used elastic net to select the subset of PFAS components most strongly related to HDP.

R9: Thank you. We have now revised this sentence accordingly.

C10. The authors spend a lot of space in the discussion laying out the potential biologic mechanism of PFBS on preeclampsia, which is nice, but I suggest providing a more concise overview of this information.

R10: We have now deleted one short paragraph.

C11. There are font differences in Table 2.

R11: We have now modified the font.

C12. There is no legend for the figure.

R12. We have added a legend and explanation for the scale.

Reviewer #2:

C1. Please be aware that I work for a consulting company and my work at the company is supported by 3M. I believe that my review was not compromised by this relationship. In addition, please bear in mind that I did see the comments from the editor that resulted in the "R1" version that I reviewed, and that I may not agree with the editor about the best way to present the results.

I find the elastic net regression approach and results interesting, but I believe that this is a relatively new technique in environmental epidemiology and that the data presentation would be enhanced by including what might, by some, be considered a standard analysis, so that the results could be compared. Also, a slightly more didactic approach, where the effect of using the elastic net rather than old-style approaches are examined and explained more, would add great value. I suggest the order of presentation be: first, logistic regression for all 8 PFAS and each outcome separately, and second, the elastic net regression. I have no objection to the author's focus on the elastic net regression findings as long as the "standard" results are available to the interested reader.

R1. Thank you very much for your suggestions. We have now added results using standard logistic regression.
C2: The authors note that preeclampsia might affect kidney function and alter the PFAS concentrations in cord blood. This effect could be especially important for a PFAS that has a short half-life, like PFBS. To improve the assessment of whether such an effect might have occurred, it would be useful to add a sensitivity analysis where the results for PFBS are adjusted for birthweight and gestational age.

R2: Thank you for your suggestions. We have now conducted this sensitivity analysis, which got similar odds ratios with the previous model without adjusting for birth weight and gestational age. The results will be submitted as supplementary data.

C3: Little information is given about the criteria used by doctors in Shanghai to diagnose, e.g., preeclampsia. In other countries, the criteria vary by doctor and do not necessarily agree with criteria published by professional organizations. The reader is left to assume that the outcome ascertainment was imprecise, meaning that the statistical power of the study would be compromised. More consideration of this likely weakness is needed in the last paragraph of the discussion.

R3: This is an interesting point. Preeclampsia is commonly diagnosed using the following criteria in China: (1) systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg after gestational week of 20 and (2) a urine dipstick measurement of at least one + dipstick. We have now added the definition for gestational hypertension and preeclampsia in the Methods. There may be individual variation among physicians in the diagnosis of preeclampsia. Such variation is likely to be non-differential and draw the results towards the null.

C4: The discussion would benefit from consolidation of the three paragraphs about potential mechanisms. If the results are replicated this level of detail and speculation might be warranted, but at this stage, a shorter summary would be more appropriate.

R4: Thank you for your suggestion. We have now shortened into one paragraph.

Minor comments:

C1: P 2, L 28 (line number in small font in left column): as noted above, would mention the standard logistic regression first.

R1: We have now modified the sentence as “Standard logistic regression and elastic net regression with logit link was used to identify independent associations between exposures and outcomes.”.

C2: P 2, L 42: rather than "incidence", "risk" seems more appropriate.
R2: We have now changed “incidence” to “risk” in that sentence.

C3: P 4, L 3: would change "is one of" to "are among"
R3: We have revised it accordingly.

C4: P 4, L 6: would change "pregnancy-related complications" to "complications of pregnancy"
R4: We have revised it accordingly.

C5: P 4, L 31: would make pollutants plural
R5: We have now revised “pollutant” to “pollutants”.

C6: P 4, L 56: PFAS is a very broad category of chemicals, and many have short half lives. Perhaps the authors mean that the most frequently studied PFAS have long half lives. Would delete "members" when the wording is revised.
R6: Thank you. We have now revised it as “Most frequently studied PFAS have a long half-life of 3-5 years in human body [14].”

C7: P 5, L 12: would insert "have" before "provided"
R7: We have now revised it accordingly.

C8: P 5, L 23: would delete "Norwegian". (It was an American study of Norwegians, done in collaboration with Norwegians.)
R8: Thank you. We have now deleted "Norwegian".

C9: P 6, L 23: would insert "As noted above," before "information"
R9: We have now added “As noted above,” before “information” in that sentence.

C10: P 6, section 2.3 somewhere: it would of interest to indicate the volume of plasma that was used for the PFAS analyses. With an LOD so low for PFBS, it suggests that an usually large volume was used, or that PFBS is, for some reason, much easier to measure precisely in low volumes.
We have now added this sentence in section 2.3: “A total of 100 μL plasma sample was used to measure PFAS.”. The low LOD of PFBS is not because of its low concentration, but is due to its structure and the high sensitivity of measuring PFBS in the machine (HPLC-MS/MS, Agilent 1290–6490, Agilent Technologies Inc., USA) we used.

I think where the authors say "PFBS" they mean "PFAS"?

Yes, we have now revised “PFBS” to “PFAS”.

This detail was probably already presented in their reference 9 and does not need to be repeated here.

Yes, the details of PFAS have been mentioned in the reference. So we have now deleted this sentence.

the LODs are missing for three of the PFAS measured.

We have now added LODs for all ten PFAS.

Perhaps at the end: the reader needs to be given information about the precision of the assay. For example, what was the between-assay coefficient of variation for each of the 8 compounds?

We have now added inter-assay and intra-assay CV at the end of section 2.3.

Please insert some citations to important examples of where this technique has been employed in environmental epidemiology studies.

We have now cited two recent publications that used elastic net regression.

where the authors say "a prior", I believe they mean "a priori", and it would be good to italicize that.

We have used other ways to describe confounders in this study as follows: “For regression models, a set of covariates that were selected based on a directed acyclic graph were set as confounders, including maternal age, educational level, parity and pre-pregnancy BMI (calculated as weight in kilograms divided by height in meters squared).”.

again, suggest "incidence" be replaced with "risk"
R17: We have changed it accordingly.

C18: P 10, L 12: please be clearer about what is meant by "college education". Does this mean a degree after 4 years? This level of detail could go in a table footnote.

R18: We used “College degree ” in Table 1. “College education” means post-secondary education with 3 or 4 years education in college or university. We have now added this information as footnote in Table 1.

C19: P 10, L 17: would insert "a" before "higher"

R19: We have now modified it accordingly.

C20: P 11, L 6: would explain that Ben means beta coefficient from the elastic net regression where this is first used in the text.

R20: We have added an explanation of it.

C21: P 11, L 17: where the authors say "unpenalized", do they mean (standard) logistic regression? This potentially confusing descriptor might be avoided by presenting the logistic regression results first.

R21: Thank you. This means standard logistic regression. We have newly added the results from logistic regression.

C22: P 12, L 60: would delete "human"

R22: We have now deleted “human” in that sentence.

C23: P 13, L 1: would change "samples in our previous report" to "in our samples"

R23: We have now changed it accordingly.

C24: P 13, L 6: would delete "bodies"

R23: We have now changed it accordingly.
C24: P 13, L 23-26: rather than "reported epidemiological investigations so far has" would say "previous epidemiologic studies have".

R24: We have now revised the sentence accordingly.

C25: P 15, L 25: where temporality is discussed, it would make sense to move the sentences about reverse causality to the same part of the paragraph, because the issues are so closely related.

R25: We have now modified the paragraph.

C26: P 15, L 31: what is the correlation of PFAS concentrations in cord serum with levels during mid-pregnancy?

R26: In response to other comments, we have newly discussed the correlation between cord level and maternal level of PFAS. PFAS with a shorter carbon chain generally have a higher transfer efficiency (defined as the proportion of cord level to the maternal level). As reported by a Norwegian study[1], PFOA, PFHxS, have a higher placental transfer efficiency (around 0.7-0.8) and PFOS, PFNA, and PFUA, and PFDA has lower transfer efficiency (0.3-0.5). Our unpublished data show similar results.

C27: P 16, L 9: The possibility of results affected by multiple testing needs to be raised. Twenty-four associations were examined, and one was statistically significant. Did the elastic net procedure somehow reduce the likelihood that a statistically significant result would be obtained by chance?

R27: Elastic net regression included all the eight PFAS in the same model, and select the most correlated variables with the outcome. There are only three associations (hypertensive disorders of pregnancy/preeclampsia/gestational hypertension) tested. Thus, the possibility of chance due to multiple comparison might be small.

C28: P 16, L 20: the dose-response pattern was present only for preeclampsia

R28: We have now modified the sentence accordingly.

C29: Table 2: The text label for PFUA needs to be corrected. Currently it says perfluorohexane sulfonate.

R29: We have now modified the term accordingly.
C30: Table 3: please add confidence intervals where a non-zero coefficient is presented.

R30: It is a good suggestion. Unfortunately, the statistical program ("glmnet" package in R) does not provide 95% confidence interval for the beta coefficient in Table 3, and we are unable to calculate it ourselves. The method is very complex. Another study published in Environmental International did not provide 95% confidence interval, either [2].

C31: Table 4: please explain how the trend test was constructed.

R31: In response to other comments, we have now added explanation in the Methods.

C32: Figure 1: I did not find the legend for this figure. It might be good to explain what the axis labels are, units, why some of the fitted red lines are not straight, what the asterisks indicate, etc.

R32: We have not added explanations for figure 1.

Yours sincerely,
Zhang Jun

References:
