Author’s response to reviews

Title: Prenatal exposure to perfluoroalkyl and polyfluoroalkyl substances and childhood atopic dermatitis: a prospective birth cohort study

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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 childhood atopic dermatitis: a prospective birth cohort study” (ENHE-D-17-00244). 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

C1. General comments

The study addresses a very relevant topic. Since the existing literature are conflicting making it difficult to draw any overall conclusion on the association between prenatal exposure to PFASs and allergy-related outcomes, more studies on this subject are needed. The discussion includes updated considerations on both epidemiologically and mechanistically issues. The manuscript is well-written, although some clarifications/modifications are needed (see specific comments). The correct abbreviation for perfluoroalkyl and polyfluoroalkyl substances is PFASs and not PFAS. Expect in instances such as e.g. PFAS levels, PFAS exposure.

R1. Thank you. We have now corrected the abbreviation for perfluoroalkyl and polyfluoroalkyl substances as suggested.

C2. Specific comments

Abstract:

The abstract stated that 811 children completed the 2-year follow-up visit. However, since 124 of these do not have PFAS measurements, it should be stated clearer that exposure-health associations was performed on a study group of 687 children - not 811.

The PFASs mentioned in the conclusion of the abstract is not the same as in the conclusion of the discussion. PFDoA is not mentioned in the abstract's conclusion.

R2. We have now revised the relevant sentence in the Abstract as follows “A total of 687 children completed a 2-year follow-up visit and had PFASs measurement. AD was diagnosed in 173 (25.2%) children during the first 24 months”.
C3. Introduction:

Page 4, 2nd section, lines 1-3: The authors mention several effects of PFASs such as immunotoxicity, hepatotoxicity and impaired thyroid function, but they end the sentence by writing mammary glands without any references to toxicity/functions of PFASs in these glands. Examples of effects on mammary glands should therefore be mentioned.

R3. Thank you for your comments. A reference has now been added to support the evidence of the effect of PFASs on the development of mammary glands.

C4. Introduction:

Page 4, 2nd section, lines 5-7: The authors should be more precise in how they cite these studies. The authors claim that in two epidemiologic studies, the exposure to PFASs was associated with asthma in adolescents, and then they refer to the studies by Dong et al. and Humblet et al. However, in Humblet, a positive association was found between PFOA and asthma, whereas there was a negative association between PFOS and asthma (and wheeze).

R4. We have rewritten this sentence as follows: “However, findings from epidemiologic studies were far from consistent”.

C5. Introduction:

The authors should also add a reference for their last statement that no associations between PFASs and asthma were found in children up to 3 years of age.

Page 5, 1st section, lines 3-6: Delete atopic dermatitis at the end of the sentence in line 6.

R5. Thank you for your suggestion. We have now deleted the statement that no associations between PFASs and asthma were found in children up to 3 years of age. Also, we have also deleted “atopic dermatitis” as suggested.
C6. Material and methods:

Statistical analyses on pages 8-9: The authors give a list of potential confounding factors. They should also add a description on the method used for choosing confounders to be included in the final adjusted statistical analyses (e.g. a priori selection, selection based on statistical significance, selection based on DAGs).

R6. The description on the method used for choosing confounders has been added as follows “Potential confounders were selected based on DAG, and included infant sex, parity (nulliparous and parous), birth weight, gestational weeks at delivery, mode of delivery, maternal pre-pregnancy BMI, maternal age, maternal education, maternal ethnicity, paternal age, paternal education, parental history of allergic disorders, paternal smoking during pregnancy, and family income”.

C7. Results:

The number of children with AD is mentioned far down in the text. This information is important and should be mentioned early in the result section and not just listed together with the other population characteristics. The number of children with AD is shown in the column headings of table 1 without any percentage. Please add the percentage also in the table.

R7. We have now added a sentence describing the number of children with AD in the text as follows “During the first 24 months, 204 children (25.2%) developed AD”. In addition, the percentage of AD in our study has now been added in Table 1.

C8. Results:

Information on maternal smoking and drinking during pregnancy are only mentioned in the text and not shown in Table 1. Please add these characteristics in Table 1.

R8. These characteristics have been added in Table 1 as suggested.
C9. Results:

Page 10, 1st section, line 8: The authors should specify that the passive smoking is due to paternal smoking as stated in table 1.

R9. We have now revised the sentence as follows: “However, 29.7% of mothers were exposed to paternal smoking during pregnancy.”.

C10. Results:

Page 11, 1st section: Please add a sentence stating that none of the other PFASs were significantly associated with AD.

R10. We have now revised the sentence as follows “PFOA concentration in cord blood was higher in children with AD than in children without AD, while no statistically significant difference in other PFASs was found between those with and without AD”.

C11. Results:

Page 11, 2nd paragraph, lines 1-4 and Table 5: The authors state that PFOA, PFNA PFDA, PFDoA and PFHxS were significantly associated with AD in girls. In addition, the associations between AD and PFNA Q4 and PFDA Q4 are highlighted in bold figures, further indicating a statistically significant association. However, the 95% CI are 0.98-4.78 and 0.99-4.49, respectively. Since the CI crosses 1, this indicate that the findings are not statistically significant. The authors should change or moderate their statement regarding what associations are statistically significant.

R11. We have corrected statements on the associations between AD and PFNA Q4 and PFDA Q4 as follows “Due to small sample size, the associations of AD with PFNA concentration and PFDA concentration, were both marginally significant”.

C12. Results:

Page 11, 1st section: The authors should add one sentence mentioning what PFAS-AD associations that are not statistically significant (e.g. for PFOS and PFBS).

R12. We have now added a sentence as follows “No other PFASs were significantly associated with AD in girls.”.

C13. Table 1:

Since the exposure-health analyses are performed on a population where n=687, you should consider adding a new column showing the figures for this population. Alternatively, deleting the n=811 and replace this by n=687.

Please add information in the table text that the figures in brackets are percentages.

Consider showing the percentages with only one decimal.

R13. We have now modified Table 1 accordingly.

C14. Table 2 and 3:

Are the analyses precise enough to give the PFAS concentrations with two decimals? The authors should consider reporting the concentrations with only one decimal.

R14. The concentrations of PFASs were measured with four decimals, so the third decimal were also precise. We have revised the concentrations with one decimal as suggested.

C15. Table 4 and 5:

In the footnote, confounding factors have been listed. Is this an overview over all the potential confounders or all the confounders included in the final statistical analyses? Please specify.

R15. It means confounders included in the final analyses. We have now specified it in the footnote.
C16. Table S1:

Since the exposure-health analyses are performed on a population where n=687, the authors should consider adding a new column showing the figures for this population. Alternatively, deleting the n=811 column and replace this by n=687.

R16. Table S1 has been revised as suggested.

C17. Table S2:

Please add footnotes similar to Table 4 and 5.

R17. The footnote has now been added to Table S2.

C18. Discussion:

Page 12, 1st section: This statement should be modified according the comments above on statistically significance and CI-intervals (Page 11, 2nd paragraph, lines 1-4 and Table 5).

R18. The sentence has been modified as follows “Our prospective cohort study suggests that prenatal PFASs exposures may increase the risk of childhood AD. In particular, we found that PFOA, PFDoA and PFHxS in cord blood increased the risk of childhood AD in girls only. In addition, the associations between AD and prenatal exposure to PFNA and PFDA were marginally significant”.

C19. Discussion:

Page 12, 3rd section, lines 2-4: I'm not sure the study be Anderson-Mahoney et al. is relevant to include in the discussion due to several risk-of-bias concerns. In the NTP Monograph (from September 2016) on PFOS and PFOA they concluded that "However, the study had a number of serious risk-of-bias concerns and was rated probably high for all three key risk of bias questions: (1) failure to consider most important confounders (e.g., smoking, body mass index, and socioeconomic status), (2) exposure characterization was based on residence in an area with drinking water contamination for at least one year with no information as to how variables such
as percent of residents reporting water consumption were used, and (3) disease outcomes were obtained by questionnaire with no indication that the questionnaire had been validated (see Figure D34). In addition, the participants were plaintiffs or potential plaintiffs in a lawsuit regarding PFOA exposure of residents near a Teflon manufacturing plant on the Ohio River in West Virginia and therefore likely knew of their exposure and potential health effects.” If the author still wants to include this study in their discussion, the bias questions from the NTP monograph should be addressed.

R19. We have deleted this reference.

C20. Discussion:

Page 12, 3rd section, lines 4-6: See comments above (Introduction, Page 4, 2nd section, lines 5-7). The authors need to be more precise when referring to the findings in other studies.

R20. The sentence has been revised as follows “A US national study found that serum PFOA was positively associated with asthma (OR=1.18, 95% CI: 1.01, 1.39) in adolescents aged 12-19 years, while PFOS was inversely associated with both asthma and wheezing (OR = 0.88; 95% CI: 0.74, 1.04, and OR = 0.83; 95% CI: 0.67, 1.02, respectively)”.

C21. Discussion:

Page 14, 3rd section: The authors should include more discussion of strength and limitations in that this is an essential part of epidemiology.

R21. That section has been rewritten as follows “The prospective cohort study design, the AD diagnosis confirmed by two dermatologists, and the reliable laboratory assessments were strengths of our study. On the other hand, 23.2% of children were excluded from analyses due to missing data or loss to follow-up, resulting in reduced statistical power for sex-stratified analyses. However, there were no significant differences in basic characteristics between those remained in analyses and those excluded. Finally, unmeasured confounding is possible and residual confounding could not be ruled out”.

C22. Conclusion:

The conclusion should be modified according to the comments above (Page 11, 2nd paragraph, lines 1-4 and Table 5).

R22. The conclusion has been revised as follows “Prenatal exposure to PFOA, PFDoA and PFHxS was positively associated with childhood AD in girls during the first 24 months of life. In addition, the association between AD and prenatal exposure to PFNA and PFDA in girls were marginal significant”.

Reviewer 2

C1. Introduction:

1) Page 4, line 18: "toxicity, liver tumor, impaired thyroid function, and mammary glands"

Please clarify "mammary gland" here. Did you mean mammary gland development or differentiation?

R1. Thank you for your comments. A reference has been cited to suggest the effect of PFASs on the development of mammary glands accordingly.

C2. Introduction:

2) Page 5, line 9-10, please modify the sentence as follows: Given that fetuses are more vulnerable to PFASs and they might impair functions of the immune system [21], we investigated the effect of prenatal exposure to PFASs on …

R2. This sentence has been revised as follows “Given the facts that fetuses are more vulnerable to PFASs and PFASs might impair immune function [22], we investigated the effects of prenatal exposure to PFASs on AD in children up to 24 months of age in a prospective cohort study in Shanghai, China.”
C3. Page 9, about potential confounders; please mention how did you select these confounders? Previous studies, factors influencing exposure or outcome assessment in current data analysis, DAGitty etc?

R3. The potential confounders were selected based on DAG, and we have added explanation of choosing confounders as follows “Potential confounders were selected based on DAG, and included infant sex, parity (nulliparous and parous), birth weight, gestational weeks at delivery, mode of delivery, maternal pre-pregnancy BMI, maternal age, maternal education, maternal ethnicity, paternal age, paternal education, parental history of allergic disorders, paternal smoking during pregnancy, and family income”.

C4. Also, why did not the authors include breast-feeding history in adjusted model which is very important covariate?

R4. According to DAG, breast-feeding is unlikely a cause of the exposure variable (prenatal exposure to PFASs in this study), thus breast-feeding is not a common cause of both the exposure and the outcome, therefore, breast-feeding is probably not a confounder in the present study and was not adjusted.

C5. Results:

1) Page 10, line 1-2: "A total of 811 children completed all the follow-up visits at 6 months, 12 months and 2 years."

For harmonization, please change 2 years to 24 months.

R5. We have changed “2 years” to “24 months” as suggested.

C6. Results:

2) Page 10, line 4-5: "The majority of pregnant women had normal weight (BMI<24 kg/m2, 82.3%)…”

What is the reason to define BMI<24 kg/m2 as normal BMI (not BMI<25 kg/m2)?
R6. BMI was classified according to the disease risk-based body mass index classification for Chinese adults, which was developed and verified by the results of the 2002 China Nationwide Nutrition and Health Survey.

C7. Results:
3) Page 10, line 8: "29.7% of mothers were exposed to passive smoking during pregnancy."
Please define passive smoking clearly in Methods.

R7. The sentence has been revised as follows “However, 29.7% of mothers were exposed to paternal smoking during pregnancy”.

C8. Results:
4) Page 10, line 9-10: "Among the newborns, 417 (51.6 %) were boys; 598 (73.9 %) were delivered by cesarean section."

It seems that number of deliveries with cesarean section is very high (73.9%), is it true? May cesarean section influence the outcome (AD)? In adjusted models, the authors included mode of delivery as a confounder, however if mode of delivery can influence risk of AD, the authors should discuss this point in Discussion.

R8. The high cesarean section rate in China was true at that time, which was mainly due to maternal demand and lack of information on the advantages of vaginal delivery. A meta-analysis included 26 studies revealed a moderately increase in the risk of asthma in children delivered by cesarean section (OR=1.20, 95% CI 1.14, 1.26) (Thavagnanam S, et al. 2008). However, the effect of cesarean section on childhood was controversial. The effect of cesarean section on AD during the first 3 years of life was not significant (OR 1.35; 95% CI 0.74-2.47) in a birth cohort study (Papathoma E, et al. 2016). The OR of having AD in adolescents aged from 12 to 18 years born by caesarean section compared with vaginal delivery was 1.50 (95% CI 1.01-2.22) in a cross-sectional study (Yu M, et al. 2015). In the present study, the effect of cesarean section on AD was not significant (OR 0.94, 95% CI 0.63-1.39).
C9. Results:

5) Page 10, line 16-17: "The median (Q1-Q3) values of PFOA, PFOS, PFNA, PFDA, PFUA, PFDoA, PFHxS and PFBS were 6.98 (4.94-9.55), 2.48 (1.82-3.24), …"

In the current study, exposure level of PFOA is significantly higher than PFOS, is it reported in China previously? Please explain your speculation about this point in discussion. One possibility can be PFOS ban since 2009. Also, different transplacental passage efficiency.

R9. The median (Q1-Q3) concentrations of PFOA in cord blood plasma were 7.00 ng/ml (4.99-9.71), which was the most abundant among all the PFAS members in our study. PFOA level in our subjects was even higher than that reported in other countries (Wang, B. et al. 2016). Also, PFAS levels in tap water in Shanghai was higher than that in 19 cities in China, Japan, India, the USA, and Canada between 2006 and 2008 (Mak YL. et al. 2009). The higher PFAS levels in Shanghai may be due to higher levels of pollutants in Yangtze river because of the fluoropolymer factories nearby, especially in the Yangtze Delta.

C10. Results:

6) Page 11, line 6-8: "In the first fully adjusted model, a log-unit increase in PFOA was associated with a 2.1-fold increase in AD risk (AOR 2.09, 95% CI 1.14-3.83). The corresponding number for PFNA was 2.17 (1.06-4.46). In the second fully adjusted model, …"

There is one crude and one adjusted model shown in Table 4 and 5. I do not see second fully adjusted model, please clarify it.

R10. PFASs concentrations were log transformed, and analyzed as linear variables in the first fully adjusted model. Then PFASs were classified into quartiles and treated as categorical variables in the second fully adjusted models.

C11. Results:

7) The authors present sex-stratified results, but there is no mention of testing the modifying effect of sex. The fact that the relationship is significant in girls but not in boys does not mean that the associations are different. An interaction test, or a difference test is mandatory to establish if the relationship really differs between girls and boys. If the authors find significant p-
value for sex*PFASs (interaction), it is worthy to discuss sex differences. Please show it in Table 4 and 5.

R11. We have now tested the significance of the interaction sex*PFASs, and showed the results in Table 4. In the manuscript, a sentence has been added as follows “The significant modification effect of sex on the associations of interest was found only for the association between PFHxS exposure and AD. Thus, the effects of PFASs on AD were generally similar in male and female children”.

C12. Results:

8) Please show p-for trend when you applied quartile analysis (Table 4 and 5), it may provide better and more clear results.

R12. Tables 4 and 5 have been revised as suggested. It has been revised as follows “In the second fully adjusted model, when PFASs were grouped by quartiles, AOR increased with increasing PFOA and PFNA levels. The strongest associations were found in Q4”.

C13. Discussion:

1) Page 12, line 11: "infants conducted in Greenland and Ukraine, reported that PC5 score, dominated by …"

PC5? Principal component?

R13. PC is the abbreviation for Principal component, which has been revised in the manuscript.

C14. Discussion:

2) Page 12, line 12-15: "In contrast, a birth cohort study in Japan involving 2063 subjects found that prenatal exposure to perfluorotridecanoic acid (PFTrDA) was associated with a decreased risk of developing eczema in female infants at 24 months (OR= 0.39; 95% CI: 0.23, 0.64) [28]."

There is another study from Hokkaido birth cohort in Japan which found contrasting results with current manuscript. They followed up children in Okada et. al report (Ref. 28) and found that in
uterine exposure to long-chain PFASs, such as PFDODa and PFTrDA, showed negative association with eczema in 4-year-old children (Goudarzi et al. Env. Int., 2016) suggesting that prenatal exposure to PFASs are immunotoxic and may suppress immune system. Please cite this paper and compare PFAS exposure level of these two studies from Hokkaido birth cohort with the current study. Also, writing few sentences regarding your speculation on longer follow up of children in your study would be interesting.

R14. The reference has now been cited as follows “After following for another 2 years, PFOS levels in the highest quartile were associated with an increased risk of total infectious diseases in all children (OR= 1.61; 95% CI: 1.18, 2.21), while the highest quartile of PFHxS was associated with a higher risk of total infectious diseases only among girls (OR= 1.55, 95% CI: 0.976, 2.45). These studies provided evidence for the potential long-lasting effect of PFASs on children’s immune system”.

However, in the Hokkaido cohort study, PFASs were measured in maternal blood taken at 28–32 weeks of gestation, while PFASs were measured in cord blood taken at birth in our study. Thus, PFASs concentrations in our study may not be directly compared with that in the Hokkaido cohort study.

C15. Discussion:

3) Page 12, line 21-23: "In addition, a case-control study with 231 asthmatic and 225 non-asthmatic children in Taiwan found that PFOA and PFOS were associated with asthma in a dose-response pattern [13]."

Please specify range of age in this study.

R15. The range of age has now been added in the text as follows “In addition, a case-control study with 231 asthmatic and 225 non-asthmatic children aged 10-15 years in Taiwan found that PFOA and PFOS were associated with asthma in a dose-response pattern”.
C16. Discussion:

4) Page 14, second paragraph: With citing Ref. 38, and 40, the authors tried to explain mechanistic effects of PFASs on allergic diseases in humans through reproductive/steroid hormones. However, these studies have been conducted in adolescents and outcome is asthma.

A previous report showed that cortisol and cortisone (glucocorticoid hormones) concentrations were significantly lower in cord blood of infants with prenatal PFOS exposure in the fourth quartile compared with those in the first quartile. The highest quartile of prenatal PFOS exposure was positively associated with a significant higher DHEA (androgenic hormone and precursor of sex hormones) level compared with the lowest quartile, whereas PFOA showed a negative association with DHEA levels (Goudarzi et al. EHP, 2017). They concluded that prenatal exposure PFASs may disrupt glucocorticoid/androgenic ratio in fetus. Additionally, animal and human data suggest that perinatal glucocorticoid levels program the fetal hypothalamic-pituitary-adrenal (HPA) axis affecting its development, resulting in changes in HPA axis function that persist throughout life (Kapoor et al. 2008; Waffarn and Davis 2012).

Therefore, I suggest the authors to cite these references before references 38-40, explanation of influence of PFASs during pregnancy and early life can change hemostasis of steroid hormones which may result in hormone imbalance in later life. This approach is more clear, reasonable and understandable. Then, citing ref. 38 and 40 suggest that the influence of perinatal exposure to PFAS on allergic diseases may be still persistent in adolescents.

R16. Thank you so much for your advice. The citations have been added in the text as follows “A birth cohort study conducted in Japan between 2002 and 2005 showed that cortisol and cortisone concentrations were significantly lower in cord blood of infants with prenatal PFOS exposure in Q4 compared with those in Q1 (β=-23.98 ng/mL, 95% CI: -47.12, -11.99 for cortisol; β=-63.21 ng/mL, 95% CI: -132.56, -26.72 for cortisone). Meanwhile, the highest quartile of prenatal PFOS exposure was positively associated with a significant higher DHEA level (β=1.33 ng/mL, 95% CI: 0.17, 1.82), whereas PFOA showed a negative association with DHEA levels (β=-1.23 ng/mL, 95% CI: -1.72, -0.25) [39]. In addition, animal and human data suggested that perinatal exposure to glucocorticoid may program the fetal hypothalamic-pituitary-adrenal (HPA) axis affecting its development, resulting in long-lasting effect in HPA axis function [40, 41]. These findings revealed that prenatal exposure may have an effect on the balance of steroid hormones, which was one of the potential mechanisms of the immunotoxical effects of PFASs [42]”.
Thank you for your consideration of our manuscript.

Yours sincerely,

Jun Zhang