Author’s response to reviews

Title: Gestational exposure to endocrine disrupting chemicals in relation to infant birth weight: A Bayesian analysis of the HOME Study

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Response to Reviewers for manuscript ENHE-D-17-00050 “Gestational exposure to endocrine disrupting chemicals in relation to infant birth weight: A Bayesian analysis of the HOME Study”

We would like to thank the editor and both reviewers for their insightful comments. These were instrumental in guiding the revision process for this manuscript.

Response to the Editor:

Both reviewers have raised important methodological issues in the analysis of the data. Given the relatively small population, I view the analytical approach as the key new point of the paper. The suggested comparison with selected other approaches including needs to be part of a revised paper. Multi-class models should be included as well.

Drawing on your comments, as well as those of the reviewers, we revised portions of the methods and results section and included additional analyses using both LASSO and Elastic Net. Additionally, we redid the BHLM analysis using a multi-class model that included all 5 chemical classes in a single model. On page 12 we show that LASSO and Elastic Net yield regression coefficients that are larger in size but with less precise 95% intervals compared to BHLM because LASSO and Elastic Net do not use shrinkage estimation. LASSO and Elastic Net also do not yield estimates of μβ which is the average association between the exposure variables, within a chemical class and the outcome, with standard deviation σβ.
Response to Reviewer 1:

Identifying which contaminants, from a large set of potential culprits, have an effect on birth weight is a challenging task for which unfortunately at this time no standardized tools are available. The authors use BHLM reasoning it is a better approach than those that have been applied so far. The limited signal in the HOME study for associations between EDC and birth weight in combination with a rather abstract description of structure of the data and the decisions made during the statistical modeling made it difficult for me to gauge what the contribution to the literature of this analysis is. The discussion provided in the current manuscript provides no real insight into whether BHLM is the way to go for analyzing this type of data, but also does not provide much in terms of biological interpretation of the (null) results. I think much can be done in terms of additional analyses and better interpretation of the results to improve the contribution of this manuscript to the existing literature.

Thank you for the recommendation to incorporate additional methods to illustrate the merits of the BHLM methods. To better understand the impact of shrinkage and the choice of prior distributions on the BHLM results, we applied both LASSO and Elastic Net to the data. The results are described in the Results on page 12 and Figures A2 and A3 of the Supplementary Materials. We found that the LASSO regression coefficients tended to be larger in magnitude than BHLM with wider and less precise 95% intervals because LASSO does not use shrinkage that borrow information between chemicals within a particular class.

The application of BHLM in this paper is motivated by 'shortage of statistical tools to explore the effects of multiple chemical exposures' and limitations of other statistical methods that have been applied. I therefore would expect at least some sort of comparison of the current approach with an alternative approach to illustrate the improved performance of BHLM in this dataset. For example one of the methods referenced in the Agier paper or the approach that was applied earlier in this dataset for a different outcome (Braun et al. 2014. EHP).

We conducted a secondary analysis with two additional methods, LASSO and Elastic Net (pg9.ln4) and compared the results to BHLM and results are given in on pages 12-13 and Figures A3 and A4. "Additionally, to better understand the impact of the shrinkage of the BHLM on the regression coefficients, we re-analyzed the data using both LASSO (least absolute shrinkage and selection operator) and Elastic Net. We used the R package ‘glmnet’ [41], and the same potential confounders from Table 3 were included in the regression as unpenalized variables."
The statement that a model with all 5 classes simultaneously was not fit because it would complicate the interpretation of model regression coefficients is in contrast with the original aim of this study and also doesn't optimally use the benefits of applying a BHLM. The current explanation provided on page 8 is insufficient. Furthermore considering that parameter mubeta reflects the distribution of the average association per chemical group I would expect a different notation.

Thank you for the modeling suggestion. In this revised manuscript, the BHLM model was re-fitted using the multi-class model that includes all 5 EDC classes simultaneously in the model for birth weight (pg5.ln15; pg8.ln9). " The objective of this study was to use BHLM to simultaneously examine the average association between exposure to 5 different EDC classes (phthalates and BPA, PFAS, PCBs, PBDEs and OCPs), 2 OPPs and birth weight and measure the heterogeneity of those associations using data from the Health Outcomes and Measures of Environment (HOME) Study [35]. "; " All 53 exposure variables from 5 chemical classes were included in the final BHLM model for analysis."

No insight is provided into the correlation between EDC within and across groups. This is crucial information for the reader to understand the potential benefits of the current approach. Suggest to add this information as supplemental material.

Thanks for the suggestion to provide information about the correlations between and across EDC groups. We built a heat map illustrating these correlations using the R package corrplot and included this heat map in Figure A2 of the Supplementary Material (pg10.ln20)."Additionally, Figure A2 in the supplementary materials provides a heat map of the pair wise correlation coefficients between EDC biomarker concentrations."

It is unclear to me why SES is included in this paper. Please provide a better motivation or remove from the manuscript.

We removed the detailed discussion of SES from the manuscript. SES is only included when discussing controlling for confounders (pg 7.ln7)."Covariates in the BHLM of birth weight included: maternal race (white vs. black/other), age at delivery, infant sex, maternal education (<high school vs. >high school), tobacco exposure (<3ng/ml vs. ≥3ng/ml serum cotinine concentrations), household annual income (<$25,000 per annum vs. >$25,000 per annum), employment, maternal insurance status (public/none vs. private), marital status (single vs. living with a spouse or partner), pre-natal vitamin use (yes vs. no), and maternal BMI (underweight, normal, overweight, obese)."
BHLM is presented as a method capable of looking at mixture effects. I don't agree with this. This method comes with the strong assumption that all EDCs within a chemical class have a similar activity per unity weight also does not take into account potential interactions between EDCs (within and across groups).

You are correct in that BHLM is not able to account for potential interactions between EDCs. We described this as a limitations of the analysis (pg15.In 18). BHLM assumes that there is an average effect within a group that has some variance. This has the advantage that it allows for different chemicals to have different effects (i.e. effect heterogeneity) when we assume a larger variance (pg 16 ln 7-8). Moreover, BHLM addresses one of the key questions related to chemical mixtures: what is the potential effect of individual chemical exposures on human health after controlling for other correlated co-exposures.

p34.l20 - Why were the organophosphate pesticide metabolites (for which a significant effect on birth weight has been reported in a previous paper on this study) not included in this analyses?

We revised the manuscript to include the molar sum of diethylphosphates (DEP) and dimethylphosphates (DMP) in the analysis (pg 6.In19). "Building on a previous analysis of the HOME data [14], we examined OPPs as the molar sum of diethylphosphate (DEP) and dimethylphosphate (DMP) metabolite concentrations."

p4.l46 - I suppose the authors refer to "(Sparse) Partial Least Squares Regression"? In general, please make a clearer distinction between variable selection methods and dimension reduction methods as they serve different purpose.

Thank you for the comment regarding clarity of method. We edited these areas of the background section to clarify which methods were being discussed (pg 4.In 15-19). "Multivariable linear regression [5, 13, 25] with selected predictors [31] is another method, but can produce unreliable estimates due to the highly correlated nature of some EDCs [32]. LASSO [31] and Elastic Net are other more sophisticated multivariable linear modeling methods that have been used for analysis of multiple correlated exposure variables."

p4.l51 - PLS was not developed to identify specific 'predictors'. LASSO was specifically developed to reduce the number of false positive associations that is identified when analyzing a large number of prediction. If in this paper BHLMs are presented as a better performing alternative to LASSO or similar variable selection methods, more justification is needed for this statement.
We deleted the statement about PLS to avoid confusion (pg 4.ln15). We revised the manuscript to include both LASSO and Elastic Net to serve as comparisons to the BHLM and illustrate the influence of shrinkage from the priors on the beta coefficient estimates and 95% interval estimates. The results are given in Supplementary Figure 1. When comparing LASSO and Elastic Net with BHLM, we found that the LASSO and Elastic Net results were larger in size with less precision than the BHLM results (pg13.ln 6-8). All of these methods have strengths and limitations; because our primary aim was to attempt to examine a whole class of EDC as a class, we used BHLM as our primary statistical method. It may be that in order to understand the interplay between the EDCs as a group and individually that more than one method is needed.

p4.l54 - I think it is very important to stress what the purpose of the current analysis was: it is to identify the joint effect of multiple exposure in a certain class on birth weight, or is it to identify which specific exposures are associated with birth weight (a variable selection approach).

Thank you for this suggestion to emphasize the purpose of the analysis. In the revised manuscript, the wording and stress around the purpose of this study was clarified at the end of the background material, which is to use BHLM to simultaneously examine the average association between exposure to 5 different EDC classes (phthalates and BPA, PFAS, PCBs, PBDEs and OCPs), 2 OPPs and birth weight and measure the heterogeneity of those associations using data from the Health Outcomes and Measures of Environment (HOME) Study (pg5.ln16). In an attempt to determine which chemicals drive those averages, or which in a joint mixture might have more weight, we also examined the individual associations between EDCs and birth weight.

p5.115 - "Which can be measured" - do you mean the prior distributions can be updated by applying the model to the data? Suggest to rephrase.

In the revised manuscript, we clarified this sentence to reflect that the hyperparameters can be measured using the data (pg5.ln13). " The prior distribution depends on hyperparameters, including a mean $\mu_\beta$ and standard deviation $\sigma_\beta$, which are informed by the data and can be quantified when fitted to the data."

p6.133 - Please provide some insight into the stability of phthalate and BPA measurements e.g. by providing an ICC.

Thanks for highlighting the stability of non-persistent chemicals including phthalates. Braun et al. (2012) examined the stability of these two chemicals, which we cite in the paper (pg6.ln15 - " If a woman provided multiple urine samples at both 16 and 26 weeks gestation,
then the sample concentrations were averaged following log10 transformation and treated as continuous variables [35, 38]. They reported an ICC between phthalates and BPA of 0.11. The fact that we chose to include both persistent and non-persistent chemicals in our study does bring with it some limitations, notably that there are different degrees of error associated with measuring each and that chemicals with larger degrees of error associated with their measurement are more likely to be found "null" in statistical models.

p6.l41 - I think it is too bad that samples at 16 and 26 weeks were pooled rather than used to explore the stability in the association or the effect of gestational age. What is the impact of using the 16 wk gestation measurements vs. the 26 wks measurements?

Thanks for your question. While Braun et al. (2009) and others from our group have examined periods of heightened vulnerability, we feel that this is beyond the scope of our work focusing on mixtures. However, we agree that this is an important issue and one that we have mentioned in the discussion. Finally, because of the potential for more measurement error of these non-persistent chemicals, the pooled measurements allow us to reduce exposure misclassification.

p8.l9 - The notation confuses me. You are using the same index for mu and sigma, while sigma can be group specific.

μβ is defined as the average association of the regression coefficients to the independent variable and σβ is defined as the standard deviation of the regression coefficients (pg8.ln11). We adjusted this section of the paper to emphasize that both μβ and σβ are group specific model parameters (pg8.ln13). For example for the PCB class, the quantity μβ is the average of the regression coefficient for the PCBs, whereas σβ is the standard deviation of the regression coefficients for phthalates. On page 5 we added a sentence to help clarify the notation. " The prior distribution depends on hyperparameters, including a mean μβ and standard deviation σβ, which are informed by the data and can be quantified when fitted to the data. The quantity μβ is the average association between all regression coefficients and the outcome, whereas σβ captures the heterogeneity of effects."

p8.l9 - Your choice for a truncated normal distribution with a mean zero and variance 105 for hyper prior sigma is not clear. Essentially, this translates to a uniform distribution, correct? By following this approach there seems to be no benefit of grouping your exposures into a chemical class. Can you show the degree to which shrinkage occurred?
You are correct that we assigned relatively uninformative prior distributions to the hyperparameter $\mu_\beta$ and $\sigma_\beta$ which take the form of diffuse normal distributions (pg8.ln11). However, it's important to emphasize that the data are very informative about the values of $\mu_\beta$ and $\sigma_\beta$ (pg5.ln6 - "The prior distribution depends on hyperparameters, including a mean $\mu_\beta$ and standard deviation $\sigma_\beta$, which are informed by the data and can be quantified when fitted to the data. The quantity $\mu_\beta$ is the average association between all regression coefficients and the outcome, whereas $\sigma_\beta$ captures the heterogeneity of effects." ). For examples, for phthalate and BPA, the posterior mean of $\mu_\beta$ is -2 g and the posterior mean of $\sigma_\beta$ was 15g. These estimated values of $\mu_\beta$ and $\sigma_\beta$ shrink the phthalates and PBA regression coefficients estimates towards 2 grams.

p8.l15 - The statement "we did not fit a model with all 5 EDC classes simultaneously..." is insufficient. Please provide a better reasoning for this decision. If you believe there was no confounding possible whatsoever between chemical groups, then why not include them in the same model?

In our response to one of your earlier comments, we indicated that we addressed this by redoing our BHLM analysis with all 5 classes simultaneously.

p10.l17 - Please rephrase "the estimate was significantly lower than zero" - are you referring to the upper limit of the 50% credible interval?

Your comment prompted a review of that section which resulted in revising it to be more explicit with regards to the 50% credible interval (pg11.ln5). "Although the 95% CI for $\mu_\beta$ for PFAS did cover zero, the estimate was significantly lower than zero based on the upper limit of the 50% CI (-21, -2)."

p10.l29 - Please specify what you mean with "information lost in the average based parameter"

Thanks for your suggestion. We edited this sentence to provide further clarity (pg11.ln16). "In order to better understand which individual EDCs within a class drive the value of $\mu_\beta$, we examined associations between individual EDCs and birth weight, given by the posterior distribution of the BHLM regression coefficients $\beta x_1 ... \beta x_p$. (See Figures 1 and 2)."

dp15.l23 - It is not clear to me what the authors try to say with this statement
The wording in this sentence (pg14.ln7) was edited to provide more clarity. "A second advantage of reporting μβ is that it prevents the investigator from only reporting chemicals that are significantly related to birth weight by virtue of it being the average association between a class and the outcome. For example, rather than sorting through 23 PCBs to identify those that are significant, we simply reported the average association of all 23 PCBs and birth weight."

p16.l4 - You have the data and the model would arguably improve by incorporating repeated biomarker measures, why did you not do it?

Thanks for asking about the inclusion of repeated biomarker measures. The Shoaff et al. 2016 study examined this question and illustrated that there is not a substantial change in the analysis results from incorporating repeated measures. Additionally, as provided in an earlier response, using the pooled averaged allowed us to decrease the risk of misclassification errors. Finally, a subset of the exposures included in the HOME data were only measured once, thus a pooled measurement was the only way to include those exposures (pg6.ln11 - "Phthalate and BPA markers were measured twice, while remaining biomarkers were only measured once.")

The last sentence of the conclusion does not reflect the work that was done, but an opinion by the authors - suggest to modify.

We deleted the sentence.

Response to Reviewer 2

The authors conclude that more studies with larger sample size than the current one are needed to explore the association between PFAS and lower birth weight; however, in the introduction they say that "There is sufficient evidence that increased PFAS (especially PFOA) exposure is associated with low birth weight" and include a reference of a meta-analysis of 9 studies with more than 4000 subjects included.

Thanks for bringing this to our attention. The phrasing around needing more studies has been removed (pg2.ln19-21). "Gestational exposure to phthalates, PFAS, PCBs, PBDEs, OCPs or OPPs had null or small associations with birth weight. Prenatal OPP, Pb, and PFAS exposure was most strongly associated with lower birth weight."

BPA is not mentioned in the abstract although it is included in the analysis.
BPA has been added to the abstract (pg2.ln8). "This study aimed to examine the
association of gestational exposure to five chemical classes of potential EDCs: phthalates and
bisphenol A, perfluoralkyl substances (PFAS), polychlorinated biphenyls (PCBs),
polybrominated diphenyl ethers (PBDEs), and organochlorine pesticides (OCPs), with infant
birth weight."

This sentence confuse the reader a little bit since it seems that all are non-persistent; please
rewrite: "Five important EDC classes are non-persistent compounds like phenols (e.g., bisphenol
A [BPA]) and phthalates, polychlorinated biphenyls (PCBs), perfluoroalkyl substances (PFAS),
polybrominated diphenyl ethers (PBDEs), and organochlorine pesticides (OCPs)."

Thank you for your suggestion to remove remarks about the persistence of chemicals. We
have removed that phrasing from the manuscript, especially in the introduction (pg3.ln7). "Among EDCs, broad classes include phthalates and phenols (e.g., bisphenol A [BPA]),
perfluoroalkyl substances (PFAS), polychlorinated biphenyls (PCBs), polybrominated diphenyl
ethers (PBDEs), and organochlorine pesticides (OCPs)."

Start describing BPA before phthalates to always follow the same order.

In re-writing the manuscript, the order of phthalates and BPA has been made consistent
through the entire paper.

In the first paragraph of the introduction I miss an explanation why we have to be worried about
EDC exposure during pregnancy (not only is because they widespread use).

Thanks for asking about this. This section of the introduction was edited to provide
greater emphasis that, in addition to the ubiquity of EDCs, the developmental vulnerability of the
fetus prompts the concerns around the effects of gestational exposure (pg3.ln15). Additionally,
some EDCs, such as PFAS, have been associated with birth weight." The vulnerability of the
developing fetus [15] coupled with the pervasive nature of EDCs has raised concerns about
reproductive health effects arising from gestational EDC exposure such as low birth weight
[15]."

In my opinion the section of the description of the previous studies is difficult to follow. The
authors do not mention BPA studies for example although the authors BPA mentioned it as the
first EDC in the introduction.
This section of the paper was revised to provide greater clarity and studies reviewed now include BPA as well (pg3.ln21 - pg4.ln.9). "Many investigators have explored the relationship between EDC exposures and birth weight [17]. Lenters et al. examined the relationships between 16 chemicals (6 phthalates, 8 PFAS, 2 PCBs and 1 OCP) and birth weight using Elastic Net Regression analyses; 2 phthalates, 1 PFAS, 1 PCB and 1 OCP were associated with lower birth weight [17]. There is sufficient evidence that increased PFAS (especially PFOA) exposure is associated with low birth weight [17, 18, 19], but mixed results are reported for other EDCs and birth weight [17, 21]. For example, Birks et al. (2016) in a meta-analysis of European birth cohorts, found that while pregnant women exposed to more than 1 EDC class were more likely to have a low birth weight infant [21]. Another meta-analysis by Govarts et al. (2016), found an association between certain PCBs and low birth weight [22]. A pattern of inconsistent associations exists for phthalates and BPA [23, 24, 25], OCPs [17, 26], PBDEs [27, 28] and OPPs [14]."

Please revise other papers assessing the association between PCBs and birth weight. I suggest the recent review of Vrijheid et al 2016 (Int J Hyg Env Health) which includes most of them.

Thank you for suggesting this article as a reference. We revised the manuscript to use this as one of the references examining the association between PCBs and birth weight.

I am wondering if it would be possible that in order to compare the results with the Lenters' study, the authors could apply the elastic net regression analysis in their study.

Thank you for your suggestion, which mirrored an earlier comment made by Reviewer 1. When we revised the manuscript, we conducted an analysis of the data with both Elastic Net and LASSO (pg9.ln4). Both our analysis and the Lenters analysis identified phthalate MEOHP during the variable selection process, but the regression coefficients associated with that EDC were different. Where Lenters found a -0.15g difference, which after adjusting for gestational age was estimated to be 0, we found a 13g increase (Figure A4).

I recommend imputing the values below the LOD rather than reply them by LOD/√2.

Cole et al. (2009) found that if <25% of the data is below the LOD, that is, the frequency of detection is >75%, replacing the values below the LOD with the square root of the LOD still provides 96% coverage of the OR (pg6.ln18). Of the 53 EDCs included in our study, the detection percentages range from 27% (β-HCH) to 100% (several). Most of the EDCs included in our study had a detection percentage of >75%.
Please, explain the method to select the confounders included in the analysis.

We drew a directed acyclic graph to select confounders based on the relationships between the covariates collected in the HOME Study and EDCs and birth weight (pg7.ln6). The diagram is given in Figure A1 of the Supplementary Materials.

In my opinion this sentence confuses the reader since the objective of the study is to analyze the effect of multiple EDCs and not each EDC group separately: "We did not fit a model with all 5 EDC classes simultaneously because it would make it more difficult to interpret the model regression coefficient".

In our response to an earlier comment from Reviewer 1, we recognized the importance of including this model in the revised manuscript (pg8.ln5)."All 53 exposure variables from 5 chemical classes were included in the final BHLM model for analysis."

The authors should explain why they perform these 5 different groups of EDC (and also why phthalates and BPA - non-persistency should not be the main reason). There are new approaches suggesting to pool them based on the mode of action (please see Sharma et al Env Int 2016 as example).

We chose to group the chemicals in that fashion because many of them are metabolites of the same parent chemical and share similar chemical composition, providing a 'cleaner' classification. Additionally, for some of the chemicals, the mode of action is unclear or there may be multiple modes of action (see review by De Coster and van Larabeke), so grouping them by mode of action would be, at least in part, arbitrary, whereas the parent chemical - metabolite relationship is known.

It would be nice to see the results stratified by sex.

We included the stratified results in the Supplementary material for the revised manuscript in Tables A2 and A3 (pg13.ln6-9). " GA is a potential mediating variables on the causal pathway between EDC exposure and birth weight, while sex is a potential modifier. Controlling for mediators may attenuate the total effect of EDCs on birth weight. Accordingly, we conducted sensitivity analyses without controlling for GA (Table 3) and stratified the BHLM results by sex (Tables A1 and A2)."
This sentence confuses since before they have mentioned associations with two phthalates (MEOHP and MCPP): "There was no overall pattern for phthalates".

That sentence has been removed and that section of the results re-written so as to improve clarity (pg11.ln19-pg12.ln2). "In the phthalates and BPA class, there was a 9g birth weight increase in response to a 10-fold increase exposure to MEOHP, and a 10-fold increase in MEHP exposure was associated with a 7g decrease in birth weight. None of the phthalates or BPA were individually statistically significant at the 0.05 or 0.5 level, which may explain why the class as a whole was not significantly different from zero."

Please, try to avoid these type of sentences: "Lastly, there was a range of associations between individual OCPs and birth weight".

Thank you. This sentence was re-written to avoid repetition with the rest of the paragraph (pg12.ln10-13). "The largest increase in the OCP class was seen with a 10-fold increase in HCH exposure (5g), while the largest birth weight decrease was seen in response to a 10-fold increase in Nonachlor exposure (10g). None of the 95% CIs for the OCP class excluded zero."

I do not know what the authors would like to mean in this sentence: "… and the factors mediating social disparities are poorly understood".

Thanks for this comment. We remove the discussion of SES entirely from the manuscript.

In my opinion I will not include ref 15 as a study assessing the association between PCBs and birth weight because it uses fish consumption as a proxy of PCB exposure.

In revising the paper to address an earlier comment regarding the references around PCBs, this was addressed as well.

Please, include a paragraph comparing the levels of the different EDCs in the HOME study with other study populations. I think it is important to emphasize that PFAS exposure in the HOME study are particularly high in comparison to other study population and maybe this is the reason why the authors only found associations with these EDCs.

We appreciate this suggestion. We have added a short paragraph that highlights the work done in a previous HOME study paper comparing the EDC concentrations from the HOME
Study to the national exposures in the NHANES study (pg10.ln12). "To provide some context of the HOME Study exposures, we compared them to the women's national average as reported in the NHANES study (as reported in [10]). With few exceptions (e.g., PFOA), median concentrations among HOME Study tended to be similar to concentrations observed in US women."

Table 2 is cited previous to Table 2. Please, consider changing the order.

In revising the manuscript, we ensured that tables and citations were in the proper order.

It would be nice if Table 2 includes a column with the LOD of each pollutant.

Thank you for this suggestion. The LODs for PBCs and PBDEs varied depending on the sample volume provided by each participant, so we have followed the example of other recent research articles using HOME (Braun et al., 2014 and Braun et al., 2016) and included the percent detected above the LOD from the total sample.

Numbers are confusing since Table 1 shows a sample size of 384 and Table 2 of 319. I recommend to impute missing values in covariates so then the different models will have similar sample sizes.

Thank you for drawing this to our attention. We revised the manuscript so that all references to the sample size for this study are uniform, including the tables.