Author’s response to reviews

Title: Using three statistical methods to analyze the association between exposure to 9 compounds and obesity in children and adolescents: National Health and Nutrition Examination Survey 2005-2010

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Reviewer #1:
The Manuscript (MS) "Using three statistical methods to analyze the association between exposure to 13 compounds and obesity in children and adolescents: National Health and Nutrition Examination Survey 2005-2010" is a study to investigate the association between 13 different chemical exposures and obesity in children and adolescents via applying three different statistical methods. The authors did a lot of work, however, there are still some problems and shortcomings in this MS. In general, the study is not ready for publication.

Major comments:
1. The association between 2,5-DCP, MEP and MiBP and obesity has been well documented, leading to the main results and conclusion without attraction. On the other hand, different models are applied to specific situations, rather than with the purpose of finding significant differences.

Response: Thank you for pointing this out. There are indeed some studies focusing on the association between 2,5-DCP, MEP and MiBP and obesity. However, most of the earlier studies only focused on only one group or singular exposure but the focus of our work is on the mixed exposures, which is more commonly in reality. Thus, we select three different models: generalized linear regression, weighted quantile sum (WQS) regression, and Bayesian kernel machine regression (BKMR). The reason why we select BKMR is that BKMR could well estimate exposure-response functions that included both nonlinear and non-additive effects, BKMR could identify important mixture components through variable selection, and the hierarchical variable selection approach could detect important groups of highly correlated exposures even in situations where individual components could not be identified, which has been reported in previous study. Bobb JF, Claus Henn B, Valeri L, Coull BA: Statistical software for analyzing the health effects of multiple concurrent exposures via Bayesian kernel machine regression. Environmental health : a global access science source 2018, 17(1):67.
WQS regression is a new method that allows the investigation of the associations between mixtures of predictors and continuous, count, or categorical data. This approach is particularly robust against outliers and extreme values because of the ranking procedure used, and is additionally robust against collinearity through the constraints imposed during weight estimation and application of an ensemble estimation procedure. Renzetti S, Gennings C, Curtin PC. 2019. gWQS: An R Package for Linear and Generalized Weighted Quantile Sum (WQS) Regression. Journal of Statistical Software.

2. The authors excluded 220 participants with missing data on BMI and covariates from analysis. Considering BMI is highly correlated to the outcome variable obesity, participants without data on BMI have to be excluded. However, it hasn't been shown exactly what covariates are the study defined, and the proportion of the exclusion is considerably low, why the imputation of missing covariates wasn't used? The reasons should have been given.

Response: age, gender, race, education level, family income-to-poverty ratio, caloric intake, serum cotinine, and urinary creatinine are treated as covariates in our study. To fully utilize the data, using imputation of missing covariates is a good idea. Considering your advice, we carefully reexamine the data and exclude the participants with missing data on covariates, which account for nearly 10%. Since our study mainly to compare the three different models when analyzing the association between mixed exposures and the obesity to highlight the using of BKMR and WQS model, we consider using the actual data would be better. And the detailed information about the exclusion process are added as follow: “We selected participants between 6 and 19 years old, with attainable measurements of urinary phenols, parabens, pesticides, and phthalate metabolites Body mass index (BMI) and waistcircumference simultaneously (n=2629) and excluded the participants whose data on covariates, including age, gender, race, education level, family income-to-poverty ratio, caloric intake, serum cotinine, and urinary creatinine, were missing (n=257). Finally, 2372 participants were included in our study.”

3. Given caloric intake contributes significantly to the development of obesity, is it necessary to divide the participants into different groups according to the level of caloric intake in the phase of experimental design? Or just like the authors do, control caloric intake as a covariate in the phase of statistical analysis? The author should discuss more about the difference.

Response: Thank you for point this out. Since we didn’t find a explicit method to deal with the caloric intake to our knowledge, we decided to control caloric intake as a covariate just like previous studies did.


Minor comment:
1. As the authors described, the BMI z-score was calculated in regards to the children's age, gender, and BMI. Is there any contradiction between age and gender being used to calculate the BMI z-score and the inclusion of age and gender as covariates at the same time? The author also need to explain the potential effect of multicollinearity.

Response: We further analyze the correlation between BMI z-score, age and gender, and the result are as follow: correlation coefficient between BMI z-score and age, BMI z-score and gender are 0.014, 0.033 respectively. Thus, the potential effect of multicollinearity is not obvious. Thus, we include age and gender as covariates like previous study: Buser, M. C., et al. (2014). "Association of urinary phenols with increased body weight measures and obesity in children and adolescents." J Pediatr 165(4): 744-749.

2. The authors defined a child to be obese when their BMI was above or equal to the 95th percentile for their age and gender. More references should have been listed to certify the reasonability of the definition.

Response: We refer to Centers for Disease Control and Prevention (CDC) and we change as follows: “We defined a child to be obese when their BMI was above or equal to the 95th percentile for their age and gender in accordance with the CDC recommendations” Defining Childhood Obesity [https://www.cdc.gov/obesity/childhood/defining.html],(accessed 31 March)

3. There supposed to be an improvement on the format of table3 and table4. It's so dizzy and unclear to put the OR effect and 95%CI in two lines. I suggest combining the OR effect and 95%CI in row just like the authors do in table1.

Response: After uploading, the format of the table may have changed. It’s our negligence not to notice this before. We have adjusted the format of tables3 and tables4.

4. The results are partly incomprehensible. For example, what statistical methods are used, why exclude these confounding factors, the explanation of the tables is not detailed enough and so on. This part needs to be improved.

Response: There are some places that we did not write clearly. We use obesity model and BMI z-score model separately referring to the outcome obesity and BMI z-score in the results. The reason for excluding these confounding factors are based on previous studies. And we add some explanation in the footnote of the table.

Reviewer #2: This study evaluated the association of 13 environmental exposures with BMI and obesity. The authors found associations between 2,5-dichlorophenol, monoethyl phthalate and mono-isobutyl phthalate exposure and obesity. This is a relevant topic given the increasing burden of obesity worldwide and the prevalence among children and adolescents. This work is also relevant given that environmental chemicals such as phthalates or pesticides have traditionally been studied individually. It includes novel statistical methods that are becoming popular in the environmental epidemiology field to address chemical mixtures rather than
individual chemicals and explore the joint effects. The manuscript is rich in content and includes state-of-the-art methods, as well as results that could be relevant for public health. The authors also provide the data used for the study, which is helpful for replication. However, from my perspective, this work needs to address important issues in order to be suitable for publication. For instance, the manuscript could be better organized and needs further details in some areas. In addition, I am particularly concerned about the model specification for BKMR. Please see detailed comments below.

Major comments:

1. Introduction: Please make clear which environmental exposures you are studying in the introduction. E.g: "We studied 13 chemical exposures including phenols (BPA, BP-3), parabens (BuP, EtP, MeP, PrP), pesticides (2,5-DCP, 2,4-DCP, 2,4,5-TC, 2,3,6-TCP) and phthalate metabolites (MBzP, MEP, MiBP)."

Response: we make clear which environmental exposures we are studying in the introduction and is as follows: "We studied 9 chemical exposures including phenols (BPA, benzophenone-3 (BP-3)), parabens (methylparaben (MeP), propyl paraben (PrP)), pesticides (2,5-DCP, 2,4-DCP) and phthalate metabolites (Mono-benzyl phthalate (MBzP), MEP, MiBP)."

2. Methods, page 6, line 15: Does NHANES have other adiposity measures that could be compared to BMI, for instance, body fat?

Response: NHANES also has waist circumference but similar guidelines to categorize obesity according to waist circumference are not available for children and are thus not reported. To our knowledge, subscapular skin fold and triceps skin fold were also measured in NHANES. But we think using BMI to evaluate obesity may be more accurate thus subscapular skin fold and triceps skin fold are not used in our study.

3. Methods, pages 7 and 8: Please include references that develop in a more detailed way the Weighted Quantile Sum regression and the BKMR (both Gaussian and probit) methods.

Response: we cite the references about Weighted Quantile Sum regression and the BKMR in the manuscript as follows:
4. Methods, page 9, line 4: 1000 iterations seems like a small number for probit BKMR, which tends to converge much slower. You don't talk about model diagnosis in the whole text, did you look at the traceplots? The traceplots should be showing a more or less homogeneously covered space to ensure the convergence is good. You can find how to get traceplots here: https://jenfb.github.io/bkmr/overview.html.

Response: We change the iterations from 1000 to 10000, and the results are showed as follows: "To ensure the convergence, we plotted the trace plots, which showed a more or less homogeneously covered space and indicated our model had a good convergence. (see Additional File 1, Fig. 1 and Fig. 2)"

5. Results, page 10, line 8: In the correlations figure (figure 1), please put the numbers (the correlations) in each square for each pair of chemicals. I would consider r=-0.06 as no correlation rather than a negative correlation. In addition, I would consider 0.81 a high correlation rather than a moderate one. Please use the word "correlation" rather than "association" consistently in that paragraph.

Response: It is our negligence to forget putting the correlations coefficient in the figure, and we add it in the figure 1. And we change the expression as follows: “We found significant correlations (P < 0.05) among 9 chemicals (Fig. 1), in addition to the correlation between BP-3 and 2,4-DCP (r=0.69). There was a positive correlation between other compounds, except for a nearly no correlation of BP-3 with 2,5-DCP (r = -0.06). 2,5-DCP was found to have a strongly correlation with 2,4-DCP (r = 0.87). Additionally, a high correlation between MeP and PrP (r = 0.81) was found.”

6. Results, page 11, line 7: Why are you considering BuP, EtP, 2,4,5-TCP and 2,4,6-DCP in a different way in Supplementary Tables S1 and S2? Why do you have two references (quartile 1 and quartile 2) for those chemicals? Unless there is a specific reason for doing this (which you should explain both in the text and in the Tables' footnotes), please consider all chemicals to have the same reference (quartile 1).

Response: The reason is that BuP, EtP, 2,4,5-TCP and 2,4,6-DCP have a low detection frequency, thus the quantile 1 and quantile 2 have the same value (replaced by the value of the limit of detection divided by the square root of 2). Considering the low detection frequency, we finally decided to delete these 4 exposures (BuP, EtP, 2,4,5-TCP and 2,4,6-DCP) to get a more reliable result.

7. Results, page 13, line 52: "In the phthalate metabolites group, MEP drove the main effect of the whole group (CondPIP: 0.403)." I would not consider that as driving the effect of the whole group as the other two chemicals have PIPs 0.312 and 0.285, which are very similar.

Response: Since we reanalyze the BKMR, and the result is showed in the Table 6, we change the content of the result as follows: “ In the phthalate metabolites group, MEP drove the main effect of the whole group (CondPIP: 0.656)”. MBzP and MiBP have PIPs 0.016 and 0.328 in the phthalate group (Table 6).
8. Results, page 13, line 59: In Figure 3 a and b, the credibility intervals are huge. Again, I am concerned about the model convergence. BKMR is a very sensitive model to initial values and number of iterations, especially in the probit version. It seems like your model did not converge properly. If you run the model with more iterations or change the tuning parameters, you might get tighter credibility intervals and more reliable PIPs. Also, you should check the acceptance rates of the tuning parameters (you can see that by setting verbose=TRUE in the function call). The r.jump2 parameter is particularly important. Ideally, r/delta2 should be between 20-45%. If the values are too high, you should try higher values of r.jump2 in the function call to see if it moves closer to the target window. This will (generally) lead to a faster convergence.

Response: We change the iterations from 1000 to 10000, and we still find the acceptance rates higher than 45%, thus we change the r.jump2 parameter from 0.1 to 0.2 in the BMI z-score model, and the acceptance rates fall into the target window. Also, in the obesity model, we change the iterations from 1000 to 10000 and r.jump2 was set 0.001 and the acceptance rates fall into the target window. Meanwhile, the credibility intervals narrow in Figure 3 a and b.

9. Results, page 13, line 59: I don't think we can talk about a positive tendency having such wide credibility intervals.

Response: After change the iterations to 10000, we get a narrow credibility intervals. Although there is still no statistical difference, we can find a positive trend.

10. Results, page 14, line 2: In the pesticides (for obesity) and phthalates (for BMI) groups, it seems like one of the chemicals drives the whole effect. It would be helpful as a sensitivity analysis to introduce those chemicals as single exposures (disregarding the others from the phthalates and pesticides groups) in common with the group of phenols and the group of parabens in a model and see what happens with the association of the mixture. If the credibility intervals tighten, it might mean that the other exposures in the pesticides and phthalates groups are not relevant for the outcome.

Response: We further model 2,5-DCP and other groups (phenols group, parabens group, and phthalate group). Besides, we also model MEP and other groups (phenols group, parabens group, and pesticides group). In Additional File 1, Fig. 3 a and b, the credibility intervals tighten a little. This is consistent with our new conclusion “Comparing the three statistical models, we found that 2,5-DCP and MEP may play an important role in obesity” for 2,4-DCP (in pesticides group) and MiBP, MBzP (in phthalate group) show little relevance for the outcome both in WQS and BKMR sensitivity analysis.

Minor comments:

11. Line numbering: Please next time make sure you enable continuous line numbering through the whole manuscript (without starting over in every page). It is easier for reviewers and editor.

Response: We enable continuous line numbering through the whole manuscript.
12. Abstract, page 1, line 39: "too much environmental endocrine disruption may increase the occurrence of obesity". Delete "too much".

Response: We delete “too much” in the former manuscript.


Response: Since we use both obesity (obesity or not) and BMI z-score as the outcome, we fit both logistic regression and linear regression. Thus, we think we use generalized linear regression may be better.

14. Abstract, page 2, line 18: You are missing a P = (P = 0.001).

Response: The missing “P” is added.

15. Abstract, page 3, line 1: "2,5-DCP, MEP, and MiBP may play an important role in the 13 exposures." They play an important role in obesity / BMI, not in the 13 exposures.

Response: We change the expression as follows: “Comparing the three statistical models, we found that 2,5-DCP, MEP, and MiBP may play an important role in obesity.”

16. Introduction, page 3, line 22: "Obesity is a pandemic disease that cannot be ignored". I would delete this sentence. I don't think we can say that obesity is nowadays ignored by physicians and scientists (as could be other diseases or health disorders).

Response: We delete this sentence in the text.

17. Introduction, page 3, line 37: "play an essential part". I would rather say "an essential role".

Response: We change the expression "play an essential part" for "an essential role" in the text.

18. Introduction: Please justify the reason why you chose those three statistical methods. There are many more that could be used for this purpose.

Response: We add additional content as follows: “Among them, WQS regression and BKMR model can resolve the non-linear and complicated interactions between chemical exposures and get more accurate results comparing with the generalized linear regression[23, 24]”


19. Methods, page 4, line 59: Please insert a reference about NHANES, especially about the multi-stage stratification probability sampling design, which people might not be familiar with.

Response: We insert a website about NHANES as follows: "https://wwwn.cdc.gov/nchs/nhanes/tutorials/module2.aspx".

20. Methods, page 7, line 11: Please use the word "multivariable" instead of "multivariate" through the text. While multivariable means you have multiple predictors, multivariate means you have multiple outcomes (See Hidalgo et al 2013: Multivariate or Multivariable Regression? (American Journal of Public Health)).

Response: "multivariate" is replaced by "multivariable" through the text.

21. Methods, page 8, line 34: "healthy outcomes". Delete the "y".

Response: We correct this word in the manuscript.

22. Methods, page 8, line 56: "We grouped the chemical exposures into three groups, according to the resource and correlation with each other". Change the word "resource" for "source". Please describe the groups and the correlations between the chemicals in each group (you list the chemicals in each group in the tables but not in the main text).

Response: We change the expression as follows: "We grouped the chemical exposures into three groups (group1: BPA, BP-3, MeP, and PrP; group2: 2,5-DCP and 2,4-DCP; group3: MBzP, MEP, and MiBP), according to their source and correlation (chemical exposures with high correlation were grouped) with each other."

23. Results, page 9, line 27: Add the % of obese people to Table 1 (in addition to the number).

Response: We add the proportion of obese people to Table 1.

24. Results, page 9, line 40: You don't need descriptives of the z-score of the BMI, it doesn't add any valuable information beyond the untransformed BMI descriptives.

Response: In order to make it convenient for readers to understand, we think it might be better to distinguish BMI and BMI z-score in the paper.

25. Results, page 10, line 1: "geometric transformation". Do you mean "geometric mean"?

Response: Yes, “geometric concentration” mean “geometric mean”.

26. Results, page 10, line 26: I would change the heading "Total" in table 3 for "Continuous" or explain that total means continuous chemical variable in the table footnote.
Response: We add “total means continuous chemical variable” in the table footnote.

27. Results, page 10, line 31: "...for children and adolescents are shown in Table 3 and 4, respectively". This looks like you have done separate analyses for children and adolescents. Please make clear that the distinction is between BMI and obesity.

Response: We change the expression of former content as follows: “The results from the multivariable logistic and linear regression models adjusted for the covariates are shown in Tables 3 and 4, respectively.”.

28. Results, page 11, line 16: Change the word "variate" for "variable".

Response: We change the word "variate" for "variable"

29. Results, page 12, line 1: Given that changes can be observed from model 1 to model 2 (especially for BMI z-score), you might want to fit three models instead of two: model 1 as you are considering it, model 2 additionally adjusting for cotinine and caloric intake, and model 3 additionally adjusting for socioeconomic status (SES) variables: education levels and family income-to-poverty ratio, to see if smoking / caloric intake are the ones that make a difference or, conversely, SES is more related to obesity. At the very least, it would be important to mention in the discussion what variable is mainly responsible for the change in the associations.

Response: We delete the chemical exposures of low detection frequency and remodel three WQS models. But at this time, we find the difference in three models is little, especially in the obesity model (all find a statistical difference). And in the BMI z-score model, all the three models find no statistical difference. Thus, it’s hard to discuss what variable is mainly responsible for the change.

30. Results, page 12, line 15: "1.61 (1.25 ~ 2.07)", change the ~ sign for ",".

Response: We change the ~ sign for ",".

31. Results, page 12, line 21: Figures should be self-explanatory. Please put a title in both figures 2a) and 2b) and an explanatory footnote.

Response: We put a title in both figures 2a) and 2b) and an explanatory footnote is in the figure legends part in the manuscript.

32. Results, page 14, line 6: "EtP, MeP, PrP, 2,4-DCP and MBzP demonstrate negative association with obesity": the curves for EtP, PrP and 2,4-DCP are flat, I would not consider that a statistically significant association. Also, it is more correct to say "inverse" association rather than "negative".

Response: We change the expression of former content as follows: “MeP demonstrated inverse association with obesity, while no other chemical exposures showed a noteworthy change in slope”. And we change the “negative” for “inverse” in the text.
33. Results, page 14, line 27: "They revealed a positive association of the mixed exposures with the BMI z-score". You need to make clear that there is no statistical evidence (the credibility intervals are too wide).

Response: We change the expression of former content as follows: “Although no statistically significant difference was found, they revealed a positive association of the mixed exposures with the BMI z-score.”

34. Discussion, page 14, line 44: "Due to the interactions between chemicals…” Rather say correlations, we don’t know about interactions, in fact, you don’t find any interactions in your study. The thing we are sure about is that some of them are highly correlated, not about interactions.

Response: In our new analysis, after excluding the low DF chemicals, we get some new findings. Since the slopes were different between 2,5-DCP and obesity, MEP and obesity while fixing MeP at the 10th, 50th, and 90th quantiles, potential interactions might exist between 2,5-DCP and MeP as well as MEP and MeP (Fig. 5a). Thus, we think using “interactions” may be better.

35. Discussion, page 14, line 48: "However, we still need to comprehensively consider the results of these three methods”. I would delete this sentence. It adds nothing.

Response: We delete this sentence in the manuscript.