Author’s response to reviews

Title: Urinary concentrations of phthalate biomarkers and weight change among postmenopausal women: A prospective cohort study

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Author’s response to reviews:

Thank you for your review of our manuscript, “Urinary concentrations of phthalate biomarkers and weight change among postmenopausal women: A prospective cohort study.” We appreciate the valuable comments of the reviewers, and we have addressed the identified concerns in the revised manuscript. All changes to the manuscript are tracked in the resubmitted version, and we also offer additional comments below.
Reviewer 1

1. When describing the study population and nested case-control study used, the authors do not clarify that the cases were included before their diagnosis. They later explain it on page 8, but it would be good to clarify early on as well as it creates confusion.

We have modified the text to clarify this point earlier in the manuscript, as requested.

2. Please comment on what types of containers were used for urine sample collection to avoid sample contamination with phthalates from plastic.

We have added this information to the Methods section, as requested.

3. Please comment on high CV for MHiBP 21.9%, and MEHP 19.5%, they are close to acceptable cut-off of 20%

The high average CVs are reflective of the very low concentrations of these metabolites (e.g. duplicate readings of 0.0 and 0.3, for a CV=141%). We have added this information to the manuscript.

4. As authors recognize themselves, there was a need for correction of significance level given multiple biomarkers tested. Why not to adjust p-value instead of saying that Type I error is possible. This would be a more sound and convincing approach.

Given the degree of measurement error already present in the phthalate biomarker data, we were concerned that adjusting for multiple comparisons would further obscure any true associations between phthalate biomarkers and weight change. Thus we prefer to keep the analyses as in the original submission, with acknowledgement of this potential limitation.

5. In the discussion, would suggest to list study strengths first and then discuss the limitations.

We have moved the paragraph describing the strengths of our analysis to come prior to the discussion of the limitations, as requested.
Table 1 - suggest presenting all variables with mean (SD) in the top part of the table and those with N (%) in the bottom; abbreviations need to be explained underneath the table

Table 2 - CI is missing in the list of abbreviations (also in Table 4)

Table 3 - suggest spelling out WHI in the table title; OR, CI are missing in the abbreviation list for the table

These changes have been made to the Tables, as requested.

Reviewer 2

1. Limitations are the use of only one baseline measurement of exposure, potential residual confounding, and over-reliance on statistical significance testing (e.g. p-values in Table 1 are not helpful; and approach to assessing potential confounding seems a bit outdated).

We have removed the p values from Table 1 as requested. We agree that other approaches to selecting confounders are available, but we have chosen a traditional statistical approach to facilitate comparability with prior work. Also, very few characteristics are predictive of phthalate biomarker concentrations (see Reeves et al Env Health 2018 169:122-130), thus minimizing the potential impact of confounding.

2. This analysis includes only observations with complete data. How many participants were excluded because of missing data on one or more covariates? How many controls dropped out of the longitudinal follow-up after the 3 year visit? Did the authors examine whether there could be differential loss-to-follow-up? Some sensitivity analyses should be conducted to evaluate this type of bias since in practice loss to follow-up is often dependent on variables such as SES (which could be related to the exposures) and BMI.
Did the authors compare the characteristics of subjects who were excluded due to missing values with those in the final study population. Did the authors consider using multiple imputation to deal with missing values and if not, why not? Please see the 2008 article by MA Klebanoff and SR Cole in AJE.

A total of 260 participants were excluded due to missing data, primarily due to missing data on physical activity (n=152), hypertension (n=168), high cholesterol (n=177), and/or cardiovascular disease (n=174). Those excluded due to missing data were more likely to be Hispanic (8% vs 4%) and have lower educational attainment (29% vs 37% college degree or higher). Small, although statistically significant, differences in DBP, MBzP were observed between those included and excluded. We reran analyses excluding the four variables with the highest amounts of missing data as covariates (stated above) and compared results between this larger sample set (n=1187) and our complete case sample set (n=997) for these models; results were generally similar for all phthalate biomarkers between these two sample sets. Thus we believe potential bias is minimal. We have chosen not to use multiple imputation as this would introduce further statistical imprecision to an analysis already affected by measurement error, as we have described in the manuscript.

A total of 67 controls did not have weight data at AV6 and were considered lost to follow-up. Those lost to follow up were similar to those included with respect to age, BMI, HEI, ethnicity, income, smoking status, although they were less likely to have a college degree or higher. Baseline phthalate biomarker concentrations were similar between those included and those lost to follow up. Therefore, we are not concerned about selection bias due to loss to follow up given that no differences were observed in baseline exposure between those included and those lost to follow up.

3. Quartiles can obscure non-linear associations and many EDCs traditionally have low-dose effects. In addition to modeling quartiles, did the authors consider using other approaches to evaluate non-linear associations and/or to choose more meaningful cut-points for analysis?

We also explored categorizing phthalate biomarkers into quintiles, and found generally similar results.
4. The very strong positive associations between \( \sum \text{DEHP} \) and overweight/obesity in the cross-sectional analyses may indicate the presence of a strong confounder or reverse causality. Some studies have suggested that certain phthalates are lipophilic; perhaps that could partially explain this strong association. In addition, the strong inverse association between MEP and BMI cross-sectionally compared with the strong positive association prospectively is very puzzling and might indicate some sort of bias, especially considering that these are based on single measurement and therefore should be biased to the null in the extreme categories. Consider controlling for baseline BMI to address this possibility in the longitudinal analysis, and consider stratifying by baseline BMI to see if the associations between phthalates and weight gain are modified according to baseline BMI category.

We thank the reviewer for this important comment. The mixed effect modeling approach we used includes baseline weight, fit as the intercept of the model, and therefore additional adjustment for BMI is not needed.

We repeated our analysis with stratification on baseline BMI, and we observed no indication of effect modification of BMI on the associations between phthalate biomarkers and weight change. We have added a description of these analyses to the revised manuscript.

5. The authors mention that underreporting of energy intake among obese women might have led to residual confounding by HEI. Consider conducting some bias analyses to determine how much this potential confounding might have affected your estimates.

Given that diet is not the primary source of phthalate exposure, we expect the effect of any residual confounding that is present to be minimal. We have clarified this in the revised manuscript.

6. Because diet is such a major source of many phthalates, consider exploring whether there could be confounding by individual dietary constituents...e.g. in addition to HEI, consider % saturated fat, fast food intake (if available) and other aspects of diet such as high fat dairy consumption that may be linked with both higher phthalate exposures and higher weight gain.
We agree that diet is potentially an important source of phthalate exposure, although exposure to personal care products, medications, and other plastics are likely the major sources of exposure; a careful analysis of dietary constituents in relation to phthalate biomarker levels in this cohort is ongoing. We believe that use of the HEI provides appropriate adjustment for dietary exposures in this analysis. We are not aware of prior studies linking dietary fat consumption to phthalate biomarker levels; WHI does not have data on fast food consumption.

Minor points:

7. p. 10, lines 46-51; the statement that effects were often attenuated in controls (supplementary tables) does not appear to be strictly accurate based on a comparison with Supplementary table 2. Consider deleting.

This statement has been revised, as requested.

8. p. 6, lines 36-41, The following sentence could be worded more simply to avoid confusion: We used as phthalate biomarkers the phthalate metabolite concentrations analyzed individually and, for certain metabolites, also grouped by their parent phthalate, by dividing each metabolite of a single parent by its molecular weight and then summing across metabolites; Consider something like:

We analyzed concentrations of each phthalate metabolite individually. For phthalates with multiple measured metabolites, we also grouped the data by parent phthalate by dividing each metabolite of a single parent by its molecular weight and then summing across metabolites.

This revision has been made, as requested.
Thank you for your time and consideration. We look forward to hearing from you regarding our revised manuscript.

Sincerely,

Katherine W. Reeves