Author’s response to reviews

Title: Tampon Use, Environmental Chemicals and Oxidative Stress in the BioCycle Study

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Please note that because we had included a Figure in our response to Reviewers, we also uploaded our Responses as a "Personal Cover" in the "Attach Files" Section. Below, please also find our responses.

Reviewer reports:

We would like to thank the Reviewers very much for their constructive comments. We have addressed these comments to the best of our ability, which we believe has greatly improved our manuscript. Below please find our responses.

Reviewer #1: ENHE-D-18-00339
This paper summarizes an exploratory analysis of tampon use, metal biomonitoring data, and biomarkers of inflammation and oxidative stress among healthy women. This analysis is based on data from an existing prospective cohort study on menstrual cycling, reproductive hormone levels, and oxidative stress. While the analysis is based on a very interesting hypothesis - tampon use can lead chemical exposures and subsequent inflammation and oxidative stress - it is not currently present-ed in a way that helps to explore that hypothesis fully.

Thank you very much. We recognize that we had not presented our hypothesis clearly and have rephrased certain parts of the introduction to better reflect our hypothesis.
Background:
While the authors present a lot of important background literature, I think the organization of several of the introductory paragraphs could be improved. For example, I suggest the authors consider a more informative first sentence like, "The vaginal route is a potentially important yet under-studied route of chemical exposure."

Thank you for this suggestion, we have added this sentence.

Page 3, Line 12: instead of 'compounds' should be 'drugs', right?

The Reviewer is correct that the reference we cite is discussing drug delivery. Nonetheless, our hypothesis is that if the vaginal route is effective for delivering drugs, it would also be “effective” for chemical compounds more generally. Nonetheless, to avoid confusion, we have rephrased this sentence to state: “Notably, the vagina has been shown to be an effective delivery route of drugs to the systemic circulation system (Hussain and Ahsan 2005), suggesting that it could also effectively deliver other compounds, like toxic chemicals, to the circulation.”

Also, I suggest a stronger topic sentence for the second paragraph. Perhaps, "Tampons are a potential source of chemical exposure." And then follow with how cotton can be contaminated because of where it is grown, chlorine bleaching may introduce dioxins, and fragrances may also be present.

We thank the reviewer for this suggestion, we have added this sentence.

The current topic sentence of the third paragraph is almost circular in logic. I suggest rewriting.

We have rephrased the first sentence of the third paragraph.

What is the background literature on inflammation and oxidative stress during the menstrual cycle? Presumably there are other influences besides chemical exposures.

There are several studies evaluating fluctuations in inflammation and oxidative stress during the menstrual cycle in an effort to identify risk factors. In fact, the BioCycle study was designed to look at estrogen and progesterone effects on biomarkers of oxidative stress during the menstrual cycle. Therefore, many of the published studies on this topic are using data from this cohort, e.g. Browne et al. Biomarkers 2008, Gaskins et al. AJE 2012, Schisterman et al. Epidemiol Rev 2014, and many more, showing that there is substantial menstrual cycle variability of these oxidative stress and inflammation biomarkers. One potential endogenous cause of these fluctuations could be the menstrual cycle variability of hormones (Schisterman et al. 2014). In fact, we believe that having 8 samples of each biomarker during the menstrual cycle is a big strength of our study, exactly because of these fluctuations. We do not believe, however, that it is likely for another factor to covary with these biomarkers and the choice of tampon use.

We have added the following text in the Discussion:
“There are substantial fluctuations in the levels of oxidative stress and inflammation biomarkers during the menstrual cycle (Schisterman et al. 2014). Although we cannot exclude the possibility that a variable exists that covaries with these fluctuations and tampon use, we do not believe that this potential source of unmeasured confounding is likely. Rather, the ability to capture these fluctuations with the multiple measurements of these biomarkers during the menstrual cycle is a strength of our
Have there been any studies on the cyclical nature of chemical concentrations in blood during the menstrual cycle? If yes, please reference.

Unfortunately, to the best of our knowledge, no such studies have been conducted. Hopefully our paper will provide motivation for such studies in the future.

Page 3 Line 60: references for "has been documented"

We apologize for the omission, we have added the reference.

It's a little strange to talk about pesticides so much and then drop them in the final paragraph. I suggest mentioning them in the background but focus only on the metals. Pesticides can be further discussed in the Discussion section.

We unfortunately were not able to assess exposure to pesticides and, therefore, we could not include pesticides in our analyses. However, we believe that tampons are a potential source for exposure to pesticides among women. Moreover, pesticides have been related to biomarkers of oxidative stress and inflammation. These provide the elements of our hypothesis to look at the association between tampon use and these biomarkers. So although we do not have information on pesticide exposure biomarkers, we think pesticides should be discussed in the Introduction as well as in the Discussion.

Methods:

Page 4 Line 42: "were planned to be collected" or "were collected"? Or is it that the study was designed to collect blood samples at certain times during the menstrual cycle and you used historic data on cycling to estimate the blood draw times?

We thank the Reviewer for bringing this to our attention. We have changed this to “were collected”.

A very large limitation to this analysis is that the metal concentrations in blood were collected before the reported tampon use and biomarkers of effect.

Yes, we absolutely agree. We include the following text in the Discussion in the limitations:

“First, and most importantly, the BioCycle Study was not designed to study tampon use and tampon-related chemical exposures. While the BioCycle Study was designed to study oxidative stress and inflammation, metal exposure was not measured in the same way as the mechanistic biomarkers. The metals were measured from a single whole-blood sample collected approximately 16 days before the beginning of the first menstrual cycle during the study and no additional collection was obtained before the second menstrual cycle. This may not have accurately represented the levels of these metals when the women were using tampons in cycles one and two. However, we believe that tampon use patterns are consistent across cycles, as also shown by the estimated ICC. Given the potentially missed critical exposure window, the fact that other sources of exposure also exist, and that – since the study was not designed for our hypothesis – no other tampon-relevant chemicals were measured (e.g. pesticides), we would expect exposure measurement error to be an important source of bias in our study. However, since there is no reason to believe that any error is related to tampon use, any bias would be towards the null, which may explain our null findings.”
It is hard to determine whether the authors have captured a reasonable list of confounders without knowing more about the association between menstrual cycle and inflammation and oxidative stress. I understand this is partly the point of this paper but are there endogenous changes that could explain the relationships and should these be considered in the model?

We agree with the Reviewer. Although we originally included some text in the limitations about potential residual confounding, we have enhanced that section as follows:

“Finally, we cannot exclude the possibility of residual confounding; although we assessed multiple variables as potential confounders, these associations have never before been examined, and we were limited to assess confounding by variables for which we had information. There are substantial fluctuations in the levels of oxidative stress and inflammation biomarkers during the menstrual cycle (Schisterman et al. 2014). Although we cannot exclude the possibility that a variable exists that covaries with these fluctuations and tampon use, we do not believe that this potential source of unmeasured confounding is likely. Rather, the ability to capture these fluctuations with the multiple measurements of these biomarkers during the menstrual cycle is a strength of our study.”

Have tampons ever been tested for metals? Just curious.

No, to the best of our knowledge tampons have never been tested for metals. Or if they have been, these results have not been reported in the literature. We hope that our study will open the door for more studies focusing on tampon-related exposures and we can have a much better understanding of this potentially very important exposure route for women that has been so far ignored.

Importantly, FDA regulates tampons as “medical devices” and, as such, manufacturers are not required to test their products. FDA only “recommends that tampons are free of [dioxins] and any pesticide and herbicide residues”. Please see https://www.fda.gov/MedicalDevices/ucm071781.htm#7a and https://www.fda.gov/MedicalDevices/ucm071781.htm#aa.

Are the biomarkers of effect temporally correlated? Was that accounted for in the modeling?

Although we do expect the biomarkers of effect to be temporally correlated, we ran separate models for each time period examined (i.e., menses, early-follicular phase, menstruating week, cycle, and cycle except menstruating week). The clustering within participant in the mixed models was referring to the use of observations from two cycles. Therefore, temporal clustering could not have impacted our results. We have now clarified this in the Methods Section.

Discussion:
Can the authors provide more biological context for the various biomarkers of effect? What is the significance of higher TBARS and lower PON1P levels?

TBARS and isoprostane are biomarkers of lipid peroxidation. Increased levels of these biomarkers, thus, indicate increased oxidative stress. Conversely, PON1P is an antioxidant enzyme. Lower levels, thus, would indicate decreased ability to combat oxidative stress. We have altered our Discussion as follows:

“In our study, we found isoprostane and TBARS, biomarkers of lipid peroxidation, to be non-significantly higher in tampon users than non-tampon users. In addition, we found lower levels of PON1P, an antioxidant enzyme known to hydrolyze exogenous organophosphate compounds (Deakin
and James 2004; Rochu et al. 2007; Shih et al. 1998). These increases in oxidative stress biomarkers and decrease in antioxidants may be due to exposure to metals, pesticides or other chemicals present in the tampons.

Page 8 Line 24: "daily intake of dioxins" from what?

We thank the Reviewer for bringing this omission to our attention. It is “dietary intake”, we have corrected this in the text.

The phthalates discussion is interesting. These should be mentioned in the Background.

We thank the Reviewer for this suggestion, we have added this in the Introduction: “The chlorine bleaching process may contribute to dioxins and furans in tampons and fragrance chemicals, such as phthalates, are likely to be found in scented products (Scranton 2013; US Food and Drug Administration 2018).”

Conclusion:
The authors may be overstating their findings of suggestive evidence without further convincing the reader of the plausibly and relevance of the metal exposures (measured before the "exposure") and inflammatory markers and oxidative stress. This is a unique study and it raises some interesting questions that need to be explored further.

We have tried to be very careful to not overstate our findings. We emphasize throughout our paper that our results are not statistically significant and that any evidence is strictly suggestive. Our first sentence of the Conclusions is: “In conclusion, we found suggestive evidence that women who used tampons had increased levels of mercury, and oxidative stress biomarkers during different times of the menstrual cycle, but these increases were not statistically significant.” However, we would like to bring this largely ignored route of exposure to attention, to hopefully encourage researchers to explore this further in better designed studies to study this association.

Reviewer #2: Comments
First sentence of abstract: Page 3, Line 7

"Tampons are widely used by up to 86% of women and are a rarely considered potential source of pesticide and metal exposure."

This statistic is true of the United States - but largely untrue of many other countries around the world where tampon use may be quite rare. Given the international readership of Environmental Health - it should be specified here in the abstract that this statistic refers specifically to the U.S.

Yes, we agree with the Reviewer, we have made this change.

Page 4, line 23
"Therefore, if tampons do contain harmful chemicals, tampon use may be a potentially important source of these chemicals via the vaginal route given the rapid absorption that occurs in the vagina and the cumulative exposure to tampons over a women's reproductive life."

Rapid absorption and systemic exposure as a result of vaginal exposure are certainly important concerns that have largely been overlooked. Also, however, vaginal exposure to harmful chemicals can
also have local effects on vaginal and cervical tissue. The best example of this is the antimicrobial nonoxynol-9. Vaginally applied Nonoxynol-9 was studied as a promising candidate to help prevent HIV transmission, but instead caused a 2-fold increase in HIV transmission due to the detrimental changes in cervical and vaginal epithelial integrity caused by the chemical.

Source: [https://urldefense.proofpoint.com/v2/url?u=https-3A__www.ncbi.nlm.nih.gov_pmc_articles_PMC3519674_&d=DwIGaQ&c=G2MiLla7SXE3PeSnG8W6_JBU6FcdVjSsBSbw6gcR0U&r=u0ZdPYS4YD_dAYlAisQDq1Z0YkxWHm2GReyKjQl2lj8&m=PGK5W7vM6QTb7XDg87cwoWjgVaiCQNn3D-zaxl0Mhk&s=PoDaq_jXAvRw_lhrIg9lbwim5OAz7PedSwAZTpFP9JQ&c=](https://urldefense.proofpoint.com/v2/url?u=https-3A__www.ncbi.nlm.nih.gov_pmc_articles_PMC3519674_&d=DwIGaQ&c=G2MiLla7SXE3PeSnG8W6_JBU6FcdVjSsBSbw6gcR0U&r=u0ZdPYS4YD_dAYlAisQDq1Z0YkxWHm2GReyKjQl2lj8&m=PGK5W7vM6QTb7XDg87cwoWjgVaiCQNn3D-zaxl0Mhk&s=PoDaq_jXAvRw_lhrIg9lbwim5OAz7PedSwAZTpFP9JQ&c=)

Thus, in addition to the potential for significant systemic exposure from the vaginal route, it would also be worth mentioning the potential risk harmful chemicals found in tampons could have on epithelial integrity of vaginal and cervical cells, and the adverse health impacts that could result.

We thank the Reviewer for suggesting this! We have edited the text in the Introduction as follows:

“… Moreover, in addition to systemic exposure, vaginal exposure to chemicals and drugs can also have local effects on vaginal and cervical tissue (Lozenski et al. 2012). Therefore, if tampons do contain harmful chemicals, tampon use may (1) be a potentially important source of these chemicals via the vaginal route given the rapid absorption that occurs in the vagina and the cumulative exposure to tampons over a women’s reproductive life and (2) may also affect the epithelial integrity of vaginal and cervical cells, potentially increasing susceptibility to sexually transmitted infections.”

Page 4 line 28
"Most tampons are made of cotton or cotton blends"

Rayon should also be specifically mentioned here as a very common component of tampons. (This may be what is implied by "cotton blends"). However, it is important to note that some leading U.S. brands of tampons are made solely of rayon, containing no cotton at all.

Examples:
Kotex:
Online ingredient listings for these three products, U by Kotex® Sleek® Tampon, U by Kotex® Click®, and U by Kotex® Fitness indicate no cotton fibers are used. Source: [https://urldefense.proofpoint.com/v2/url?u=https-3A__www.kimberly-2Dclark.com_en-2Dus_brands_ingredients_consumer_kotex&d=DwlGaQ%c=G2MiLla7SXE3PeSnG8W6_JBU6FcdVjSsBSbw6gcR0U&r=u0ZdPYS4YD_dAYlAisQDq1Z0YkxWHm2GReyKjQl2lj8&m=PGK5W7vM6QTb7XDg87cwoWjgVaiCQNn3D-zaxl0Mhk&s=aTlA3P0b0Bq6PkY2amUEW6NhLyc7FwGNgZK3OSj10&e=](https://urldefense.proofpoint.com/v2/url?u=https-3A__www.kimberly-2Dclark.com_en-2Dus_brands_ingredients_consumer_kotex&d=DwlGaQ%c=G2MiLla7SXE3PeSnG8W6_JBU6FcdVjSsBSbw6gcR0U&r=u0ZdPYS4YD_dAYlAisQDq1Z0YkxWHm2GReyKjQl2lj8&m=PGK5W7vM6QTb7XDg87cwoWjgVaiCQNn3D-zaxl0Mhk&s=aTlA3P0b0Bq6PkY2amUEW6NhLyc7FwGNgZK3OSj10&e=)

o.b.
Online ingredient listings for all o.b. tampons state they are made solely of two types of ray-on. Source: [https://urldefense.proofpoint.com/v2/url?u=http-3A__www.ob-2Dtampons.com_faq_about-2Dtampons&d=DwlGaQ%c=G2MiLla7SXE3PeSnG8W6_JBU6FcdVjSsBSbw6gcR0U&r=u0ZdPYS4YD_dAYlAisQDq1Z0YkxWHm2GReyKjQl2lj8&m=PGK5W7vM6QTb7XDg87cwoWjgVaiCQNn3D-zaxl0Mhk&s=ptkW5htM_6tNc3_83LsREsK9DZIesFv-yUb9pBVz4&e=](https://urldefense.proofpoint.com/v2/url?u=http-3A__www.ob-2Dtampons.com_faq_about-2Dtampons&d=DwlGaQ%c=G2MiLla7SXE3PeSnG8W6_JBU6FcdVjSsBSbw6gcR0U&r=u0ZdPYS4YD_dAYlAisQDq1Z0YkxWHm2GReyKjQl2lj8&m=PGK5W7vM6QTb7XDg87cwoWjgVaiCQNn3D-zaxl0Mhk&s=ptkW5htM_6tNc3_83LsREsK9DZIesFv-yUb9pBVz4&e=)
Tampax is the only major brand that currently manufactures a tampon that is 100% cotton (Tampax Pure and Clean - a new product which was introduced in 2018). Most of their products are listed as including "rayon and/or cotton".

Source: https://urldefense.proofpoint.com/v2/url?u=https-3A__tampax.com_en-2Dus_tips-2Dand-2Dadvice_period-2Dhealth_tampon-2Dingredients&d=DwIGaQ&c=G2MiLlal7SXE3PeSnG8W6_JBU6FcdVjSsBSbw6geR0U&r=u0ZdPY54YD_dAY1AisQDq1Z0YkxWHm2GRytKjQ1i2j8&m=PGK5W7vM6QTb7XDbMW7cwoWjgVaiCQNN3D-zaxl0Mhk&s=44zF4UIHaBT5MX4-4CyMTz8UOJQ0cylQ1uI5CsqDPKo&e=

Mercury has been used in the rayon manufacturing process historically - leading to legacy mercury pollution at rayon manufacturing plants. Source: https://urldefense.proofpoint.com/v2/url?u=https-3A__www.ecowatch.com_dupont-2Dmercury-2Dpollution-2Dvirginia-2D2150827849.html&d=DwIGaQ&c=G2MiLlal7SXE3PeSnG8W6_JBU6FcdVjSsBSbw6geR0U&r=u0ZdPYS4YD_dA1AisQDq1Z0YkxWHm2GRytKjQ1i2j8&m=PGK5W7vM6QTb7XDbMW7cwoWjgVaiCQNN3D-zaxl0Mhk&s=-BiS6-fT00szBnyc_2Zv1gBNTtH71vF2oeRZXRamURY&e= This could be worth mentioning as a possible source of mercury in tampons.

Indeed, by “cotton blends” we meant cotton and rayon blends. The brand that is by far most commonly used in the US is Tampax, followed by Playtex, both of which use cotton and/or rayon (please see Figure; source: https://www.statista.com/statistics/287354/most-used-brands-of-tampons-in-the-us-trend/). By “most”, therefore, we were referring to the tampons sold and not most brands. However, to avoid confusion and be clear, we have added the following text: “Most tampons are made of cotton or cotton blends, although some tampons are made solely of rayon.”

We thank the Reviewer for mentioning the possibility of mercury residue in rayon. In fact, many toxic chemicals are used during rayon manufacturing. Rayon is also commonly bleached resulting in dioxin production (although some brands like O.B. now say they do not use bleached rayon). However, we were not able to find a study characterizing the residue of chemicals, like mercury, in ray-on. So even though we do agree with the Reviewer that rayon tampons could also be potential sources of chemicals, we do not feel comfortable stating this in the paper, at least until further studies are conducted.

It is also worth noting the significance of this paper as it reflects a first of its kind to attempt to investigate exposure to heavy metals from the use of tampons, and the first to investigate biomarkers of inflammation and oxidative stress in association with the use of tampons. These are key concerns that have long been ignored and are worthy of further research, given the significant population of women in the U.S. using these products regularly throughout their reproductive years.

Thank you very much!