Author’s response to reviews

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

Authors:
Jessica Singh (jsingh.ehs@gmail.com)
Sunni Mumford (mumfords@mail.nih.gov)
Anna Pollack (apollac2@gmu.edu)
Enrique Schisterman (schistee@mail.nih.gov)
Marc Weisskopf (mweissko@hsph.harvard.edu)
Ana Navas-Acien (an2737@cume.columbia.edu)
Marianthi-Anna Kioumourtzoglou (mk3961@cume.columbia.edu)

Version: 2 Date: 31 Jan 2019

Author’s response to reviews:

Reviewer Reports

Editor Comment

Your manuscript "Tampon Use, Environmental Chemicals and Oxidative Stress in the BioCycle Study" (ENHE-D-18-00339R1) has been re-reviewed. Based on these reports, and my own assessment as Editor, I am pleased to inform you that it is potentially acceptable for publication in Environmental Health, once you have considered some relatively minor revisions suggested by one reviewer. While we do not like to ask for repeated revisions we feel in this instance some of the changes would improve the clarity of the final ms, so we hope you will respond, either with revisions or a reason why you think a change is not needed.

RESPONSE: We thank the Editor and the Reviewer for reviewing our revised manuscript. We have responded to these comments and made the corresponding edits in the manuscript. Below please find our responses.

Reviewer #1
The authors present an interesting preliminary investigation of the association between tampon use and metal concentrations in blood and biomarkers of oxidative stress.

The authors have adequately addressed previous reviewer comments, although some of the detailed explanations in the response to comments could be added to the manuscript to improve the overall discussion.

RESPONSE: Thank you!

Abstract:
Suggest changing "metals" to "metal concentrations" in the third sentence.

RESPONSE: We have added the word concentrations.

Background:
The Background is much improved and now motivates an examination of the potential importance of tampon use and chemical exposure and biomarkers of oxidative stress and inflammation.

RESPONSE: Thank you!

Page 4, Line 7: "Exposure to dioxins.. " this sentence is awkward as written. The bleaching process can create dioxins to which women can then be exposed. Right now it reads that the exposure occurs through the bleaching process.

RESPONSE: Thank you, we have made this change: “The bleaching process can create dioxins to which women can then be exposed,… “

When the authors discuss other potential chemical exposures, it would help the reader if they could more clearly distinguish between what they are actually assessing in this study - metals - and those other chemicals that maybe relevant - pesticides, dioxins, fragrance chemicals.

RESPONSE: We thank the Reviewer for this comment. We believe that the Reviewer is referring to the Introduction Section. In this Section, we discuss in general the toxins to which women could be exposed when using tampons. We believe that adding a clarification distinguishing in those paragraphs might not be appropriate. However, we do agree with the Reviewer that there should be a distinction, which is why we clarify that we will only be looking at metals in the last paragraph of the Introduction: “For this study, we used existing data from the BioCycle Study to assess whether tampon use is related to increased metal concentrations in blood. As pesticides are not available in BioCycle, this hypothesis could not be directly tested in this study. However, we hypothesized that any potential exposure to metals and pesticides through tampon use can be related to increased inflammation and oxidative stress. We thus assessed the association between tampon use and blood biomarker levels for inflammation and oxidative stress.”

Page 4, Line 18: Suggest adding "in the body" to the end of the sentence starting with "To our knowledge…"

RESPONSE: We thank the Reviewer for this suggestion. However, we are not aware of any studies that have assessed metal or pesticide concentrations in tampons, even not in the body. Therefore, we think it is important to not restrict this sentence to biomarkers. However, if the Reviewer knows of such a peer-
reviewed study, we will be happy to change our sentence.

Methods:
Did the authors explore the relationship between measured metal concentrations and oxidative stress and inflammation biomarkers? I realize these outcomes were not measured at the same time. If metals increase inflammation (and not oxidative stress?) then looking at the correlation between metals and concentrations may support their original hypothesis (tampon use * metal exposures * inflammation). Also, I am assuming that a similar hypothesis (tampon use * pesticide exposures * oxidative stress) also exists and discussion of this analysis would help the authors justify their investigation of tampon use * oxidative stress in the absence of pesticide exposure measurements.

RESPONSE: The author is correct that we hypothesized tampon use * metal exposure * inflammation/oxidative stress, and a similar pathway for pesticides (for which we had no information on exposure). We thank the Reviewer for suggesting this additional analysis. However, there is no information to date to assess the tampon use contribution to metal or pesticide biomarkers compared to other sources of exposure. If we observe an association, therefore, we cannot solely link it to tampon use. Nonetheless, this association (metals/pesticides * inflammation/oxidative stress) has been consistently reported in the literature and we provide several references both in the Introduction and Discussion Sections. Furthermore, the metals were not measured concurrently with the inflammation/oxidative stress biomarkers, which do vary during the menstrual cycle, potentially substantially impacting our ability to detect an association. We thus believe that due to these two factors (that an observed positive association in our data would not necessarily allow attributing the observed metal effects to tampons, and the quite reduced chance of observing an association), and the already numerous models that we have run and sensitivity analyses, including the additional suggested analysis would be more confusing for the readers than helpful. We do, however, discuss all limitations of our study in the Discussion and we clearly state our hypothesis in the Introduction; we believe that adding this new analysis will not add clarity. No study like ours has ever been conducted before. With our study we are trying to highlight a potentially severe route of exposure that has been grossly overlooked and we hope that our findings will result in the design of better studies to more comprehensively evaluate these associations.

Discussion:
While adequately addressed in the Reviewer comments, further explanation of the significance of the specific oxidative stress biomarkers (e.g. TBARS and PON1P) should be included in the Discussion.

RESPONSE: We thank the Reviewer for this suggestion. We have added some more information in the Discussion: “In our study, we found isoprostane and TBARS, biomarkers of lipid peroxidation, to be non-significantly higher in tampon users than non-tampon users. Increased levels of these biomarkers, thus, could indicate increased oxidative stress related with tampon use. In addition, we found lower levels of PON1P, an antioxidant enzyme known to hydrolyze exogenous organophosphate compounds,36–38 which could indicate decreased ability to combat oxidative stress among tampon users. 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.”

The second paragraph seems out of place. I suggest switching it with the third paragraph since that paragraph is focused on the study findings (metals and oxidative stress). Then, the paragraph on other chemical exposures (dioxins, phthalates, etc) needs a topic sentence. For example, something like the last sentence of that paragraph, "Tampons may be a source of exposure to other chemicals that are related to oxidative stress and inflammation."
RESPONSE: We thank the Reviewer for this suggestion. We have changed the order of the second and third paragraph in the Discussion, and we have added a topical sentence in the beginning of the (now) third paragraph.

The limitations of the study are adequately addressed; however, I suggest starting with the strengths of the study and then discussing the limitations.

RESPONSE: We thank the Reviewer for this suggestion. We have added: “Our study is the first one, to our knowledge, to investigate tampons as a potential source of exposure to metals and other chemicals that may result in elevated inflammation and oxidative stress. We were able to use data from a well-characterized cohort with detailed information on tampon use and oxidative stress and inflammation biomarkers measured at multiple points during the menstrual cycle.”

Conclusion:
I agree that chemical exposures from tampon use are something that needs additional attention. The language in the last paragraph could be tightened and made stronger. Tampon use isn't a public health concern rather tampon use is a potentially important yet understudied source of chemical exposure that could be associated with adverse health. We need to test products like tampons for chemicals and conduct additional sufficiently-powered studies of tampon users to assess the importance of tampon use as an exposure pathway.

RESPONSE: We thank the Reviewer for this suggestion. We have changed the last sentence as follows: “Tampon use is a potentially important, yet understudied, source of chemical exposure that could be associated with adverse health. This potentially important public health issue requires additional research efforts, including the chemical assessment of tampons and the conduction of larger and sufficiently-powered biomarker studies of tampon users to assess the importance of tampon use as a chemical exposure pathway.”

Table 1:
I suggest presenting the demographic data in Table 1 not as characteristics among tampon users and non-tampon users but rather to compare the tampon users and non-tampon users. For example, among white women, what percent are tampon users versus non tampon users? This aligns better with the overall analysis that compares users and non-users.

RESPONSE: We thank the Reviewer for this suggestion. However, we wanted to keep the 100% within variable (eg the race levels among tampon users should sum to 100%), because otherwise this might be confusing to the readers. Also, this break down better reflects changes in the distribution of confounders among tampon users vs. non-users (instead of individually comparing each level of each variable). All the numbers together, therefore, do provide the appropriate information for the readers to compare tampon users and non-users.

Reviewer #2

Appreciate the changes made to the manuscript - it is much improved.

RESPONSE: Thank you!