Author's response to reviews

Title: A retrospective study of PBDEs and PCBs in human milk from the Faroe Islands

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Author's response to reviews: see over
Dear David Ozonoff,

We appreciate very much the constructive comments regarding our manuscript 1170664577666432 by Fängström et al. We have therefore carefully revised the manuscript according to the suggestions made by the reviewers. The revised manuscript will now hopefully meet the requirements for publication in Environmental Health: A global access science source.

The comments/suggestions given by reviewer 1, 2 and 3, respectively, were numbered and each one of them has been dealt with according to the responses given separately.

Yours sincerely,

Britta Fängström
Comments from reviewers on

Manuscript 1170664577666432 in Environmental Health: A Global Access
Science Source
"A retrospective study of PBDEs and PCBs in human milk from the Faroe
Islands"

Reviewer 1, Dr. Olaf Paepke*, Ergo Research

1. The paper gives important information regarding the development of PBDEs and PCBs in
selected individual mother child pairs. This information is reported here for the first time.

Additional to these components data for Hydroxy-PCBs are reported for first time for this
population. It could be demonstrated that the metabolites represent about 1 % of the
individual parent components.
The development of the human exposure situation for components in question are well
defined and compared to developments in other countries, especially with Sweden. An open
question is still the different development of the time trend observed for Faroe Island and
Sweden.
A: We agree with the reasoning of Olaf Paepke.

2. In general the methods are appropriate especially with respect to all laboratory work. The
measurement part has been done for PBDEs by GC/LRMS (NCI) inclusively the
determination of PBDE 209. PCBs and Hydroxy-PCBs have been measured by GC/ECD. The
best available technique is the application of GC/HRMS. This technique allows the
application of 13C labelled standards for all congeners (if available). It is our impression that
especially the determination of BDE 209 is quite sensitive regarding a potential influence of
external contamination and/or possible selective losses/discrimination. These losses are
sometimes very difficult to explain. It is our personal experience that the use of 13C labelled
PBDE standards is extremely helpful for the analytical procedure.
A: 14C can be used as stated and we have studied its usefulness as well. The agreement

It is our impression that the data are sound and plausible. On the other hand there are 2
remarks which need to be checked.

3. Page 10, first paragraph: The recovery experiments are performed by using cow milk. The
results for these experiments are given in Table 3. I is our impression that at the "low spiking
level" the results for BDEs 153,154 and 209 are highest for both experiments. This can
especially be observed for BDE 209. Is there any influence possible coming from the cows
milk directly or from the blank? If any this should be expected most prominent for the "low
spiking level".
A: The cow milk was analysed in a small pilot study before the recovery study was
performed to evaluate any PBDEs in the milk, the study confirmed that there were no
PBDEs in the milk. Blank milk samples were analysed in parallel with the spiked
samples and are just before analysis on the GC/MS spiked with the PBDE mixture at the
two levels, therefore any background interference during the extraction and cleanup are already corrected. The low spiking levels are always most difficult to determine and the uncertainties are biggest for the low levels, therefore the results from the low level experiment is not as good as the high level.

4. Page 11, Line 8: The ratio between metabolite and parent substance of the two hydroxy components is given at below 0.002 in all cases. This statement can not be followed by comparing the data given in Table 2. A recalculation of the values is recommended.
A: The sentence has been revised to avoid misunderstanding

The manuscript adheres to the relevant standards for reporting and data deposition.
5. The discussion and the conclusion are well balanced and supported adequately by the data. Quite a few literature data are used for comparison. Helpful might be to refer to the paper of Schröter-Kermani et al./* */Organohalogen C., 47, 49 - 52, 2000, as well.
A: We have tried to avoid to many references and to concentrate to relevant publications on human milk. It is not necessary to include this reference in the manuscript.

6. Due to the importance of the measurement of Hydroxy-PCBs, the authors may include these components in the title of the paper.
A: We do not agree with this comment. It is important to focus the title and that is what we have tried to do.

As a non-native speaker it is hard for the reviewer to give a clear statement on the writing of the paper. Taking this into account and summarizing the reading, everything is very clearly expressed, good to understand and easy to follow.
Reviewer 2, Dr. Thomas Webster, Boston University School of Public Health

Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached):

None

Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

1. pgs. 10 (PBDEs), 11&13 (PCBs), 12 (deca): Because the earlier time points were pooled data, they have no measure of variation; this makes assessment of trends more difficult. Please provide more explanation of this limitation.
   A: We agree with the comment but since no other milk was available it is not possible to do more than indicating the problem.

Discretionary Revisions (which the author can choose to ignore)

1. pg. 6: Explain that participants are all long-term residents of the Faroe Islands, if that is correct.
   A: Unfortunately adequate data on each individual is not available to us.

2. pgs. 10, 15: Where was the cow milk obtained, in the Faroe Islands? Dairy products in the USA contain PBDEs, although the sampling has been very limited (Schecter et al 2004, ES&T 38:5306-11).
   A: The cow milk was bought in Sweden. Before the recovery study was performed the cow milk was checked for any PBDEs, and there were no PBDEs in the cow milk.

3. pg. 11: The conclusion about diet could use more explanation, e.g., was there no association at all or one that was not statistically significant? You may have low power to detect such an association.
   A: Since the number of samples are very limited in the study it is difficult to make any conclusions about association between diet and concentrations. However the material was checked for this but no such association was observed.

4. Table 1: Although the summaries are suitable for the paper, it would be useful to present the congener-specific data for each data point. The reader could then examine associations between congeners.
   A: The individual results have been excluded since the amount of space compared to the information it gives is limited. The authors think that the value in the results from the individual analysis is within the mean/median and ranges and are reported in the tables.
Reviewer 3, Dr. Per-Ola Darnerud, Swedish National Food Administration

Reviewer's report: General

The article shows the levels of PBDEs, PCBs and PCB-OHs in breast milk from Faroese women, sampled at different time points. As regards environmental pollutants the Faroese population has been extensively studied, especially pregnant women and mothers with small children and in particularly in connection with fish consumption and potential health effects. However, there are some details that needs to be dealt with before the manuscript should be published. They are listed below and if followed I believe they will improve the quality of the article.

_________________________________________________________________

Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

As a general comment on the document, the authors should include a discussion about the representativity of the women recruited in the study, adding some comments about possible selection bias. In 1st paragraph of the Discussion, the time trends for BDE-47 and BDE-153 are discussed in relation to other studies. The facts that the present samples are rather few, that they are pooled and that they are sampled in a way that they may not be representative for the Faroese pregnant women (see note under Materials and Methods, Sampling) should make the authors careful to draw too far conclusions on time trends for these two, and perhaps also for other, congeners. This comment is valid for several paragraphs in Discussion. Moreover, the expression "trend" used in this manuscript may implicate that the study has been using a number of data points, producing a regression line on basis of values adjusted for important factors, such as age etc. We know from earlier studies that the age of the mothers is an important factors to adjust for, at least regarding PCBs. This has not been done and therefore the term may be changed to "changes in levels over time" or some similar term.

A: We have revised the manuscript and made changes to improve the quality according to this comment

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Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

(Referee's comment: I have not divided my comments into MER or DR)

Abstract

Background

1. line 4: ".the temporal trend." Consider another term (see above).
A: The authors agree

Results

2. line 7: The high levels reported, in relation to other European countries, must be taken with some caution, as the number of samples is relatively small.
A: Agree, but still we do point out that individuals may have high levels also in Europe.

Background
3. 2nd para., lines 9-10: A question - are the marine mammals mentioned in this sentence that stationary, being considered to specifically represent industrially parts of the world with respect to PBDE levels?
A: The sentence has been changed omitting any comments on the degree of industry density in the mammalian environment.

4. 3rd para.: In this paragraph, the authors review present data on PBDEs in human breast milk. Regarding the review of Swedish data, I miss the study of Lind and co-workers who monitored PBDE levels in individually sampled breast milk from Uppsala mothers (Lind et al., Environ. Res. 93 (2003), 186-194). In this study a comparison to the Stockholm study by Norén and co-workers was made and the time-related changes observed were shown to be similar to each other.
A: A sentences has been included and the paper from Lind and co-workers are now included in the manuscript.

Material and Methods
Samples
5. lines 2-3: The term transition milk is unknown to me and should preferably be explained.
A: Since the time for milk sampling is given it is no reason to use the term “transition”

6. In this paragraph, the sampling strategy is explained. How can the authors be sure of that the strategy to pick certain numbers of women eating different amount of whale meat will not in some other way lead to sampling skewness as regards levels of PCBs and PBDEs. Also, why was it not possible to analyse all the samples individually in order to study the distribution in levels also at the earlier time-points?
A: Sampling volumes that were stored were too small to make individual analysis from the first two time points. The women selected are not representative for the whole population at the Faroe Islands as stated in the material and method section.

Instruments:
7. The description of the GC and GC/MS conditions seems too detailed and may be shortened. On the other hand, no information is given on how the quantification was performed; for instance, was internal standard used, was one- och multipoint calibration performed? Also, where blanks used and what was the results from the blank analyses?
A: Changes has been made to meet all comments regarding standard, calibration and blanks. The authors think that the GC(ECD) and GC/MS conditions are difficult to shorten.

Cleanup procedure:
8. The title should preferably be altered to Extraction and cleanup procedure.
A: The title has been changed

9. In the 1st para., it might be informative to mention that the original method (Hovander et al.) was used for analysis of serum samples for PCBs and OH-PCBs.
A: The sentence has been changed, and serum is now included in the sentence

10. In the 2nd para. data on two of the four used surrogate standards are presented - why not on all four? Perhaps these data should be presented in Results?
A: The missing recovery from 4-OH-CB193 are now included in the ms. The authors think that the recovery results belongs to the material and method section in the ms.
Recovery experiments:
11. The text tells us about a recovery study performed in triplicate on two spike levels. However, in Table 3 data on the higher level is given in duplicate.
A: The recovery study was performed in triplicates for both levels, however unfortunately one sample was lost in the last analytical procedure. Therefore there are only results from duplicates in the high level.

Discussion
12. 1st para. The wide distribution range is discussed and reference is made to US data. In this case, it should be of interest to include the Swedish data on individual samples that are present (Lind et al., 2003), as these represent the Nordic body burden situation.
A: Agree, changes have been made in the text and the reference by Lind et al is included.

13. 3rd para., lines 10-end of para.: These sentences should perhaps be somewhat more developed, to increased the clarity for the reader. For instance, if the same congeners are reported from time to time, is it important whether the levels are reported on molar or weight basis?
A: The text has been revised

14. 6th para.: The discussion in this paragraph is somewhat difficult to follow regarding the possible sources to PBDEs in breast milk. Is the traditional Faroese diet of importance for PCB/PBDE levels found in breast milk and what data are supporting this?
A: The PCB levels in the milk are most likely related to the traditional diet, however there might be other still unknown sources. For the PBDEs in the milk the source is still unknown and it is a very complex matter. The text has not been changed. The author’s intentions are to comment on this complexity and looking at different possibilities.