Author's response to reviews

Title: Fetal loss and maternal serum levels of 2,2',4,4',5,5'-hexachlorbiphenyl (CB-153) and 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene (p,p'-DDE) exposure in Greenlandic and European populations

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Author's response to reviews: see over
Response to reviewer’s report

Title: Fetal loss and maternal serum levels of 2,2',4,4',5,5'-hexachlorobiphenyl (CB-153) and 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene (p,p'-DDE) exposure in Greenlandic and European populations

We would like to thank the reviewers for the large effort they have put into specifying parts of the manuscript that can be improved. We have corrected the text and answered to the reviewers comments. We have included the reviewers comments in italic and our response is given below each comment in normal writing.

Reviewer 1: Eva Cecilie Bonefeld-Jorgensen

General comment
This manuscript studied the relationship between the exposure of CB-153 and DDE and fetal loss. The study design and methodology is acceptable. But there are still some problems needed to be explained and corrected and the text is in some sections unclear. The manuscript may be published after corrections and modifications.

Minor essential revisions

Background:
Page 3, line 7: give the full name of DDT
Page 3, line 16: give the full name of DDE
Page 3, line 19: give the full name of BHC

The full names of these compounds have been given in the text.

Methods:
Page 5, line 6: “(420 Inuits…”: that do not match with the number in table 2 where the number of previous pregnancies is given as 429.

Thank you for noting this – the correct number is 429 which have been corrected in the text.

Page 6, line 8 “…with analysis of results found to be within the tolerance limits”: please give the corresponding reference.

It has been included in the text that “The tolerance limits were set as plus/minus three times the standard deviation of the results from a number of reference laboratories.”

Results:
Page 8, section 2, line 9. “---odds ratios were approximately ---“

Thank you for noting – corrected.

Discussion:
Page 10, line 1: In the sentence “Although not statistically---- indicating should be
replaced with suggesting.

OK – indicated replaced with suggesting.

Page 10, line 9: substitute words with these given in bold “CB-153 correlated very well (r=0.9) with previous reported total PCB----“

The sentence have been corrected as suggested.

**Major compulsory revisions**

**Methods:**

Page 5, line 1-2: low participate rate of Kharkiv may give bias problems and this should be stated and discussed.

The following sentence have been inserted in the discussion to address this:

“Another problem that potentially may affect the results is the low participation rate especially in the Ukrainian population. However, we have no reason to believe that participation in Ukraine is related to the exposure, since the exposure level is unknown at the time of entering the study, and therefore the limited participation would most likely only limit generalizability of the study, and not the validity of the exposure response associations. “

Page 7, line 3-7: State the reasons why these factors are choosen as potential confounders.

The following have been included in the paragraph on potential confounders:

“due to previously reported strong associations of these covariates to spontaneous abortion and/or organochlorine exposure.”

Page 7, 12-13 “Data are presented both as crude and adjusted for above mentioned potential confounders”: however, in Table 2 and Table 3, only the adjusted data were given.

Thanks for noticing. The adjustment have been specified in the methods section:

“Data are presented both as adjusted for number of previous pregnancies in the models containing more than one previous pregnancy and fully adjusted for the above mentioned potential confounders.”

Page 7, line 14-17: The two sentences “In addition the average POP exposure…and. The group with no ---by least squares means tests” are not clear and should be rewritten.

The section has been rewritten and now reads:
“To evaluate whether not only the chance of ever experiencing a loss, but also increasing number of fetal losses was associated to POP exposure we performed a general linear regression analysis on the association between POP exposure level and number of losses classified as 0, 1 and 2 or more fetal losses. The models were adjusted for potential confounders mentioned above. For individual comparisons of exposure level in the three specified groups we used the group with no fetal losses as reference and compared that group to the 1-2 and 2 or more losses, respectively, by least squares means tests.”

Page 7, Statistics section: the method used for comparison of POPs, fetal loss among countries should also be given.

I hope the statistical analysis performed are more clear after the revision. We believe to have described the statistical comparisons made. If the reviewer refers to comparisons in table 1; only basic statistics are calculated here and no significance test on differences between countries.

Page 7, line 18-19 “For both the logistic regression model and the general linear regression model we made a final model combining the data from the three populations adjusted for populations”: did authors analyze the homogeneity of association between POPs and fetal loss across the three populations to check whether the data of three populations can be combined?

Homogeneity of associations were checked by inclusion of an fetal loss by country interaction terms in the models. We found no statistical significant indications of interaction and therefore combined models without the interaction term are presented. This has been specified in the text.

Results
Page 8, line 3-5: “The exposure level varies ……whereas about 10 fold higher mean exposure level was found in Greenland”: did these difference statistically significant?

Since the evaluations of exposure levels are not included in the main aims of the present study, we did not perform significance testing on that. The differences of exposure between countries among all women in the Inuendo population have been described in detail in a previous publication (Jonsson et al 2005, Environmental Health.). We believe significance testing should be limited to tests of the main hypothesis.

Page 8, line 9. Text concerning Table 1 must be introduced into the Result section concerning the higher level of urogenital and chronic diseases for Warsaw.

The following sentence have been included:
“Furthermore, the population from Warsaw seemed to have a higher level of urogenital disesases and cronic diseases.”

Page 8, section 2: In table 2 “per 1 log unit” for Warsaw finds significant OR given in bold – but that is not described in the text and should be included. Moreover per 1 log unit must be defined in the text as well as in the Table Legends
The following have been included in the results section:
“However, among the population from Poland the pregnancy adjusted odds for fetal loss and odds for fetal loss among women with only one previous pregnancy increased with increasing CB-153 exposures.”

In the methods section on statistics “per 1 log unit” have been explained:
...and data form these analyses are given as the odds ratio per unit increase on the log scale of the exposure (per 1 log unit). In the tables we include “continuous” in the description of the columns “per 1 log unit”.

Discussion:
Page 9, line 11-14: the sentences “The data did not show significant dose response…. analysis based on the single countries” is not consistent with page 8, 2nd paragraph and table 2,3 and should be explained more in detail.

The following have been included in the first paragraph of the discussion to explain in detail:
“although the odds ratios suggested a weak association, and a higher risk of foetal loss was found at high level of CB-153 in the combined estimate.”

Page 10, line 10-11: please give the reference for the sentence “… and with the 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) equivalent (TEQ) in plasma from PCB (r = 0.9)”

The results refer to Gladen BC 1999, Correlations among polychlorinated biphenyls, dioxins, and furans in humans. The references has been inserted.

Page 11, line 7-9: the sentence “since our main …for lactational loss of POPs” is not clear and need to be rewritten.

The sentence have been revised:
“The main analysis in our study differ from the study by Longnecker et al [5], by using the women as the statistical unit and not the individual pregnancies. Therefore, we were able to include number of liveborn children as a proxy for lactational loss of POPs and thereby adjust for a marker of reduction of organochlorines due to lactation.”

Comments to Tables:
In general bold numbers and per 1 log must be defined in the table legend
In the tables it has been specified in a footnote that bold numbers refers to statistically significant results.

The reference level used to calculate the OR should be explained in the legend
The reference (lowest exposure level) is explained in the heading of the tables.

Table 2 :
1) The “0” and “-“ must be defined. Does it mean different things??
“0” means that data has been evaluated and no observations in the specific category was obtained.

“-“ refers to missing results due too low number of individuals in the strata to perform the desired analyses. This have been specified in the tables.

2) For Warsaw, odds ratios of different categories of CB-153 are “-“, please explain
This is due to too low number of individuals at higher exposure levels as explained above.

3) Explain in the table legend how the significant OR for Warsaw at per 1 log can be obtained when no numbers are given?
In the analysis of CB-153 on a continuous scale (log transformed) – the results can be calculated since differences in fetal loss risk is calculated as a function of increasing exposure levels (although all individual measurements are below 50 ng/g lipid).

The significant OR for Warsaw should also be mention in the text of the Result section.
The results for Warsaw has been mentioned as explained before

4) Explain in the table legend what bold numbers means.
Bold numbers have been explained as mentioned above.

Table 3:
1) The “0” and “-“ must be defined. Does it mean different things??
See response to the same question to table 2.

2) For Greenland significant OR is found for 500-1000 ng/g lipid DDE – that is neither mentioned in the result nor in the discussion sections. That must be explained in detail.
The following sentence have been included in the results section:
“Analyses stratified on countries indicated that the risk of spontaneous abortions was increased at the highest level of DDE present in Greenland. However, this result was only statistically significant among data restricted to one previous pregnancy.”

3) Explain in the table legend what bold numbers means.
See above

Table 4.
Within the table P´,p´- DDE should be corrected to p´,p´-DDE
Corrected
Reviewer 2: Ann Aschengrau

Reviewer's report:
This manuscript describes research of great interest to EH readers. However, retrospective studies of fetal loss, such as the present one, have important limitations resulting from possibly erroneous outcome reporting. The authors can improve the manuscript by addressing this major limitation as well and the minor ones described below.

We agree that misclassification of outcomes is a major limitation of the present study.

We have included a section in the discussion referring to potential misclassification of both exposure and outcomes. The section is initiated with “A major limitation of the present study….”
We have modified this section slightly to specify even further this problem.

The section now reads:
A major limitation of the present study is the potential misclassification of both outcome and exposure. Previous studies have shown that when women are asked about previous spontaneous abortions, about 75% of the cases is recalled [23] and similar results are found in validation studies comparing recalls and medical records in of fetal loss [24]. In our case this would most likely cause nondifferential misclassification, since the exposure level is not known, resulting in attenuation of any true effects.

Compulsory Major Revisions
1. The high miscarriage and zero induced abortion rates in Poland are not credible. It appears that some women erroneously reported a fetal loss when they, in fact, had an induced abortion. The authors should consider validating the self-reported losses using reports in other sources such as medical records, and conducting a quantitative sensitivity analysis to assess the impact of erroneous reports of fetal loss. At the very least, they should present combined results for Greenland and Kharkiv alone in Tables 2, 3 and 4.

Unfortunately medical records would not solve the problem, since medical reports would not represent induced abortions (since they are illegal), but some of the illegal induced abortions may end up as medical registered abortions since the women may end up in hospital after having the abortions induced illegally.

As stated in the discussion removal of the population from Poland in the analysis only marginally change the results. We believe it would take up too much space in the paper to include these results in the tables but have analyzed the data and below the results are presented.
**Minor Essential Revisions**

**Background**

1. Give some numerical results for the prior studies that are described.

Odds ratios for some of the studies mentioned in the introduction have been included

**Methods**

1. State when during pregnancy the subjects were recruited. It is possible to include the current pregnancy outcomes in this analysis study?

We considered including the current pregnancy, but since the women were on average 24 weeks pregnant in Greenland and Ukraine, and 33 weeks pregnant in Poland, the current pregnancies ended in only very few pregnancy losses, - and the risk of losses were of course highly dependent on when in

### Table 1: Odds ratios for some of the studies mentioned in the introduction

<table>
<thead>
<tr>
<th></th>
<th>0-25</th>
<th>25-50</th>
<th>50-100</th>
<th>100-200</th>
<th>&gt;200</th>
<th>Continuous (Per 1 log unit)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DDE</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preg adj. odds ratio (95%CI) a</td>
<td>1.1 (0.7-1.7)</td>
<td>1.5 (0.9-2.4)</td>
<td>1.0 (0.5-2.1)</td>
<td>2.1 (0.9-5.4)</td>
<td>1.1 (0.9-1.3)</td>
<td></td>
</tr>
<tr>
<td>Adj odds ratio (95%CI) b</td>
<td>0.9 (0.5-1.6)</td>
<td>1.3 (0.7-2.3)</td>
<td>0.9 (0.4-2.1)</td>
<td><strong>2.7 (1.0-7.4)</strong></td>
<td>1.0 (0.8-1.3)</td>
<td></td>
</tr>
<tr>
<td>Adj. OR one prev. pregnancy</td>
<td>1.5 (0.6-3.8)</td>
<td>2.0 (0.7-5.3)</td>
<td>0.5 (0.1-2.9)</td>
<td>2.9 (0.5-15.6)</td>
<td>1.1 (0.7-1.6)</td>
<td></td>
</tr>
<tr>
<td><strong>PCB</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preg adj. odds ratio (95%CI) a</td>
<td>1.6 (0.9-2.9)</td>
<td>1.4 (0.7-2.5)</td>
<td>1.2 (0.6-2.4)</td>
<td>1.8 (0.9-3.6)</td>
<td>1.1 (0.9-1.4)</td>
<td></td>
</tr>
<tr>
<td>Adj odds ratio (95%CI) b</td>
<td>1.8 (0.9-3.7)</td>
<td>1.8 (0.9-3.7)</td>
<td>1.3 (0.6-2.9)</td>
<td><strong>2.3 (1.0-5.2)</strong></td>
<td>1.2 (0.9-1.5)</td>
<td></td>
</tr>
<tr>
<td>Adj. OR one prev. pregnancy</td>
<td>1.4 (0.5-3.8)</td>
<td>1.4 (0.4-4.1)</td>
<td>1.2 (0.3-4.9)</td>
<td>2.4 (0.6-9.2)</td>
<td>1.2 (0.8-1.9)</td>
<td></td>
</tr>
</tbody>
</table>

### Table 2: CB-153 and p,p’-DDE

<table>
<thead>
<tr>
<th></th>
<th>No fetal loss</th>
<th>1-2 fetal losses</th>
<th>&gt;2 fetal losses</th>
</tr>
</thead>
<tbody>
<tr>
<td>CB-153 (ng/g lipid)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Combined</td>
<td>57 (51;65)</td>
<td>66 (57;75)</td>
<td><strong>115 (78;170)</strong></td>
</tr>
<tr>
<td>p,p’-DDE (ng/g lipid)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Combined</td>
<td>443 (392;500)</td>
<td>476 (410;552)</td>
<td><strong>779 (519;1171)</strong></td>
</tr>
</tbody>
</table>
pregnancy the women were recruited. Therefore we could not simply add the few losses from the current pregnancies, and the sample size was too low to run a separate analysis. The following sentence is included in the methods section: “The women were on average 24 weeks pregnant in Greenland and Kharkiv, and 33 weeks pregnant in Warsaw, when interviewed and the blood sample was drawn.”

2. **Give more information on the selection and enrollment process.** Give your assessment whether selection bias was possible and likely?
As noted in the paper the selection and enrollment have been described in detail in another paper (Toft et al 2005, Environmental Health). We have included a section, with discussion of the potential selection problem based on low participation rate in the Polish population, as requested by reviewer 1, and believe this covers the assessment on whether selection bias was possible and likely.

3. **Consider conducting analyses that divide the losses by trimester.**
Unfortunately, we do not have information on when in pregnancy the previous losses occurred, and therefore we are not able to analyze losses divided by trimester.

4. **Describe exactly when the blood samples were drawn.**
The blood samples were drawn at the same day of the interview of the recruited women. This has been specified.

5. **State whether confounder data were collected for each pregnancy.** For example, it is unclear which age was used in the analysis.
Confounder data were not available from each pregnancy. That is one of the reasons why we used the women and not the individual pregnancies as the statistical unit.
It has been specified that the age used was: the age at conception of the present pregnancy.

6. **Give the reason why the variables urogenital inflammation, urogenital diseases and chronic disease were grouped?** I think that it would be better to control for each component individually, if there are sufficient numbers of subjects with these conditions.
Since urogenital inflammation, urogenital diseases and chronic diseases are all diseases that may affect the outcome, we choose to group them – due to low numbers in some of the specific groupings. From table 1 it can be seen that these outcomes are generally not very common – except for urogenital inflammation in Greenland.

7. **Give the categories for all confounders that were controlled.**
The categories or statement of use of the confounder as a continuous variable have been given in the section on potential confounders on page 7.

8. **Justify why the five exposure categories were selected.**
The five exposure categories were previously used in analysis of male semen quality in the men of the women in the present cohort. (Toft et al 2005). However the PCB categories were slightly changed to better represent the somewhat lower concentration in women (0-50 subdivided) and >200 combined. Overall the division of the cohort aim at presenting the data in approximately quintiles (based on the overall number of subjects) with reasonable stratification within each population.

9. It would be useful to the reader if the authors conducted dose-response trend tests and reported their associated p values.
We performed linear regression analyses and believe this would identify dose response if present. Trend tests based on the odds ratios in the different strata would have lower power, and would depend on the strata chosen. We therefore prefer to only present the results from the linear regression analyses.

10. Provide the rationale for conducting analyses among women with only one previous pregnancy

We include a model with only one previous pregnancy, since the number of previous pregnancies may affect the fetal loss rate, and although we adjust for this, the adjustment can not exclude residual confounding. Furthermore, since exposure is measured in the present pregnancy, using women with one previous pregnancy would give less misclassification of exposure.

This information is included in the method section.

Discussion
1. The authors place too much emphasis on statistical significance when interpreting the results. They should instead place greater emphasis on the epidemiological measure of effect (e.g. odds ratios).

In the discussion we have included some reference to odds ratios and do not only present statistically significant results. However, especially in the light of reviewer 1, who puts much emphasis on statistically significance testing, we believe it is important to specify if the presented results reach statistical significance, although from an epidemiological point of view we agree that the magnitude of the odds ratios may be more interesting. We hope both reviewers can accept the presentation of a combination of odds ratios and statistical testing.

On behalf of the authors,
Yours sincerely,
Gunnar Toft