Author's response to reviews

Title: Inter-population variations in concentrations, determinants of and correlations between 2,2',4,4',5,5'-hexachlorobiphenyl (CB-153) and 1,1-dichloro-2,2-bis (p-chlorophenyl)-ethylene (p,p'-DDE): a cross-sectional study of 3161 men and women from Inuit and European populations

Authors:

Bo AG Jonsson (Bo_A.Jonsson@med.lu.se)
Lars Rylander (lars.rylander@med.lu.se)
Christian Lindh (christian.lindh@med.lu.se)
Anna Rignell-Hydbom (anna.rignell-hydbom@med.lu.se)
Aleksander Giwercman (aleksander.giwercman@med.lu.se)
Gunnar Toft (gutof@as.aaa.dk)
Hennning S Pedersen (hsp@gh.gl)
Jan K Ludwicki (k.ludwicki@pzr.gov.pl)
Katarzyna Goralczyk (k.goralczyk@pzr.gov.pl)
Valentyna Zvyezday (dimusic@ic.kharkov.ua)
Marcello Spano (spanomrc@casaccia.enea.it)
Davide Bizzaro (d.bizzaro@univpm.it)
Eva C Bonefeld-Jørgensen (EBJ@MIL.AU.DK)
Gian-Carlo Manicardi (manicardi.giancarlo@unimore.it)
Jens Peter Bonde (JPBON@as.aaa.dk)
Lars Hagmar (lars.hagmar@med.lu.se)

Version: 2 Date: 10 October 2005

Author's response to reviews: see over
Dear Editor,

Thank you for the opportunity to submit a revised version of the manuscript. Below we give a point-to-point response to the reviewer’s comments. Overall we find the reviews to have been constructive and we find that they have helped us to make the revised manuscript a better one as compared to the original one. We hope that it now will be acceptable for publication.

With best regards,

Lars Hagmar, MD, PhD
Professor

Response to comments from reviewers and editors:

Dear Dr. Hagmar

We have now received two reviews of your manuscript (above). Both reviewers were impressed with the work you and your colleagues have done. As you will see, however, the reviewers had a number of comments which I feel need to be addressed (in the form of a point by point response in a cover letter) in order to make a final decision on this paper. In particular I hope you will address how this paper differs from your other submitted paper in ways to require separate publication and what its specific message is to the reader. Perhaps some attention to organization and phrasing would clarify this.

Re: We have made a number of revisions in the manuscript, also based on the comments from the reviewers, which we think has clarified how this paper differs from the other papers from the Inuendo project, and we have also specified the main messages.

Here are the reviews:

Reviewer #1: Prof. Philippe Grandjean, University of Southern Denmark

This is an impressive study of subjects from four different populations, and a substantial number of serum analyses have been carried out. This MS describes the serum analyses only, and it seems that this report provides documentation for use in a separate study on reproductive effects. Thus, the selection of study subjects was based on pregnancy (except for the Swedish subjects). If this MS should stand alone, the authors should justify the validity of the comparisons and also discuss how the selection criteria may have influenced the findings.

Re: The participants, both pregnant women and Swedish fishermen’s wives, had no possibility to know their individual POP exposure levels, and it is therefore highly unlikely that this could have caused any selection bias with respect to exposure. This was stated already in the original manuscript in the Discussion: “We have, however, no reason to suspect that the subject’s decision to participate was affected by any knowledge of their individual POP exposure.” We have in the statistical analyses adjusted for known POP determinants in order to facilitate comparisons. We are aware of that the participation rates differ between the populations but it is not possible to adjust for participation rates as this is a population characteristic and not an individual characteristic. We have added this reasoning to the Discussion.
Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

1. The abstract says that this study is part of a larger project, and that the specific aims were to describe serum concentrations of PCB and DDE in four populations, to examine inter-population variations and exposure determinants. The conclusions refer to differences in average concentrations and variations in the correlations between PCB and DDE. The first sentence of the Discussion also refers to the differences in the PCB-DDE correlations. The authors need to specify clearly what they were looking for, what they learned, and what the most important message to the reader is. This ought to be reflected also in the title of the manuscript.

Re: We appreciate this comment and we have revised the manuscript being more specific in aims and conclusions in both the Abstract and in the main text. Moreover we have changed the title in accordance with the recommendation: “Inter-population variations in serum concentrations, determinants of and correlations between 2,2',4,4',5,5'-hexachlorobiphenyl (CB-153) and 1,1-dichloro-2,2-bis (p-chlorophenyl)-ethylene (p,p'-DDE): a cross-sectional study of 3161 men and women from Inuit and European populations”

2. The authors refer to the subjects as a single cohort in the abstract, and elsewhere they use the plural term. Because this is a cross-sectional study, where different selection criteria were used, I would prefer not to use the word 'cohort', but suggest population groups or similar wording.

Re: OK, done.

3. The results section in the abstract is very difficult to read because of the wealth of data presented, and needs to be rephrased so that the reader can better follow the logic.

Re: It has been rephrased.

4. The Introduction is rather general and too lengthy, and it needs to state more clearly why the authors focused on PCB-153 and p,p-DDE. One serious shortcoming of this study is that the two indicators were not validated in regard to their associations with other POPs. The reference to Vietnam veterans in the Introduction may not be relevant to this study and should be substituted or deleted.

Re: The original Introduction has been reduced in size and we have deleted the reference to Vietnam veterans. On the other hand, we have giving more arguments for the choice of POP biomarkers and for the choice of study populations (see comment # 5).

5. The Methods section outlines the selection of study subjects. What I miss here is a statement why the four populations were selected in regard to the study objectives. Did the authors anticipate particularly large differences between these populations that would throw light upon the issues mentioned as the study aims? Please provide some information why the four study locations are of interest.

Re: This is a good point, and we have in re-structuring the Introduction considered this. Already in the Introduction we described in the original manuscript that there are previous data supporting that both the Inuit population and the Swedish fishermen’s population are relatively highly exposed. We have now elaborated on this and also giving argument for that the PCB/DDE ratio in the Ukrainian population differs from
that of the Inuits and Swedish fishermen. The new sentences follow: “In the Arctic, the combination of environmental conditions and biomagnification in the aquatic food chains result in accumulation of POP in local food at levels which are often in excess of contaminants in the mid-latitudes where these contaminants originate [1]. Also Swedish fishermen’s families at the east coast at the Baltic Sea, with a high consumption of herring and salmon, constitute a highly exposed group [2,3]. However, in both these populations there are large inter-individual exposure contrasts. Both among Inuits and Swedish fishermen the serum levels of e.g. 2,2′,4,4′,5,5′-hexachlorobiphenyl (CB-153) and 1,1-dichloro-2,2-bis (p-chlorophenyl)-ethylene (p,p′-DDE) were in the same order of magnitude [1-3]. Much less is known about exposure levels for the general populations in Central and Eastern Europe [4]. However, previous data support that the p,p′-DDE levels was one order of magnitude higher than the CB-153 levels [23].”

6. Also, the inclusion (and exclusion) criteria are not clear. Were the subjects identified at the time of verification of pregnancy, or later? Were women who miscarried also included? Any age restrictions? The time of serum sampling may be important, e.g. because the size of the distribution volume for lipophilic substances may change during pregnancy.

Re: I. Concerning internal validity:
We have clearly stated that the participants were included, by visit to antenatal clinics (Warsaw and Kharkiv) or midwives (Greenland), after that the pregnancies had been verified. The Warsaw women were on average 33 weeks pregnant (25-75 percentiles: 31.0-35.7), the Kharkiv women were on average 24 weeks pregnant (25-75 percentiles: 12.1-33.6 weeks), and the Inuit women were on average 24 weeks pregnant (25-75 percentiles: 16.7-32.4 weeks) at time of interview and blood sampling. This later information has now been included in the manuscript. We agree that the size of the distribution volume for lipophilic substances may change during pregnancy. Thus, we have used gestational length as a determinant for variation in POP serum levels. The result of this analysis was presented in the Results of the original manuscript: “A significant negative association between gestational length at blood sampling and POP in serum was observed only for women from Kharkiv.” The magnitude of the effect is given by the β-coefficients in Table 8. However, this information has now been explicitly given in the Results. Concerning the question about age restriction, the applied criterion was clearly described: “A general criterion for eligibility was that the participants had to be born in the country of study and to be at least 18 years of age.”

II. Concerning external validity:
We have now been more explicit in the Discussion:
“Concerning the external validity of the present study, our conclusions are restricted to the age groups studied. Thus, we cannot say anything about serum levels of POP biomarkers in children, adolescents or in women past their reproductive age (except for Sweden). Three of the female study populations comprised pregnant women, which could hamper a comparison with non-pregnant women of the same age. However, gestational length affected the POP biomarker levels only in the Kharkiv population. Adjusting for the other determinants, each extra week of pregnancy in Kharkiv women was associated with a decrease of the POP biomarkers with 1 %. We think that the participating men from Greenland, Warsaw and Kharkiv were representative for the general population in the same geographical area, whereas the Swedish fishermen were more highly exposed to POPs than the general Swedish population.”

7. It’s curious that population in Sweden was not selected using criteria similar to those used elsewhere. The authors have published several impressive studies on different groups of
fishermen, but I'm concerned about the entirely different selection procedures for the purposes of the present study. The Discussion is very brief on how they may impact on the findings, except that to mention (p. 15) that age is an important factor.

Re: As stated before in this rebuttal, we are not concerned that selection bias has affected the exposure distribution in each population. However, it is obvious that caution is needed for direct comparisons between the Swedish populations and other populations. This was stated already in the original manuscript. But this sentence has now been moved to the first paragraph of the Discussion: “It has to be borne in mind that the age distribution in the Swedish fishermen’s populations differed a lot from the age distributions among the populations from the other countries, and direct comparisons between the Swedish and the other populations must therefore be interpreted with caution.”

8., In regard to the statistical methods, the authors used standard parametric methods, which seem appropriate, but then switched to Spearman’s correlation coefficient. I would suggest that the authors use the parametric Pearson coefficient on transformed data, perhaps best log transformed.

Re: We don’t agree. The reviewer gives no argument for why he considers the non-parametric correlation analyses using raw data is inferior to calculating Pearson’s r for log transformed data. We think both options are correct and see no reason to make any revisions.

9. The tables provide both arithmetic means and medians, and the 90% ranges indicate substantial variation and skewness. I would suggest using geometric means (the Methods section says that the validity of such transformation was checked, but not the outcome).

Re: The geometric means are generally very similar to the medians. We would prefer to keep the medians because for variables like “seafood” for which there are a number of zero values, the geometric means will become “0”, which looks strange as the 5th percentile value will be “>0”. We will delete the arithmetic means from the Tables, also to facilitate to change them to portrait format, as requested.

With respect to the concluding remark within brackets, which we suppose refer to log transformation, we write at the end of the Results: “By log transformation of the POP variables the model assumptions were somewhat better fulfilled in the Swedish population. However, in the three other populations no such improvements were achieved by log transformation of the POP variables, and we do therefore present the results for the untransformed variables for all four populations.”

10. In the Discussion (p.15), the use of CB-153 and p,p-DDE as biomarkers for POP exposure is claimed to be validated by previous studies that include Vietnam veterans. This statement is not reasonable, because the present study has clearly documented that the correlation between CB-153 and p,p-DDE varies between populations. Why would correlations with other POPs then not vary between populations? It may be a serious limitation if the present study that no verification of correlations with other POPs (e.g. in a small number of pooled samples) was included. At least, this possible shortcoming deserves proper attention in the Discussion.

Re: We agree and have revised the Discussion accordingly. See also response to comment # 4.

11. The final paragraph of the Discussion (indicating that the descriptive data presented are valuable and contribute to a study of reproductive effects) should be deleted. The reader of the present manuscript will have no background (without the other MS) to judge the validity of this statement.

Re: OK, done.
12. The Conclusions do not mention the differences in PCB-DDE correlations. This section should highlight the key message to the reader, and it needs to be coordinated with the abstract and the rest of the MS.

Re: The Conclusions have been revised accordingly.

-----------------------------------------------------------------------------------------------

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. Some detail could be left out, e.g., the specific names of Greenland communities (repeated in a footnote to Table 3).

Re: OK, done.

2. The choice of a p value of 0.15 appears unusual and should be justified.

Re: The choice of a p value of 0.15 is, of course, arbitrary. The reason for such a high p value was that we also would like to present estimates for variables that were relatively close to significant, normally defined as a p value below 0.05. In addition, the confidence intervals were always presented for the estimates in the full models, and the reader could therefore easily judge whether the p values were above or below 0.05.

3. The beginning of the Results section (p. 11) provides overall results for the four populations and refers to the Tables.

Re: This paragraph has been revised accordingly.

4. Please clarify whether current seafood intake (number of seafood dinners?) in Greenland included both fish (with minimal PCB and DDE content) and marine mammals. Is there any way to separate these sources?

Re: On lines 387-388 in the original manuscript it was stated: “Among Inuits seafood comprised both sea mammals and fish, whereas for Sweden it comprised locally caught fish from the Baltic Sea (east coast) and the North Sea (west coast), respectively.” We have unfortunately no possibility afterwards to separate sea mammals and fish for the Inuit population, even if we fully agree that it should have been preferable. In order to give this message even clearer we have now added the following sentence to the Methods section: “For the Inuits seafood comprised sea mammals as well as fish, whereas for the other populations it only refers to fish.”

5. The presentation of these complex results is difficult to follow. I think it would be easier for the reader to follow, if you presented the regression models for one population (males and females) at a time, and highlighted the most important determinants along the way. It appears that the men and women from one location are similar, while subjects of the same sex but from different locations differ substantially.

Re: We agree that the manuscript my gain from restructuring the Results section. We have in the revised version three subheadings, coherent with the three aims and in the same order. However, we have chosen not to mix the section on determinants with the other text. We mean that in this way the Results will be more easily understood. We have also used population and not gender for structuring the paragraphs on determinants.
6. I would suggest adding a plot of PCB vs DDE, perhaps with the four different regression lines.

Re: In principle we agree, but on the other hand we think that a plot with more than 2000 data points (women) and four regression lines (and 95 % CI’s for the regression lines) will not be very informative (or readable) for the readers.

7. ‘DC’ should be removed (or explained) at the end of the first full paragraph on p. 15.

Re: Thanks, it was just a typo that is now removed.

8. The Discussion of temporal trends (p.16) should refer to other studies that have shown decreased serum concentrations. Because this issue has been well documented already and was not a main aim of this study, the Discussion could be abbreviated.

Re: We agree and this section has been revised abbreviated accordingly.

9. The differences in PCB-DDE correlations (p. 17) should be moved forward in the Discussion and emphasized, assuming that the authors agree that this is the key finding of this study.

Re: We have in accordance with given recommendations restructured the Discussion, bringing up the findings in order the aims have been stated, and first after that we have Discussed methodological issues and potential biases. As a consequence the differences in PCB-DDE correlations have been moved forward in the Discussion.

10. The statement on top of p.18 in regard to differences between cross-sectional and longitudinal studies is interesting, but deserves a fuller explanation. Because age has been reported as a significant predictor in many studies, it may be a less important issue here, so the discussion could be abbreviated.

Re: We find this comment somewhat contradictory. We have tried to show that the relation between age and POP is not that simple. The reviewer asks both for a fuller explanation and an abbreviation of the paragraph. We have tried to explain the relation in a somewhat more detailed way.

In regard to breast-feeding and parity, one must also consider weight changes during (and after) pregnancy. Again, these factors are already well described and less important here.

Re: Theoretically, weight increase during pregnancy could result in lower POP levels in serum due to an increased distribution volume (adipose tissue). We think that we considered this by both adjusting the analyses for gestational length and for BMI at blood sampling. It is unclear what the reviewer means that we have not considered.

11. The authors report their results in terms of serum lipid weight. Although widely accepted, some other authors have questioned this approach. This issue may also relate to the discussion of BMI in regard to POP kinetics, distribution volume, and the possible impact of BMI on elimination of the POPs. Some further attention to this matter would be useful.

Re: We suspect that the reviewer might think of the recent paper: Schisterman EF, Whitcomb BW, Louis GM, Louis TA. Lipid adjustment in the analysis of environmental contaminants and human health risks. Environ Health Perspect. 2005 Jul;113(7):853-7. However, this article gives a firm support for our way of standardize POP levels for serum levels. In a simulation study, applying the causal scenario that adipose tissue PCB causes serum PCB per unit serum lipid and causes the outcome, the percent bias of estimates was lowest for our way of standardization compared to e.g. lack of adjustment or two-stage models.
12. Smoking has received much more attention than other more important determinants, and the paragraph on p. 20-21 could be substantially abbreviated.

Re: Smoking has been a more controversial determinant of POP exposure than many other and deserves therefore more attention, but we have anyhow abbreviated the paragraph somewhat.

13. The paragraph on the middle of p. 21 is largely repetitive and could be left out.

Re: The paragraph was deleted when restructuring the Discussion. Information not given elsewhere has been integrated in other part of the Discussion.

14. Most of the Tables will not fit the journal design in portrait format. Rather than moving them to separate files that can be accessed by the reader, I would suggest that they be redesigned to fit the required format. For example, Tables 1 and 2 could fit if they just included (geometric) mean and 90% range for each of the four populations.

Re: OK, we have followed the recommendation.

15. Table 4 appears superfluous

Re: OK, Table 4 has been deleted.

and Table 5 should give Pearson correlations, or they should be provided in a Figure (with the r in the legend).

Re: Based on the reply to Grandjean’s major point # 8, we don’t agree and would like to keep Table 5 as it is.

16. Tables 6 and 7 could be additional files, but it may be easier for the reader to appreciate the results if split according to location, rather than sex.

Re: We have in accordance with the recommendations split the tables according to location and not to sex, and also changed them to portrait format. Thus, the previous voluminous Tables 6 and 7 have been changed to four much slimmer tables. We would like to keep them in the manuscript as they comprise crucial results.

17. Figure 1 is difficult to read. Could it be redesigned as a generalized additive model with a non-parametric regression line, or some similar function to illustrate the association and its possible deviation from linear?

Re: We have deleted Figure 1. There is information in Results about the difference between Inuits and Swedish fishermen in CB-153 exposure both before after age adjustment.

Reviewer #2: Dr. Birger Heinzow, LGASH

I like the paper and especially appreciate the opportunity of electronic publishing to provide a wealth of details of this multicenter study. I am impressed how the obstacles of analytical variance between laboratories have been taken care and solved.

Re: Thanks

Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)
Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

line 50 female inut is lacking
Re: Thanks. The Result section of the Abstract has, however, now been rewritten after recommendation by the other reviewer.

Discretionary Revisions (which the author can choose to ignore)

Line 368:
Another PBK explanation of a gender difference in POP body burden, besides "exlactation" of POPs (excretion by breastfeeding), should be considered the higher energy turnover (high input) of fishermen, which would result in concentration of POPs in a small volume of distribution.
Re: We think that we have already corrected for this by assessing the impact of BMI, which was surprisingly low.

Finally, should you choose to revise and submit this as a separate paper, there are a number of formatting issues that would need to be addressed to allow online publication:

Please remove any unnecessary capitalization from the title. Also, please change "" to "cross-sectional."

Re: OK, done.

Street addresses and zip codes are needed for all affiliations.

Re: Street addresses, when relevant, are added. Zip codes for all are added

Also, please remove web address for affiliation #11 and replace with full street address.

Re: As asked we have removed the web address, but there is no street address to replace it with. We don’t agree with you. It had been better to leave the web address

List of abbreviations is missing.

Re: Is now added (seems not to be optional anymore to full descriptions of abbreviations in the text, which you actually write in the instructions!!)

Et al cannot be used in the reference list. All authors need to be mentioned.

Re: OK, done

Tables need to be properly formatted according to EH instructions at http://www.ehjournal.net/info/instructions/.

Re: OK, done

Figures need to uploaded as separate files according to EH instructions.
Re: OK, done

Please recheck your spelling.

Re: OK, done.

When you have prepared a revised paper, please upload to the website with the point by point response in the cover letter.

Thank you for submitting to the journal. We look forward to your response.

With Best Wishes

dave ozonoff

cuo-Editor in Chief

David Ozonoff, MD, MPH
Professor of Environmental Health
Boston University School of Public Health
715 Albany Street, T2E
Boston, MA 02118
USA
tel. 617 638-4620
fax 617 638-4857