

## **Author's response to reviews**

**Title:** Immune cell counts and risks of respiratory infections among infants exposed pre- and post-natally to organochlorine compounds: a prospective study

### **Authors:**

Anders Glynn ([anders.glynn@slv.se](mailto:anders.glynn@slv.se))  
Ann Thuvander ([ann.thuvander@socialstyrelsen.se](mailto:ann.thuvander@socialstyrelsen.se))  
Marie Aune ([marie.aune@slv.se](mailto:marie.aune@slv.se))  
Anders Johannisson ([anders.johannisson@afys.slu.se](mailto:anders.johannisson@afys.slu.se))  
Per Ola Darnerud ([poda@slv.se](mailto:poda@slv.se))  
Gunnar Ronquist ([gunnar.ronquist@akademiska.se](mailto:gunnar.ronquist@akademiska.se))  
Sven Cnattingius ([sven.cnattingius@ki.se](mailto:sven.cnattingius@ki.se))

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Anders Glynn  
Toxicology Division  
National Food Administration

Editor-in-Chief  
Environmental Health

**Dear Editor-in-Chief**

We resubmit the manuscript “Immune cell counts and risks of respiratory infections among infants exposed pre- and post-natally to organochlorine compounds: a prospective study” to EH after revision. We have revised the ms as directed by the EH Editorial Team in their e-mail.

Response to reviewers comments**Referee 1**

*“Also, since PCBs in mother’s blood and milk correlate closely, it is difficult to separate the effect of prenatal and postnatal PCB exposure. These strong correlations make it difficult to give to firm conclusions on which PCBs cause what effects on the immune system. Maybe this could be stressed a bit more in the manuscript.”*

-We have revised the Abstract so that this problem with correlations is more evident (Conclusions). Furthermore, in the revised ms this problem is discussed in the first paragraph of the Discussion.

*“3) Thus the PCB(28,52,101) are “immunosuppressive” and 4) PCB mono and di ortho are “immunoactivating”. The two last conclusions seem not very well founded.”*

These “pre-mature” conclusions have been omitted in the Abstract and Discussion. In the second paragraph of the Discussion we point out that our study design do not allow for conclusions about immunoactivation or –suppression.

*“The authors go a long way in order to discuss their - to some extent - conflicting findings. Although, I like most of the discussion the authors should not distance them selves so much from their results, if they reevaluated them according to the above.”*

We did not really understand this wish for “Major Compulsory Revisions” but we think we have done this by addressing the comments above in the revised ms.

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**Referee 2**

*“Based on the findings presented, the authors suggest that prenatal exposure to tri- to pentachlorinated PCBs may be associated with immune suppression, whereas exposure to mono- and di-ortho PCBs may cause “immunoactivation.” Given that the only read-outs were the percent and number of different leukocyte subsets in the infant's serum and the incidence of reported respiratory illness, the data do not strongly support these conclusions.”*

We agree that our study design do not allow for conclusions about immunoactivation and immunosuppression. These “pre-mature” conclusions have been omitted in the Abstract and Discussion. In the second paragraph of the Discussion we point out that our study design do not allow for conclusions about immunoactivation or –suppression.

*“An additional weakness that the authors are encouraged to incorporate into their thinking is that the study was not designed to assess immune enhancement (i.e., Reduced incidence of respiratory infection doesn't necessarily mean that the immune system is more stimulated. Indeed, there are many other factors that could account for this--especially when examined only at a single point in time).”*

Se answer to first comment.

*“Another concern is that disease diagnosis was not confirmed using medical records (as an aside, perhaps using the term influenza is therefore misleading as no confirmation of this specific illness was included).”*

We agree on this comment and this concern is discussed in the Discussion in the end of page 16 and beginning of page 17. We have also revised the term “influenza” to “influenza-like symptoms” on page 9, last para.

*“In Figures 1 and 2, it is not clear why mean lipid concentrations (ng/g lipid) are divided up into <3, 3.1-5.7 and #5.8. The middle group represents a rather small range while the upper group reflects a rather large range. An explanation of this division would be welcome (perhaps this relates to the next comment?)”*

As now stated in the Methods section, page 9, second paragraph, and in the Figure legends, an effort was made to have equal numbers of participants in the categories formed. The rather narrow exposure interval in the middle group of pre-natal CB 28+52+101 is a result of this categorization.

*“Table 2 is somewhat confusing. In particular, the sub-division of mean organochlorine concentrations in serum and breast milk into the 3 columns “Infections,” “white blood cells” and “lymphocytes” is not clear.”*

The Table has been revised with a new heading (Study groups) and a clarification in the footnotes.

*“It is a bit difficult to reconcile the data in Figures 1 and 2. In Figure 1, the number of lymphocytes is elevated in serum from subjects in the highest PCB level group; however, when the different lymphocyte subsets are analyzed, none of the subsets account for this increase (nor is there a slight increase in all subsets).”*

This is what we found in the statistical analysis. As indicated in Figure 2 the numbers of B- and T-lymphocytes were increased in the highest exposure group (in line with the increased lymphocyte numbers in Figure 1), but this increase was not statistically significant at the significance level of  $p \leq 0.01$ .

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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)”*

We think we have found these mistakes and have done the changes.

*“(1) All of the figures and tables present findings from "3 month-old infants exposed to organochlorines post-natally." It is not clear to me how prenatal exposure is removed from these data. Were not all infants exposed both pre- and post-natally? Explanation of this could be more explicit.”*

We have looked through the Tables and Figures and do not understand this comment. Table 4 gives the results of pre-natal exposure and WBC/lymphocyte subsets, except CB 28+52+101 which is given in Figure 1 and 2. Table 5 gives the results of pre- and post-natal exposure and infection risks. Table A in the Additional files gives the results for WBC numbers and post-natal exposure, Table B WBC percentages and post-natal exposure, Table C lymphocyte numbers and post-natal exposure, and Table D lymphocyte percentages and post-natal exposure.

*“(2) Overall the paper is well written; however, there are some minor grammatical errors throughout the paper.”*

We have looked through the ms and corrected the errors we found.

*“While the authors' candor about the limitations of this study is a strength of this manuscript, they may want to consider revising the way in which this is presented. For instance, on page 14, the statement that these results do not allow for firm conclusions (which I agree with) comes across so strongly that it dampens enthusiasm for the study all together. In its current form, the Discussion runs the convincing the reader that this report is too premature to be informative.”*

We have revised the Discussion and put more enfasis on discussing our findings in the beginning of the Discussion and discussing the weaknesses in the end.

*“The idea that in utero exposure can enhance some aspects of immune function while impairing others is supported from work in animals, but this work is not cited here. For example, see Vorderstrasse et al (2004) Journal of Immunotoxicology (vol 1(2): 103-112) and Vorderstrasse et al (2006) Journal of Toxicology and Environmental Health, part A (vol. 69: 1-19). These findings may bolster observations reported here because, although in mice, they directly examine respiratory immune responses to influenza virus infection.”*

We thank the reviewer for making us aware of these articles. We have added this information in the Discussion on page 15, first paragraph.

### **Referee 3**

*“1. It is not entirely clear how the 86 infants for blood sampling were selected. It is unclear whether or not a selection bias has occurred, although the authors mention the similarity between the groups. The authors should stipulate how the infants were selected.”*

In the Methods section, page 6, paragraph 2, we report the selection process of the 86 infants. The results of the analysis of selection bias is presented in the first paragraph of the Results section, page 11. In the Discussion section these results are discussed on page 18, paragraph 3.

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*“The interviews were taken 3 months post partum. Did the mothers keep a diary or were the questionnaires based on recall? The former is more reliable than the latter.”*

As stated in the Methods section, page 7, paragraph 1, the questions about infant disease was based on recall. The problems with this is discussed in the Discussion section, end of page 16-beginning of page 17.

*“Were the interviewers and mothers blinded to the OC concentrations during the study period or were they aware of the concentrations? In the case of the latter: could this play a role in the reporting of infections, etc?”*

The interviewers and the mothers were blinded, as stated in the Methods section, page 7, end of paragraph 1. This is also mentioned in the Discussion section, beginning of page 17.

*“The results section makes for difficult reading. I would suggest rewriting the section in a manner that makes it easier to understand.”*

We did not change this section since the referee did not give any specifics about what was difficult to understand. We do not think this section is difficult to read.

*“Figures: not completely clear how to interpret them. Scatter diagrams may be more illustrative.”*

We did not change the figures, since we cannot see how scatterplots would improve the presentation of the results.

*“Tables: not completely clear how to interpret them. I would suggest presenting the data in a clearer manner.”*

We have not made any major changes in the Tables, since the referee did not give a hint on what was difficult to interpret.

#### **Referee 4**

*“Could the association with infection history actually be more closely related to timing of infection (i.e., how recently an infection occurred) and thus with white blood cellcount)? If so, what does that mean? What was the a priori expectation or hypothesis regarding each of the other immune markers? More discussion of how we should be interpreting the various lymphocyte measures would be useful.”*

The referee is right about the possibility that the increased WBC count could be related to more recent infection. This possibility is now discussed on the Discussion section, page 17, second paragraph.

*“Abstract: It would be helpful to include the definition of mono-ortho and di-ortho PCBs (i.e., which congeners are included)”*

This has been included in the Abstract, results part.

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*“Background, 1st paragraph. What do you mean by “immunotoxic”? Can you be more specific – i.e., is this literature predominately focusing on immunosuppression, or is it broader? Similarly, in the 2nd paragraph, you mention “alterations in markers of immune function”. What kind of changes (direction) are seen, and is there any consistency?”*

The literature on PCBs and DDE has concentrated on suppression of the immune system, and this is maybe clearer now in the first paragraph of the Background section. Paragraph 2 has been revised with a few sentences illustrating the divergent results of studies of immune cell counts.

*“Methods: It would be clearer if you explicitly stated in the methods section that women who did not breast feed were included in the study and that in these cases, postnatal exposure was assumed to be zero.”*

This has been added on page 8 after the equation for mother’s milk exposure.

*“The details of the information about infection history are a little unclear to me. It would be useful to provide the specific questions that were asked.”*

We have included more information about the questions asked regarding infant diseases on page 7 first paragraph.

*“I’m not sure of the value of summing across the different exposure groups. Are there other examples of this kind of analysis in the literature, and if so, is this method (summing categories) the usual procedure that is used? I’m not sure how to interpret this summation variable.”*

More information about the summation about the procedure for exposure has been added on page 10 second paragraph. The usual procedure that we have found in the literature is a summation of the absolute concentrations into a sum organochlorine concentration. This procedure assumes that the studied compounds have the same potency to modulate the immune system. We avoided this assumption by summing up the categorized exposure variables. Thus the exposure to the different compounds got the same weight in the summation. We also now give an example of how the summation was done. We hope that the procedure is more clear now.

*Results: page 11 – white blood cell and lymphocytes analysis – Although Figure 1 is a clear visual display of the relevant data, it doesn’t allow the reader to easily describe the results. Please provide the actual data values for each of the PCB 28+52+101 categories corresponding to the bars in Figure 1 by adding this information to the figure legend, or to a table, or to the text.*

We did not do this since we wanted to avoid double-reporting of results. We think the Figure as it is now gives enough information for the reader.

*“Table 2. It would also be useful to add a descriptive label, such as “Study Population” for the columns”*

This has been done.

*“Table 4. Another column, including the data for the PCB 28+52+101 congener grouping would be useful.”*

This could unfortunately not be done since the CB 28+52+101 data was categorized. The results are instead given in Figures 1 and 2.

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*“Tables 4 and 5. It would be easier to follow if the order of the columns was consistent with how the data have been presented previously (i.e., the 228+52+101 group, then the di-ortho group, then mono-ortho, then p,p'-DDE).”*  
The Tables have been revised.

*“Table 5. Footnote c is missing – is it a definition of the categories? If not, it would be useful to include that information a footnote.”*  
Information about the categories have been added under footnote c.

*“page 9 – spelling error (navel infection instead of nasal infection)”*  
It is not a spelling error. Should be navel infection.

Best regards

Anders Glynn