

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)

Ann Thuvander (ann.thuvander@socialstyrelsen.se)

Marie Aune (marie.aune@slv.se)

Anders Johannisson (anders.johannisson@afys.slu.se)

Per Ola Darnerud (poda@slv.se)

Gunnar Ronquist (gunnar.ronquist@akademiska.se)

Sven Cnattingius (sven.cnattingius@ki.se)

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Author's response to reviews: see over

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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. Below are the answers to the comments included in the e-mail from the Editorial Team.

1. "Please explain why the age of three months was chosen."
The explanation is now available in the ms on page 6, beginning of paragraph 2.

2. "Please explain how time since vaccination was taken into account (covariate in the regression analysis of blood cell data?)."

On page 10, second paragraph a more detailed description of the independent variables included as potential confounders in the regression analyses of immune cell counts/percentages is given. We only had information about if the infant had been vaccinated or not, we did not have information about time since vaccination.

3. "Likewise, were infants with current infection excluded from the data analysis regarding blood cells?"

Infants with an ongoing infection at time of sampling were not excluded. In the Result section, Organochlorine compound exposure and WBC, and Organochlorine exposure and lymphocyte subsets, on page 14 we have however included results of an analysis of associations between counts/percentages and organochlorine compound exposure after exclusion of infants with an ongoing infection at the time of sampling. The results did not change much.

4. "On p 11, you say that more children with recent vaccination were included in the study sample, so that would also suggest that this parameter be included in the data analysis."

Vaccination status of the infants was included as a potential confounder in the statistical analysis and this is more clearly stated on page 10, second paragraph. This information is also given in Tables 4, 5, and E.

5. "On p 17, you say that adjustment of immune cell results for respiratory infections did not alter the findings. This information needs to be presented in the Results section, with emphasis on recent or current infections."

This information is already there in the Methods and Results sections and the Tables, since infant respiratory infections during the period before sampling was included as a potential confounder in the multivariate regression analyses of the WBC and lymphocyte subset results. See also answer to Comment 3.

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6. "How many refusals did you receive, or, in other words, what was the participation rate?"

The participation rate in blood sampling of infants is now given on page 6, second paragraph.

7. "It seems from the information provided on p 7 that you had information about the age, the duration, the severity, and the need for medical treatment, but it is not clear whether this information was examined in the data analysis. Since you see associations with a past history of infection, it would seem appropriate to examine whether this affected infections at a particular age (e.g. when maternal antibodies would provide less protection), as well as frequency and severity."

We have now included results from statistical analyses where a more strict definition of infection is used (3 days or more with symptoms) in the Result section, Respiratory infections on pages 15, end of first and second paragraph, and Table E. As now stated on page 11, end of first paragraph, it was not possible to use the information about infection in combination with information about body temperature in the statistical analysis, since there were too many missing values for the latter variable. Too few had more than 1 infection, and this was also the case for treatment of symptoms by a physician.

8. "The organochlorine concentrations are expressed per gram. Please explain if lipid adjustment was not possible, and what the implications might be."

Serum organochlorine concentrations were lipid-adjusted. We have made this clearer in the MS. For the calculation of post-natal organochlorine exposure, the concentrations on a fresh weight basis were used since this more correctly represents the amount of the compounds that is ingested by the infant during nursing (see page 9 beginning of first paragraph).

9. "Under the results, you refer to TEQ-weighted mono-ortho PCBs, but there is no explanation of this calculation in the Methods section."

We think that the use of the TEF system for dioxin-like compounds is well known by researchers in the field, and we give the reference to the most important TEF article in the Tables. Therefore we did not include an explanation in the text, due to limited space.

10. "On p 9, you refer to categorization of results, and you need to explain how that was achieved, e.g., as tertile groups."

We have explained our categorization better now, we hope, on end of page 9 and beginning of page 10.

11. "A section is needed to describe the covariates and how you selected those that were included in the final regression equations."

On page 10, second paragraph, a more detailed description of the selection of independent variables included as potential confounders in the regression analyses is given with references.

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12. "Please clarify whether 'respiratory infection' refers to past history - but not current - of any airway infection."

We do not really understand this comment, and what it refers to. We hope that our answer to Comment no. 3 also covers this comment.

13. "You explain that you use two different p-values to judge significance, please explain or use the same value (or, better, provide exact p values). On p 11, you provide exact p-values, but in one instance you just say >0.05. This does not seem logical."

As already stated in the Methods, section Calculations and statistics, a stricter level of significance ($p \leq 0.01$) was used for the statistical analyses of immune cell counts/percentages, since multiple comparisons were made. This was not the case for the infection results, so in this case the significance level was $p \leq 0.05$. The differences in presentation of p-values have now been revised on page 12-13 (former page 11).

14. "The summation of prenatal and postnatal scores appears like a crude approach. Please explain why the relative contributions could not be analyzed using non-edited data."

We have revised the text explaining our procedure for summation of exposures on page 11 and 12, hopefully making it more clear why we did not sum up absolute concentrations.

15. "The description of the ranges of PCBs on top of p 12 should take into account problems with results below the LOQ."

We do not understand this comment. We dealt with problems of CB 28+52+101 results below the LOQ in the statistical analyses by categorizing the exposure, with study participants with concentrations below LOQ in the lowest exposure category, as stated in the Methods section.

16. "Since much of the literature refers to total PCB, we recommend that you include total PCB in the data analysis and then supplement with the analyses of individual groups of PCB congeners."

We do not think that inclusion of total PCB in the data analyses will give any more valuable information. As discussed in the Discussion section, different studies have measured total PCB in different way, some using CB 153 as a marker substance, some summing up three or four different PCB congeners. Comparisons of results between studies on basis of total PCB is therefore not meaningful. We think that our separation of different PCB compound groups gives a better description of the PCB exposure situation.

17. "In the discussion, you state that dietary exposure to low-chlorination PCBs is low, as indicated by the low serum concentrations. However, if they show a different association with the risk of infections, wouldn't they originate from a source different from the other PCBs? If they come from indoor sources, could they reflect poor housing, poor ventilation, or other factors that could confound the relationships?"

We have included this possibility of confounding by exposures, other than CB 28+52+101, related to PCB-houses in the Discussion on page 17 about uncertainties on page 17.

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18. "The conclusions section of the main text does not appropriately reflect your study, and one sentence reviews past studies elsewhere. Please rephrase."

The conclusions have been revised.

19. "In the abstract, the headers should be in line with the abstract with a colon, instead of a period before the section text. The last section is Conclusions (plural). The words prenatal and postnatal are both one word, without a dash. Likewise, unadjusted without a dash. The pagination should be removed before submission of the final version. Authors' contribution should read Authors' contributions. All tables must be changed into a closed cell format with all borders showing."

These revisions have been made.

Best regards

Anders Glynn