Author's response to reviews

Title: Inter-individual variations of human mercury exposure biomarkers

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Author's response to reviews: see over
Dear Drs. Grandjean and Ozonoff,

Here is a summary of what changes have been made in our revised manuscript “Inter-individual variations of human mercury biomarkers” by Marika Berglund, Birger Lind, Brita Palm, Karolin Björnberg, Östen Einarsson and Marie Vahter. Response to reviewer’s comments is also included. We appreciate very much the comments raised by the reviewers and have changed the manuscript accordingly.

Reviewer Thomas W. Clarkson:
1) Page 4 line 2: We agree that kinetics also is included in the term toxicology of a toxic agent and the words “kinetics and” have been deleted. The sentence should read: “People are exposed to different forms of mercury (Hg), which differ with respect to toxicology.”

2) Page 4: As the reviewer points out the reference (1) “NRC: Toxicological effects of methylmercury. Washington, DC: National Academy Press; 2000” concerns methylmercury. However, in chapter 2 “Chemistry, exposure, toxicokinetics and toxicodynamics” all chemical species of Hg are reviewed.

3) Page 4, line 3: It is pointed out that the kidney is also a target organ for inhaled mercury vapour. Accordingly, the following has been rewritten and should now read: “The target organ for methyl mercury (MeHg) toxicity is the brain, which is especially susceptible during development [1]. Target organs for elemental mercury vapour (Hg[0]) are the brain and kidney and the target organ for inorganic Hg compounds (IHg, Hg[II]) is the kidney [1].”

Page 5, lines 4-6: Yes, reference (1) says that THg in hair reflects inorganic mercury exposure in non-fish eaters (chapter 4, page 118).

4) Page 5, last paragraph of the Background section: In line with the reviewers comment the phrase “...often with high exposure levels..” has been changed and the sentence should now read: “The available information on Hg kinetics is based on 25-35 years old experimental studies, sometimes with high exposure levels, involving a limited number of volunteers.” In the study by Kershaw et al 1980, the dose was 1400 µg Hg per person, which is pretty high.

5) Page 9, paragraph 1: The source of the radiolabeled MeHg has been provided.

6) Table 2: The same volumes (1.0 mL) of plasma, RBC and whole blood were used in the analysis.
Yes, CV% changes with Hg concentration. In general, the lower concentrations the higher CVs. However, in this case there was not a big difference in CVs in the low and high Hg concentration range (mainly because of the high sensitivity of the analytical method and the low detection limits). For example for plasma, CV was 6.9% in the concentration range 0.048-0.7 and 4.4% in the concentration range 0.7-1.3.

We didn’t calculate CVs for OHg because the concentrations were calculated as the difference between THg and IHg. Since the OHg concentrations were generally higher in whole blood and RBC, we expect the CVs to be lower, or in the same range as for THg and IHg. For example, in blood CV for OHg was 4.5% (in the concentration range 0.3-6.9). In plasma, where OHg concentrations were lower than IHg concentrations, the CV was 14%. This information was not included in the table, since it does not add substantially to the description of the analytical method, and since there is already a large number of figures in the manuscript.

7) Page 12, Table 4: The reviewer wonders if some of the low significance correlations in table 4 may be due to chance. Yes, it is possible. However, after looking at scatter plots of the data, it can not be excluded that the correlations are true associations.

8) Page 14: The reviewer suggests us to use the same concentration units for Hg in hair and blood. However, we chose to use the same units as (most) others do, for comparison reasons.

9) Page 17: The reviewer writes that there is a recent survey saying that hair treatments have no effect on levels of mercury in hair. We were not able to track this paper, but we have rewritten the sentence, which now should read “However, it should be borne in mind that artificial waving and other hair treatments may reduce Hg concentrations within the hair strand”.

Reviewer Alan Stern:
1) Page 2, second to last line: We are not sure of what seems to be missing in this sentence, however we have rewritten it in order to make it more clear. The sentence should now read: “The use of THg concentration in whole blood as a proxy for MeHg exposure will give rise to an overestimation of the MeHg exposure depending on the degree of IHg exposure, why speciation of mercury forms is needed.”

2) Page 5 and ff: We obtained the information about fish intake and number of dental amalgam fillings by use of questionnaires. The information was self-reported by the participants. The distributions of these parameters were described in the Results section (page 11) but according to the reviewer’s comment this information is now provided in the Methods section (page 6).
On page 6 it should now read: “Information regarding fish intake (usual number of meals/month) and number of dental amalgam fillings was collected via self-reported questionnaires. A usual number of 0-22 fish meals/months and a total number of dental amalgam fillings between 0-15 were reported.”
3) Figure 1: Directional arrows has been included in Figure 1.

4) Page 10: The reviewer thinks that the percentage of IHg in the reference material seems unusually high. However, it should be pointed out that in these reference materials only the concentration of total Hg is provided as a recommended value by Seronorm. Furthermore, to our knowledge there is no commercially available reference material for IHg in blood. Our results for IHg in the Seronorm samples are well in agreement with our previous results using the same samples which we have done for several years. In addition, repeated analysis of a cow blood spiked with low concentrations of IHg and MeHg were performed in parallel with the Seronorm samples in a number of analyses. This gave average recoveries of 101% and 96% for IHg and total Hg respectively (Bjornberg KA et al 2003). This reference is also referred to within the text (reference 33).

Table 1: Seronorm 404107X and Seronorm 404108 were used as reference material for analysis of both blood samples and RBC samples.

Table 3, about LODs: The reagent chemicals are cleaned in the reaction tower prior to the addition of mercury content in the samples. In the case of the chemical blanks, deionized water (and solubilization solutions) is added instead of the biological materials to be analyzed. This means that LODs are calculated based on the determination of mercury in the chemical blanks after the cleanup step. We have not measured blanks and calculated LODs without performing the cleanup step. However, the areas under the curve (AUCs) for chemical blank readings were smaller than the AUCs for the cleanup step readings, when judged by the eye.

5) Page 11: The intent of “distribution of OHg and IHg” is the partitioning or division of mercury forms in whole blood between RBC and plasma. The sentence has been rewritten and should now read “The distribution of OHg and IHg in whole blood between RBC and plasma was calculated as the percentage of total OHg or IHg in whole blood according to equation 1 and 2 (below), using individual hematocrit values (B-EVF, %).”

The abbreviation B-EVF stands for Blood Erythrocyte Volume Fraction, i.e. hematocrit.

6) Page 12, Equations 1 and 2: Using these equations we have calculated how much of the total OHg and IHg in whole blood that is present in RBC and plasma respectively (in percent of total).

1. CI means the 95% confidence interval and this is now explicitly stated within the text.
2. The following sentences (on page 12, 2nd paragraph) have been rewritten due to the reviewer’s linguistic comments:
   - “RBC-IHg was on average 6.8% of RBC-THg (median; range 3.3-24%), and…..”

The following sentence was deleted: “The average percentage of RBC-IHg of RBC-OHg was 7.2% (median; range 3.4-32%).

   - “In a person with no dental amalgam fillings RBC-IHg was 4.6% of RBC-THg.” This does not refer to the average percentage as it is only one person.
7) Page 13, 2nd paragraph:
- “In our previous study of women with a high fish intake…” Although we have used an “old” study population these results have not been published previously. Therefore we cannot simply refer to the earlier study (which is reference 31). As described in the Methods section (page 6) data from our previous study was included for evaluation of Hg in hair.

“The difference was not statistically significant (Students t-test, p=0.4).” We don’t agree with the reviewer’s comment that this sentence was superfluous.
- Last sentence: This has not been stated earlier in the paragraph.

8) Page 14, 3rd paragraph:
In line with the reviewer’s comments, we have inserted the word moderately with respect to the association dental amalgam fillings and IHg in urine.

9) Page 15, 2nd paragraph, 2nd line: As the reviewer writes demethylation is part of the MeHg kinetics. However, demethylation during sample treatment and analysis is concerned in this context. Therefore, “as well as” is proper to write.

10) Page 15, 3rd paragraph: The value 15% represents the full range among all individuals, i.e. 76-100% in blood and 5-20% in plasma (See Results section). The subsequent descriptions of inter-individual variation refers to the full range 15-54% in blood and 30-81% in plasma. The words “total range” has been included.

11) Page 16, 2nd paragraph: “This can explain the larger inter-individual variation in the distribution of IHg…” For clarification, according to the reviewer’s comment, this sentence has been rewritten and should now read “Thus, the variation in MeHg exposure from fish adds to the variation in RBC-IHg, which can partly explain the larger inter-individual variation measured in the distribution of IHg between RBC and plasma.”

12) Page 17, 1st paragraph: We found that about 9% of THg in hair was in the form of IHg and our data indicates that this is a result of MeHg exposure and demethylation of MeHg (in blood, hair, and analysis) rather than a result of IHg exposure (described on page 16). As the demethylation of MeHg in the hair analysis is about 5% (Results, page 11), this leaves about 4% demethylation in the hair follicle. This is not a rate but rather a “degree of demethylation”.

13) Page 17, 2nd paragraph: In order to guide the reader in this direction we included a reference to Figure 3 in this paragraph.

14) Page 18, 2nd paragraph: The sentence has been rewritten according to the reviewer’s comment and should now read: “The demethylation taking place during sample preparation and analysis with this method will lead to…”.
15) Page 19: We agree with the reviewer’s comment that H-THg is the best measure of long-term average MeHg exposure, and have added the sentence “THg in hair seems to provide the best measure of long-term average MeHg exposure” on page 19.