

Author's response to reviews

Title: A preliminary study of mercury exposure and blood pressure in the Brazilian Amazon

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Response to the reviewers' comments

We would like to thank the reviewers for their thoughtful and helpful comments that will serve not only to improve the present manuscript but also our subsequent studies.

Major Compulsory Revisions

Comment 1: The results are not based on a random sample of the population studied. A convenience sample has been used, and recruitment into the study was carried out using house-to-house surveys and at village meetings. Inclusion and exclusion criteria are not given. Thus, the sample of the study subjects might not be representative, and the risk of bias is considerable. This is a limitation of the study and should be mentioned clearly in the discussion.

Response to comment 1: The inclusion criterion is explained in the text: we state we accepted the volunteers recruited through the village meeting or house-to-house survey. Inclusion criteria: at least 15 years of age. Exclusion criteria for the present analysis was reported diabetes. We have included a section on convenience samples in the discussion.

Comment 2: Blood pressure level is estimated by a casual blood pressure measurement. However, the reliability of a single measurement is rather low. Several measurements are demanded to clarify the blood pressure level, and 24 hour ambulatory blood pressure measurement is far the best method to obtain reliable determination of a person's blood pressure. The circumstances of blood pressure measurements should be more clearly presented and the limitation of casual blood pressure measurement should be clearly indicated in the discussion.

Response to comment 2: For this exploratory study, we used a single measurement. This probably underestimates the effect of mercury since it adds "noise" to the response. Since we did have positive results, our subsequent studies will include several measures of blood pressure for each participant, as well as other measures of cardiovascular functions (eg. R-R variability). Blood pressure measurement is now explained in more detail in the "Methods" section, and we indicate the limitations of using a single blood pressure measurement in the discussion.

Comment 3: Sodium intake is an important factor in the evaluation of blood pressure level. Some populations in South America have a low intake of sodium, a high intake of potassium and their blood pressure level is lower than in people from Europe and USA and Canada due to a difference life style regarding diet. A measurement of 24 hour urinary sodium output would have given information about the sodium balance. The study would be improved if these data are available and can be included in the

analysis. If these data are not available, it is another limitation which should be mentioned in the discussion.

Response to comment 3: We thank the reviewers for the suggestion and will try to assess sodium urinary output in our subsequent studies. We have added this limitation to the discussion.

Comment 4: The authors have used a limit of systolic hypertension at 130 mmHg, and they use this level in their analysis. However, the usual limit for casual blood pressure is 140 mmHg, which the authors also use for discussion. The analysis of the present results should be done with the international recommended level for casual blood pressure which is 140 mmHg systolic.

Response to comment 4: We decided to use the limit for "borderline hypertension", which is 130 mmHg, as blood pressure is relatively low in this population, and too few individuals are above 140 mmHg to carry out valid statistical analyses. We also added that information to the discussion.

Comment 5: The conclusion of the abstract is out of focus. Only the first sentence "The findings of this preliminary study add further support for Hg cardiovascular toxicity" is relevant.

Response to comment 5: We took out the irrelevant sentences, and let only the first sentence in that section.

Comment 6: The introduction is rather long and could be shortened. Focus of the paper is mercury and blood pressure. It is the authors' hypothesis that the people around Tapajos River consume fish with a high content of mercury due to pollution. However, no relationship was demonstrated between fish intake and blood pressure, and no information is given about mercury content in the fish from the Tapajos River. Consequently the introduction and discussion regarding the influence of the consumed fish on blood pressure and mercury content in the blood is speculative and not supported by data given in the paper.

Response to comment 6: We shortened the intro and refocused it on fish, Hg and blood pressure. There are numerous studies on Hg levels in fish in the Tapajos region and other areas of the Brazilian Amazon. We have now included some of these in the introduction. In addition, the relation between fish consumption and bioindicators of Hg exposure has been repeatedly demonstrated. In the results section, we included the association between hair Hg and fish consumption from the present study.

Minor Essential Revisions

Comment 1: Table 2. Both SD and range are used for description of the peripheral tendency in the study sample. One of the parameters is sufficient.

Response to comment 1: We decided to use only SD and we took out the range.

Comment 2: Table 3 can be omitted. The three data sets could be given in the text.

Response to comment 2: Table 3 was deleted.

Other comments

Comment: Further to these comments, we believe that it would be worthwhile to note if blood pressure was measured without knowledge of mercury exposure and other predictors.

Response: We added the information that blood pressure was measured without knowledge of mercury exposure and other predictors in the "Methods" section.

Comment: Was blood pressure measured in increments of 5 mm Hg?

Response: We added the information that blood pressure was measured by increments of 10 mmHg in the "Methods" section.

Comment: Also, we think that a scatter plot or perhaps an additive model showing blood pressure (preferably adjusted for covariates) in relation to mercury exposure would be useful to the reader.

Response: We added Figure 4 to show scatter plot of blood pressure (adjusted for the significant covariates) in relation to mercury exposure.

Summary of the changes made

We revised the format of the entire manuscript, according to the guidelines available on the website. We included in the text the responses to each of the comments.