Author's response to reviews

Title: Chronologically Matched Toenail-Hg to Hair-Hg Ratio: Temporal Analysis within the Japanese Community (U.S.)

Authors:

Tom Hinners (hinners.tom@epa.gov)
Ami Tsuchiya (tsuchiya@u.washington.edu)
Alan H Stern (alan.stern@dep.state.nj.us)
Tom M Burbacher (tmb@u.washington.edu)
Elaine M Faustman (faustman@u.washington.edu)
Koenraad Mariën (koenraad@doh.wa.gov)

Version: 2 Date: 27 August 2012

Author's response to reviews: see over
27 August, 2012

Dear Drs. Ozonoff and Grandjean:

Enclosed please find a revised version of a manuscript previously submitted for your review. The manuscript number is MS:1117524891762524 and the submission title for publication as a research article is, “Chronologically Matched Toenail-Hg to Hair-Hg Ratio: Temporal Analysis within the Japanese Community (U.S.).”

We have addressed the editorial comments presented in the email provided and describe below what was done.

We want to thank the reviewers for their comments as we consider the manuscript improved with the recommendations they made. Our responses to the comments provided by the reviewers are provided below each comment in italics. We have changed the manuscript to reflect all the recommendations of the reviewers.

Thank you again for considering our manuscript for acceptance. I look forward to hearing from you in the near future.

Sincerely,

Koenraad Mariën
Response to editorial comments and reviewer’s reports.

Editorial comments pertaining to the format of the submission were addressed as follows:

1) On the title page, each institutional address now begins on a new line.
2) Title of the last section of the Abstract and of the main text has been changed from Conclusion to Conclusions.
3) The list of abbreviations has been formatted so as to reflect journal requirements; abbreviation:term separating the pairs with semi-colons in sentence format.

Reviewer 1.

Comment 1:
Methods Section
Hair- and Toenail-Hg Analysis Subsection
The discussion of quality control should be expanded to include identification of the standard reference materials and a summary of the results obtained compared to the certified mercury concentrations. Depending on the number of different SRMs analyzed this may best be done as a data table.
Completed. The discussion of quality control has been expanded to include source of standard reference materials and a synopsis comparing results with certified concentrations.

Comment 2:
Table 1
I find the ratios in the far-left column of Table 1 to be confusing; for example:

a. Where there are multiple ratios given, each should be better defined. For example, the first entry has 2 ratios both described by footnote a. However, this footnote does not differentiate these 2 values.
b. Where there are 3 ratios given the first entry, the entry in [ ] and the entry in ( ) are not adequately differentiated.
c. In footnote c the ratio one calculates from the data given for the instant study gives a value of 3.133:1 but it is given as 3.16:1.
d. In the second entry, the 29 should precede (toenail) to be consistent with 30 (hair).

We acknowledge the concern of the reviewer here. The reviewer basically indicates that the left column of Table 1 is a mess. We have reworked the left side of Table 1 to improve clarity and to address all but one of the issues raised.

The remaining issue pertained to footnote c (now footnote d) where the reviewer indicates that the hair-Hg to toenail-Hg ratio should be 3.133 instead of 3.16. The value of 3.16 is correct as values in the footnote were rounded to nearest hundredths for publication, but the ratio was based on values measured to nearest thousandths. To rectify this, we now provide values to the nearest thousandths in the table.
Comment 3:
Background Section
¶1 Where the authors state: “Roman et al. [12] do not address the findings of a subsequent study that failed to observe an association between MeHg exposure and coronary heart disease or stroke [11].” This reviewer has 2 suggestions:
a. The wording leaves open the interpretation that Roman et al. knowingly chose not to address the findings in reference 11. The Mozaffarian et al. paper (reference 11) was published in the New Eng J Medicine in March 2011 and the Roman et al. paper was published in May 2011 in Environ Health Perspect. It is likely that these authors were not aware of the Mozaffarian paper when theirs was submitted. This reviewer suggests that the statement be revised to clearly allow for the likely event that Roman et al. were unaware of Mozaffarian et al.
b. The use of “failed to observe”, while strictly speaking is correct, leaves open the distinct possibility that the Mozaffarian study missed an existing effect. This is possible but, given the large number of observations in both women and men, it is far less likely than the conclusion made that there was no effect. This reviewer suggests language “…that a subsequent study [11] did not observe an effect in a study of over 9000 subjects”.
Both a&b completed. This section pertaining to Roman et al. has been changed to address the issues raised by the reviewer.

Comment 4:
Background Section
¶3 The authors state that the Korean and Japanese women studied “…consumes substantially more fish than the national average…” This reviewer suggests expanding the discussion of the influence of dietary fish relative to toenail and hair mercury levels, for example: The toenail Hg concentrations measured in this study are significantly higher than those reported in several of the other studies referenced. References: 8, 11, 17 & 18. In at least 2 of these studies (8 & 11), the toenail-Hg concentration was directly correlated with fish consumption. In the instant study, the authors state that the subjects provided comprehensive data on fish consumption. Was a correlation found with toenail-Hg or hair-Hg? Does this higher fish consumption account for the significantly higher toenail mercury levels found in this study?
Completed. Additional text has been provided although we did not provide details on the relationship between intake and body burden as a) that would take away from the two focus areas of this paper and b) details on that subject matter have already been published by us previously.

Comment 5:
Results Section
¶3 The authors measured the hair-Hg to toenail-Hg ratio using both chronologically matched and unmatched hair and nail specimens. The methodology by which the hair sample was chronologically matched to the nail clippings was described in detail. This resulted in a hair segment that was selected in such a way as to hypothetically include the full growth period represented in the corresponding nail clippings. The 2 Hair-Hg to toenail-Hg
ratios, based on the regression analyses, were 2.79 and 2.39 for the chronologically matched and unmatched samples, respectively. The authors attribute the difference in these ratios to differences in the body burden measured by the chronologically-matched hair segment compared to the unmatched full-length hair sample taken at the same time as the toenail clippings. This fluctuating body-burden hypothesis may indeed account for all or part of the observed difference in the ratios. If so, it may correlate with consumption of fish and that may vary with seasons and could possibly be teased from the fish-consumption data. In the alternative, this reviewer has reported [J Radioanal Nucl Chem (2012) 291:409-414] that the mercury concentration in scalp hair, at least in 3 urban subjects, gradually increases from the root to the distal end of hair strands. This is presumably due to exogenic mercury that binds to sulfhydryl groups. If this phenomenon is generally true, then a hair segment that is more representative of the distal end of the hair strand, such as the chronologically-matched sample, will have a systematically higher mercury concentration compared to the full-length strand from which it was subdivided. As a consequence the chronologically-matched hair-Hg to toenail-Hg, based on a hair segment, will be greater than the unmatched hair-Hg to toenail-Hg ratio based on a full-length hair sample. If environmental mercury significantly contaminates scalp hair, this monitor may be compromised for determination of the mercury body burden. To avoid, or at least reduce, the impact of environmental contamination it might be preferable to restrict the hair sample to the first centimeter or two out from the root even though doing so would render unfeasible chronologically-matched hair and toenail samples. In the final analysis, environmentally-sheltered toenail samples, that are inherently less susceptible to this mechanism of contamination, may be the better choice for measuring the body burden of mercury in epidemiological investigations.

Acknowledged and text changed. The reviewer provides a citation of their work indicating that for three individuals investigated across a period of 400 to 700 days, exogenous deposition of Hg may play a role in the concentrations observed within samples of hair nearing the distal end of the hair strand. In the cited study, the distal and proximal ends of strand equivalent to approximately 2.5 cm at each end were compared. As the time periods compared between the distal end proximal ends were not the same for the three individuals (460, 570 and 710 days) we cannot determine what the possible influence would be on a per unit time basis. Regardless, deposition may play a role in the total Hg concentration observed in hair; especially when considering time periods of a year or two as was the case with these three individuals. In our study, we had multiple hair samples from which to choose. Accordingly, nearly all our segmented hair samples were obtained within the first 8 cm of hair growth and as our samples came, not from the crown as was the case in the cited study, but from the nape of the neck, the hair strand may have been further protected from exogenous sources. We do not have a means by which to test this hypothesis in our sample results. We did compare the hair-Hg to toenail-Hg ratio from segmental hair results found entirely within the first four cm of hair from the proximal end of the scalp (n=9) with those segments found entirely beyond the 4 cm mark (n=8). Those samples entirely beyond the 4 cm mark from the proximal end of the hair strand had a slightly higher ratio than those found within the first 4 cm; 3.4 versus 3.1 (based on arithmetic mean values).
The mean for the total sample was 3.1 (3.08) so the average from the remaining samples (n=24) which encompassed the 4 cm mark had an average mean ratio below the mean. It is plausible that exogenous deposition could impact hair-Hg values but we cannot infer it from our results. We have included text within the Methods indicating that nearly all of the samples (39 of 41) were obtained within the first 8 cm from the proximal end of the hair strand (with 25 of the 41 coming from within the first 6 cm of growth).

An experiment that could be considered to address this issue consists of obtaining hair samples from participants, measuring hair-Hg levels at the proximal ends of the strand and then in 12 or 14 months obtain another sample of hair containing that same hair segment (which has now had environmental exposure), and measuring hair-Hg level within that segment again. Hair growth rate could be determined by measuring the increase in hair length over time using the remaining scalp hair where the first sample was obtained.

Comment 6:
Discussion Section
¶6 The authors state: “Thus, attempts at identifying stability in body burden within a study population should be made if biomarkers chronologically removed from disease manifestation are to be used to investigate a dose response relationship.” This is obviously a very important point and of considerable concern in the trace-element epidemiology community. Very few studies have been reported addressing this issue. In this context it is worth mentioning that Garland et al. (see reference 17) has reported the reproducibility of 14 trace elements, including mercury, in toenails collected from the same subjects six years apart in the Harvard Nurses’ Health Study. Comparing these time points spanning 6 years, the Spearman correlation coefficient for toenail mercury was 0.56 (p < 0.003) indicating that the mercury body burden is relatively stable in these subjects.

The reviewer is correct in that Garland and co-workers did investigate Hg levels in toenails in 127 women from the Harvard Nurses’ Health Study at two time points, six years apart, with average toenail-Hg levels being 0.87 and 0.67 µg/g (r = 0.56). We did not discuss this at length as discussion would take away from the primary focus of this paper. Also, there were two additional factors that played a role in us not delving further into a discussion. First, the time periods between our study and the Garland et al. work were not similar and cannot be readily compared. Second, we are unable to resolve the discrepancy in average toenail-Hg values across studies. Specifically, our study consists of a Japanese cohort that is overexposed to Hg and our results are similar to results obtained by Ohno and co-workers from within the Japanese population (Reference #25). However, the average toenail-Hg values from both our study and that of Ohno et al. were less than those observed by Garland et al. in the nurses who do not consume fish with frequency and are not considered to be overexposed. We considered discussion on these matters to be outside the scope of this article.
Methods Section
Hair and Toenail Sampling Subsection
Comment 7:
¶4 Change: clipping to clippings
Completed.

Results Section
Comment 8:
¶1 Change: Interquartile ranges… to Interquartile Hg-concentration ranges….
Completed.

Comment 9:
¶1 Rearrange the 2 sentences beginning with “Respective 95th…” and “Arithmetic mean…” so that the coefficients of variation follow the mean and standard deviation. For example, 0.60 ± 0.42 (0.70).
Completed.

Comment 10:
¶1 Change: …relative small sample… to relative small number of samples…
Completed.

Comment 11:
¶3 Change: Regression model…. to A regression model…..hair-Hg samples, reflecting….exposure, gave a slope of 2.79 ng Hg/mg hair….
Completed.

Discussion Section
Comment 12:
¶2 Change: …pharmacokinetic… to …toxicokinetic
Completed.

Comment 13:
¶3 Change: …deleterious effect;… to …deleterious effects
Completed.

Comment 14:
¶3 Change: …even from single… to …even from a single
Completed.

Figure 4
Comment 15:
Add concentration units to x and y axis labels to be consistent with Figures 1 & 2
Completed.
Reviewer 2.

Abstract

Comment 1.
Line 1. P1, Toenail Hg levels are used as a biomarker of methylmercury (MeHg) exposure. MeH is lacking.
Completed.

Comment 2.
The authors should mention that the participants were females. Difference in growth ratios hair and toenail is not examined.
Completed. Abstract indicates that the Japanese participants were women, but we have rewritten this to improve clarity.

Comment 3.
The authors showed Hg value by arithmetic mean. However, the distribution of toenail-Hg looks more logarithmic normal from Figure 1. They should use geometric mean or median.
We very much welcomed this observation and want to thank the reviewer for raising the issue. In four of the six manuscripts associated with this MeHg study, we have had a reviewer ask about the distribution and if it should be log transformed. When we initiated the study we expected that log-transformation would have been required as the literature has provided results suggesting that fish consumption (and therefore, possibly Hg intake and body burden levels) are not normally distributed but are skewed right. These data presented in this paper along with other biomarker data on hair and blood previously published did not lend themselves to log-transformation. This was the case even though, as can be observed in Figure 2 for example, variability is dependent on the mean and log transformation could lead to a homogenous error that is independent of the mean. Our efforts though suggest that log-transformations does not provide any beneficial conceptualization or formalization of characterizing the variation. Our study initially had a sample in excess of 200 but results quickly indicated that the two populations being examined (Japanese and Korean) could not be combined. Accordingly, the sample numbers we are dealing with now may be too small to allow for proper elucidation of the actual distribution. However, we have now also included the hair-Hg and toenail-Hg geometric mean values within the Results for the reader to use for comparative purposes. Previously these data were difficult to find as they were only in a footnote within Table 1.

Comment 4.
Lines 5 and 7.P2, Hg should be MeHg. The authors measured total Hg in toenail or hair to estimate MeHg exposure.
Completed.
Background

Comment 5.
Lines 8 and 13, mercury should be MeHg.
*Completed.*

Comment 6.
Line 1 from the bottom. Should be “for MeHg exposure”.
*Completed.*

Comment 7.
Lines 1 and 14 P3, mercury should be MeHg.
*Completed.*

Comment 8.
Line 15 P3, why only between cardiovascular effects. How about the neurological effects?
*Completed. Text has been added to include neurological effects.*

Results

Comment 9.
(p>0.05) is not necessary because the authors mentioned that the significant level was set a 0.05.
*Completed. Text has been removed.*

Comments 10-14.
The authors used arithmetic mean. However, the figure shows median and the quartile values, and the toenail-Hg does not show normal distribution.

All the arithmetic means are bigger than medians.

They should use geomean or median.

In the same reason, correlation coefficients should be calculated by their log changed figures.
*For comments 10-14, please refer back to the response provided for Comment 3 as this all pertains to the same topic.*
Discussion

Comment 15.
In a lamination of this work, they should add the sentence that these results are obtained from females.
Completed.

Comment 16.
Mercury exposure, Hg level, and Hg body burden should be changed to MeHg.
Completed.

Reviewer 3.

Minor Essential Revisions

Comment 1.
- In the background, I would suggest cutting the first paragraph and replacing with a statement about the need for improved exposure assessments and the importance of biomarkers in assisting this. The background should also include discussion of the previous studies compiled in the table and highlight how this study fills a gap in the literature.
We have added language to the Background that discusses previous studies as mentioned in Table 1 and clarified how the goals of this study as they pertain to exposure assessment and biomarkers significantly add to the existing literature base. We do request, however, that the first paragraph of the Background remain as it frames the importance MeHg as a public health concern and provides an introduction to MeHg exposure and incidence of myocardial infarction; the endpoint most frequently investigated using toenail-Hg levels as endpoint.

Comment 2.
- I am primarily concerned by the inconsistent use of MeHg and Hg (total Hg?), and the lack of discussion of Hg species/metabolism. This needs to be clarified in the article and the metabolism of Hg/Hg incorporation into the nail should be discussed (e.g., is it MeHg the primary form incorporated into the nail?). Furthermore, exactly what is being measured (total Hg) should be clearly stated. Is this the same for both toenails and hair? Is the form or Hg incorporated the same way in both materials?
Both Reviewer 2 and Reviewer 3 have made reference to the inconsistent use of MeHg and Hg within the document. We have made corrections addressing this issue as previous language was obviously confusing. Within the background section we have made reference to the existing relationship between the various biomarkers (blood-Hg, toenail-Hg and hair-Hg) and fish consumption; which is the primary source of exposure to MeHg. Text has also been included on the use these compartments as biomarkers and specifically how the toenail compartment has developed into a useful marker of exposure to MeHg. The concerns raised in this comment, and in similar comments by reviewer 2, in reference to mercury species were not only valid but very useful. The results of changes based on these comments have improved the manuscript. Thank you.