Author's response to reviews

Title: Prenatal Mercury Exposure, Autism, and Developmental Delay, Using Pharmacokinetic Combination of Newborn Blood Concentrations and Questionnaire Data: A Case Control Study

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Author's response to reviews: see over
Dear Dr. Grandjean,

Thank you for the opportunity to submit a revised version of our manuscript. In addition to the requested format changes, we have made several edits and clarifications throughout the manuscript in response to the referees’ questions and suggestions. Below, please find the referees’ comments followed by our responses, which also include where we have altered the manuscript when applicable. Thank you again and please let me know if you have additional questions.

Sincerely,
Stephen McKean

Response to editors’ comment:

Comment:
One of our concerns is whether adjustment for hematocrit or hemoglobin might be appropriate and feasible.

Response:
Unfortunately we do not have values for hematocrit or hemoglobin levels, and therefore adjustment for these factors was not possible. If hematocrit and hemoglobin levels are associated with blood mercury concentrations, the factors will only confound the association between the exposure (i.e. seafood consumption) and the blood mercury concentration if hemoglobin and hematocrit levels are also associated with the exposure; we do not have a strong reason to believe this is the case. Additionally, we found that higher levels of reported seafood consumption during the third trimester significantly predicted higher blood mercury concentration; therefore, in general, higher bloodspot mercury concentrations reflect an increased level of exposure via seafood consumption rather than solely being a function of hemoglobin and hematocrit levels.

Responses to Referee 1:

Referee Comment:
Therefore I think the paper would benefit by repeating the analyses of the different outcomes for (A) the spot urine measurements alone, and (B) an index of exposure constructed from the reports of the amounts of fish consumed in specific trimesters of pregnancy and information on their relative mercury. Reports of these separate analyses will help allay possible concerns that the null results reported in the paper are attributable to mixing relatively more reliable information (e.g., the blood spot mercury measurements) with the likely less reliable questionnaire-based data.

Response:

We are planning to perform an additional study focusing on the association between blood spot mercury concentrations and autism. However, it is clear in the current study that the median bloodspot mercury concentrations are nearly identical across all three developmental groups, indicating there is likely no association with the bloodspots alone.
Referee Comment:
It appears from this that a continuous measure of cognitive function was generated. In this case, it would be far preferable to assess the effects of mercury and other influences on cognitive function as a continuous variable rather than the “developmental delay”--parameter which I understand as a quantal measurement. I realize that this would create a lack of parallelism in the analysis with the treatment of the autism spectrum findings, but the question of effects on neurodevelopment indexed by the most sensitive measure of cognitive function is important enough to warrant a separate analysis.

Response:
The authors agree that cumulative mercury exposure and its effect on a sensitive measure of cognitive function (such as the Mullen score) is an interesting and important question. However, the aim of this study was to assess how cumulative mercury exposure impacts the risk of Autism and Developmental Delay, which is a different but also important question. We added a sentence to the discussion mentioning mercury and its association with cognitive function as an important topic for future research.

Referee Comment
p. 12 “Frequency of consumption during each time period (i) was assigned the following values: 0 (none); 0.5 (more than 0 but less than 1 serving per week); 1 (1 serving per week); 2 (> 1 serving per week).” This categorical treatment of the fish consumption rate destroys the proportionality assumed in the derivation of the formula for the regression analysis. I would suggest revising the treatment to better estimate likely mean values for fish consumption within each category.

Response:
Respondents were not asked to report the number of fish servings per week, but rather, which category if fish consumption they fell into. The values assigned are our best estimates of the average frequency of consumption for each category (e.g., 0.5 servings per week is the midpoint for the category of 0-1 servings per week); if the frequencies were correctly assigned then proportionality holds. Unfortunately it is impossible to know if the assigned average frequencies are correct, and particularly difficult to estimate the mean fish consumption rate for the category of greater than one because of the way the dietary question was phrased. Therefore, we assumed that people who ate more than one serving per week, on average, tended to eat two servings per week.

Referee Comment
p. 4—“The toxicokinetic model described in this paper yielded fish MeHg concentration estimates that are consistent with fish species containing lower levels of MeHg. Overall, cumulative MeHg exposure does not appear to elevate the risk of autism or developmental delay.” I would suggest adding “detectably” before “elevate”? It also might be helpful to quantify confidence limits on detection—how large would the effect have to have been in order to have been detected with 95% confidence for the combined model and for the blood spot and questionnaire data considered separately.

We have added "detectably" to this sentence, as well as the following sentence: "Based on the regression standard error for the association between ASD and TD, we would have reported statistical significance for an adjusted odds ratio of 1.09 or larger." The point estimates of the odds ratios are, in additional to not being statistically different from one, not meaningfully different from one (1.03 for AU/ASD vs. TD and 1.00 DD/AtD vs. TD).
Responses to Referee 2:

Referee Comment:
In theory, this is an interesting approach. However, it suffers from the unsupported assumption that dietary recall of fish consumption is a highly reliable tool under the best of circumstances (overall correlations between MeHg intake reconstructed from recall and MeHg biomarkers generally fall around 0.3-0.4), much less, 2-5 months post-partum. This means that participants were asked to recall specifics of fish consumption up to 17 months prior to the interview and to distinguish the specifics of fish consumption patterns at that time from the patterns one and two months subsequently. This seems beyond any reasonable use of dietary recall data.

Response:
We agree with the referee that food frequency questionnaires are prone to imperfect recall, and this limitation is discussed in the background section of the manuscript. However, it is important to recognize that using bloodspot mercury as a surrogate for exposure throughout the entire pregnancy is also likely to be inaccurate, because mercury exposures and blood concentrations change during pregnancy. Rather than assuming that bloodspots are highly reliable indicators of past mercury exposure, we assume that bloodspots and dietary questionnaires are both prone to some exposure measurement error. We combine them in an attempt to take advantage of both types of information.

Referee Comment:
This conclusion is supported by the finding that the model's prediction of unrealistically low MeHg concentrations for the categories of fish for which consumption data were collected. The authors attempt to address this by collapsing the fish categories. This results in an estimate of an overall average MeHg concentration in the fish of 42 ppb. However, this also seems unrealistically low since the FDA's reported mean value for canned tuna (which, depending on the study, is either the most commonly consumed seafood, or the second most commonly consumed seafood after shrimp - all other fish rank much lower) is 350 ppb for albacore and 128 ppb for light tuna (http://www.fda.gov/food/foodborneillnesscontaminants/metals/ucm115644.htm).

Response:
Our estimated MeHg concentration is low, but as pointed out in the discussion, consistent with fish species low in MeHg (the species that pregnant women are advised to consume). The referee points out that the average mercury concentrations in albacore (which pregnant women are advised not to eat) and canned light tuna are 350 and 128 ppb, respectively, but the table the referee referred to us also shows that the range of plausible values is large and and can even include a non-detectable concentration of mercury. Unfortunately, we cannot distinguish among the specific types of tuna in this study. However, our estimated concentrations fall within a plausible range if mothers in our study followed guidelines and consumed species with low mercury levels (we added this argument to line 467 of the discussion). As we also point out in the background section and now reiterated in the discussion, there is evidence suggesting that MeHg exposures based solely on food frequency questionnaires are overestimated, possibly resulting from the over report of fish consumption. If fish consumption was over reported, then our concentrations would tend to be underestimated. We should note that this is one of the way in which our method has advantages over using questionnaire data alone; when estimating cumulative fish concentrations, a systematic tendency to over report fish consumption is counteracted by a reduction in the estimated MeHg dose per serving (this argument is now included on line 424).
Referee Comment:
Furthermore, this corrective procedure addresses only the recall for the type of fish consumed. It does not address inaccuracy in recall of the amount of fish consumed during each of the distant recall periods. This creates a fundamental problem in the overall analysis since the study, in part, was designed to refine the investigation of the association between gestational MeHg exposure and autism/developmental delays from the single post-partum blood spot measurement by estimating exposure separately for the second and third trimester.

Response:
We agree with the authors that collapsing the individual fish categories into one overall category does not correct for the inaccurate reporting (due to dietary recall) of the amount of fish consumed. We now discuss this beginning on line 464 of the limitations section of the discussion.

Referee Comment:
The title of this paper addresses only the estimate of MeHg exposure. However, the MS, itself, in addition to constructing the model and using the model to estimate temporal trends in exposure, significantly addresses associations between exposure and the autism/developmental delay diagnoses. The title is, therefore misleading and needs to be rewritten to reflect the key epidemiological aspect of the MS.

Response:
We have developed a more comprehensive title that reflects all aspects of the paper: Prenatal Mercury Exposure, Autism, and Developmental Delay, Using Pharmacokinetic Combination of Newborn Blood Concentrations and Questionnaire Data: A Case Control Study

Referee Comment:
Line 89 - "High doses" is not defined here and associations have been found between what are generally considered low-moderate levels of MeHg exposure and neurologic developmental effects.

Response:
We removed “high doses” from the sentence, as the dose itself is not why the developing brain is especially sensitive to mercury.

Referee Comment:
Lines 199-212 - The analytical method is not adequately described here. Is this analytical method laser ablation? It is not clear exactly how the laser was used and the connection between the laser and Hg quantitation.

Response:
We have clarified that laser ablation was used as the analytical method of Hg quantitation (Line 204).

Referee Comment:
Lines 216-217 - Why were the blood spot Hg concentrations log-transformed? This is not obvious and needs explanation. Log-transformation should not be an a priori default approach. Most statistical procedures do not require normality and log-transformation comes at a price in terms of interpretation of the results.
Response:
While not required statistically, by taking the log transformation, we can easily observe that increasing cumulative mercury exposure by orders of magnitude has very little effect on the predicted odds of ASD and developmental delay/atypical development (DD/AtD).

Referee Comment:
Lines 238-240 - It should be stated that this is the Cb at steady-state.

Response:
We do not rely on any steady-state assumptions regarding the blood mercury concentrations (C_b). In contrast, both C_b and mercury intake (I_j) are assumed to vary over time. The equation shown here reduces to the usual steady state equation only when I_j is constant.

Referee Comment:
Line 243 - "a" is not defined in words. Based on the equation that follows, it is a weighting variable, but that is not sufficiently descriptive and it is not clear what quantities it is weighting and why.

Response:
The variable “a” as the reviewer points out is a weight that accounts for the elimination of mercury from blood over time; we now denote that in the previous line of text. Fish consumption occurring further away in time prior to bloodspot collection carries less weight while fish consumption that occurred closer to bloodspot collection (e.g. the third trimester) carries more weight in terms of predicting bloodspot mercury concentration. We have added text to the manuscript to clarify the purpose this weight and how it functions.

Referee Comment:
Lines 248-249 - "Notably, the blood mercury concentration is thus expected to be a linear function of the fish mercury concentration." This is only the case at steady, and if the intake rate does, in fact, vary by the month pre-conception (and if the data collected can, in fact, reflect that variation) then the assumption of achieving steady-state is questionable. Given the relatively long half-life of MeHg in mother and fetus and given the fetal homogenation of blood Hg over time, this may not be critical, but it needs to be discussed.

Response:
There is no steady-state assumption in our method. In fact, the method is designed to avoid the unrealistic steady-state assumption when attempting to relate the bloodspot to the exposure history. We now state this more clearly in the background (line 99) and methods sections (line 260).

Referee Comment:
Line 274 - The value assigned to f requires a citation.

Response:
We have added the appropriate citation.

Referee Comment:
Lines 300-314 - Stern (2005), ""A revised probabilistic estimate of the maternal methyl mercury intake dose corresponding to a measured cord blood mercury concentration.” Environ Health Perspect. 2005 Feb;113(2):155-63. Provides a detailed discussion of the appropriate values for the variables in the
one-compartment model. Furthermore, as discussed in that paper, these quantities are distributed in the population. At a minimum, this should be acknowledged and its implication addressed.

**Response:**
We agree with the reviewer that the ratio for maternal blood mercury concentration versus newborn blood mercury concentration will vary across maternal-child pairs. We are unable to reasonably estimate what this ratio would be for each specific mother-child pair level; instead, we assumed an average ratio and used this value for all mother-child pairs. This reduces the predictive ability of the model (depending on how much the ratios vary around our assumed average ratio) and will increase the variance of the fish concentration parameter in the regression model. We added this as a limitation in the discussion on line 445.

**Referee Comment:**
Lines 351-352 - In addition to the structural problems underlying these estimates of fish MeHg concentration that result in extremely low estimates (discussed above), the relative concentration among the fish types produced by the model doesn't make sense. Tuna Hg concentration tends to be higher, not lower than average ocean fish.

**Response:**
We agree that tuna would be expected to have a higher mercury concentration, and this point was elaborated upon in the discussion:

“Because the consumption of tuna is associated with the consumption of other types of fish, the other fish types may have subsumed the contribution that tuna has to blood MeHg concentration when they are included in the model. Additional error could stem from the imprecision associated with recalling specific types and quantities of fish.”

**Referee Comment:**
Line 366 - Table 4 and Fig. 2 are presented in terms of "exposure," but the estimates are given in pbb (i.e., concentration.)

**Response:**
The labels have been changed from ppb to ppb-days.