Author’s response to reviews

Title: Arsenic Exposure and Risk of Non-Alcoholic Fatty Liver Disease (NAFLD) Among U.S. Adolescents and Adults: An Association Modified by Race/Ethnicity, NHANES 2005-2014

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Environmental Health

Dear Dr. Karagas,

Thank you for your interest in our manuscript and the opportunity to revise it in response to the very helpful comments and suggestions provided by the reviewers. We believe that the attached version of the manuscript is a substantially improved. We believe that the findings of this study are very important, and that they will be of great interest to readers of Environmental Health. We hope that you will agree and find the attached version worthy of publication.
Attached you will find two versions of the manuscript, one with the changes since our initial submission tracked for easy identification, and a clean version with all changes accepted. Also attached is a clean version of all Tables.

Thank you for your time and consideration.

Regards,

-Jean

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Reviewer #1:

1. Methods, Other Covariates Line 163, overweight (18.5-<25) , Overweight (25-<30) need further correction.

Thank you for pointing out this error. It has been corrected.

2. In Table 2, Geometric mean ALT level is lowest in non-Hispanic black, But in Table 3, it ranked the 3rd of the geometric mean arsenic level among different groups, which is not consistent with the overall trend of correlation of elevated ALT level and arsenic exposure. What's the possible explanation and please put it into discussion.

Please note that arsenic levels are presented in Table 2 and ALT levels in Table 3. We have revised the heading to make these differences more obvious to the reader.

3. Although Table 4 has already adjusted for age, gender and BMI etc., it would be better to have the BMI figure among different ethnicity groups to see if there is any different distribution among different ethnicity groups which may confound the result.

We examined the distribution of obesity by race/ethnicity and found it to be significantly higher among non-Hispanic blacks and lower in the other/mixed race group compared to the others. We have reported this finding in the Results section, Lines 204-206. Given this we also stratified our logistic regression analysis by weight category to determine if the association varied by weight. We also examined the association between arsenic and elevated ALT among those obese only. These results are presented in Table 4 and in the results section.

4. Since high BMI is the major contributing factor, the role and impact of arsenic exposure may be best delineated in lean male in this cohort just as Taiwanese study. I wonder that if you can show this in another figure, and better in separate race/ethnicity groups.

We have revised Table 4 to include results stratified by weight status. There we see that regardless of weight status, there is a rise in the odds of elevated ALT as arsenic exposure increases, though the magnitude of the increased risk is greatest among those obese.
Reviewer #2:

This is a well written manuscript with the underlying hypothesis that exposure to inorganic arsenic is associated with non-alcoholic fatty liver disease and that race/ethnicity modifies the effect. The authors used a cross-sectional biomonitoring survey design, NHANES, to assess levels of urinary arsenic and alanine aminotransferase as a biomarker of non-alcoholic fatty liver disease. This study has important strengths that include a large representative sample from the U.S. population as well as detailed ascertainment of exposure, biomarker of disease and covariates. Some important limitations are inherited in the cross-sectional design and therefore the interpretation of results must be scaled to match the design. In addition, I have some concerns about the analytical and methodological approach and I think sensitivity analyses are in order. The discussion is brief and the introduction could be strengthened. I have series of comments and recommendations that should be addressed prior to considering this manuscript for publication:

1. Is there any underlying hypotheses (genetics, environmental or cultural) that explain racial differences in non-alcoholic fatty liver disease? A stronger argument must be made for testing effect modification by race in the introduction, as it is a premise for the study. As of now it appears that the given that only one race was deemed significant it was included post hoc in the title/hypothesis. We have added some text to the background to make our rationale for wanting to determine if there were race/ethnic differences in the association between arsenic and elevated ALT more clear. A key question regarding NALFD is why risk varies so much by race/ethnicity. Arsenic exposure may, in part, explain some of this difference. We’ve revised the text to state this more clearly. See lines 118-122.

2. The authors state that it occurs almost exclusively in obese individuals (line 88). In light of this, testing for the association of iAs and ALT in the obese category is in order might help support their findings. Yes. This suggestion was appreciated. We have done as suggested. The results are presented in Table 4 and in the Results section of the manuscript, Line 224-231

3. Include rationale for excluding Hepatitis B and C in the body of the manuscript. What about hepatitis A? It has also been shown that Hepatitis A serology is associated with iAs exposure in NHANES (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4855991/) this might be an important confounder. Though many people have Hepatitis A antibodies, the virus is not associated with elevated ALT levels outside of the acute phase. Given that those with active hepatitis A would be unlikely to participate in the NHANES study, and that excluding subjects positive for Hepatitis A antibodies would result in excluding a large portion of the sample unnecessarily, we excluded only those positive for Hepatitis B and C. Text describing the rational for excluding those with Hep B and C has been added to the manuscript at lines 130-131
4. A categorical indicator for survey cycle should be included in analyses to control for
temporal trends in both iAs exposure and prevalence of non-alcoholic fatty liver disease (or
ALT) in the U.S. population. Temporal changes in both exposure and disease biomarker by
survey cycle might be a very important confounder of the association. We agree that the
addition of an indicator of survey cycle is important. We have included the analyses done for
this revisions and have noted so in the methods. See lines 191-192

1. 5. After combining multiple cycles, were the survey weights scaled to match this
approach? If not, this needs to be done in order to accurately capture the sampling design
as explained here: https://www.cdc.gov/nchs/tutorials/nhanes/surveydesign/weighting/task2.htm. If this was
already done please state in the methods. Yes. This was done. Text indicating this has
been added to the Methods, lines 183-184.

6. In the methods please provide the % of samples below the LOD and discuss how this might
influence the results. The change in LOD by survey cycle supports the inclusion of cycle as a
covariate in multivariate adjusted models. A total of 90 samples had arsenic levels before the
LOD. This and the method for assigning a value is described in the Methods, Lines 144-145.
Text explaining that the relationship between the log-transformed iAs and elevated ALT
levels was not linear for some race/ethnic groups, therefore necessitating that exposure be
categorized (and eliminating any impact of having values below the LODD) is included on
Lines 187-188.

7. The authors should briefly state whether the ALT cutoffs used to classify individual are
clinically used or used in research? The cutoffs are commonly used in the clinical setting to
screen for NAFLD. This is now indicated on Lines 178-179.

8. I couldn't find anywhere the number of individuals both total in the study and by race that
had elevated ALT. This number must be provided. Given the small sample size for some
races/quartile combinations I'm concerned that this might lead to some spurious associations.
Could be beneficial to include in table 4 the number of "cases" or positive individuals for
high ALT. This is a good suggestion. We have added this information to Table 4.

9. Along the same lines, when stratified analyses have small sample sizes the survey weights
tend to yield unstable results. I encourage the authors to run non-weighted sensitivity
analyses and report in a supplement. In general results should be robust in direction and
association but the magnitude might differ. Thank you for the suggestion. We have done as
suggested. The results in the unweighted analysis, included in a supplemental table, are
similar to those from the weighted analysis presented in Table 4.
10. I'm not sure what the rationale is for stratifying by quartiles of exposure, it would very beneficial to see results by continuous iAs exposure measurement. If a dose-response is relevant for ALT why didn't the authors model exposure and ALT as continuous? Or even continuous iAs exposure and elevated ALT in logistic models? While we agree that using iAs as a continuous variable in the model would be preferred, there are stricter assumptions for modeling a continuous predictor (i.e., linear relationship with log-odds of elevated ALT) that were violated for many race groups. As a result, we chose to categorize iA into quartiles to better understand the effect of elevating iA in each race group. As you can see from table 4, the odds are not always increasing as we move to higher quartiles for some race groups. The rational for grouping iA exposure into quartiles is now provided in Lines 214-215.

11. It would be very valuable to present joint analyses (all races together). Along the same lines if effect modification is hypothesized I would encourage the authors to conduct a formal interaction test in a model that includes all the races (i.e. continuous iAs exposure and race interaction) and report the F-test p-value. I assume that the borderline test for effect modification by race (page 8) is by categories of exposure and elevated ALT? If so, please clarify. –Yes, effect modification by race/ethnicity (and weight status) was assessed using arsenic exposure categorized. This is described in Lines 215 and 223.

12. Please consider changing the title as non-alcoholic fatty liver disease was not objectively measured. However, if there's enough clinical evidence that ALT is a good surrogate that this might be OK. Otherwise replacing non-alcoholic fatty liver disease with ALT works and better reflects the scope of this work. Because ALT is a good surrogate for NAFLD when other liver diseases are excluded, we believe it is appropriate to refer to NAFLD in the title. We have made a slight change to refer to increased “. . . risk of NAFLD. . . “.

Minor:

1. In the title, non-alcoholic fatty liver disease includes a hyphen, but not in the manuscript. Please make sure there's consistency throughout the manuscript. Done. Thanks.

2. Page 6, line 131-132. "These measurements reflect approximately the last three days of exposure", please add reference. Or explain how was this estimated. Added

3. Page 8, line 181: "Due to race and ethnicity differences in iAs and ALT, we ran the fully adjusted model, presenting stratified ORs…” It is not clear if estimates in Table 4 are from one full model and then predicted ORs are presented from that model or multiple similar models stratified by each race. Please clarify if this table are from predicted ORs or truly
stratified by race (i.e. multiple regression models). The results presented are from stratified analysis. See Lines 254-255.

4. Urine dilution adjustment methods appear under covariates on page 7 and in the methods for iAs exposure on page 6. The wording here is a little confusing please consider consolidating in one place. Was urinary creatinine included as a covariate in models or by dividing exposure/ creatinine? This was condensed into the Arsenic Exposure section on page 6. See lines 165-167 for the description of how arsenic levels were divided by creatinine levels to adjust for urine dilution.