Author’s response to reviews

Title: Fluoride exposure and sleep patterns among older adolescents in the United States: A cross-sectional study of NHANES 2015-2016

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Responses to Editor and Reviewers

Editor:

Dear Dr. Malin,

Your manuscript "Fluoride exposure and sleep patterns among older adolescents in the United States: A cross-sectional study of NHANES 2015-2016" (ENHE-D-19-00281) has been assessed by our reviewers. Based on these reports, and my own assessment as Editor, I am pleased to inform you that it is potentially acceptable for publication in Environmental Health, once you have carried out some essential revisions suggested by our reviewers.

Thank you. We have addressed the reviewer points in our response below and the changes are highlighted in the revised text with tracked changes. We have also made additional minor edits to the manuscript for clarification.
Reviewer reports:

Reviewer #1: This study examines the association between exposure to fluoride and sleep patterns among American adolescents (n=419 with water fluoride data). Participants were recruited between 2015-16 as part of the NHANES. The study measured plasma fluoride and tap water fluoride concentration at the same visit as the questionnaire evaluating sleep patterns. Results show a 1.97 times higher odds of reporting symptoms suggesting sleep apnea. Higher water fluoride concentration was also associated with a later wake time and bedtime. Plasma fluoride did not associate with any of the outcomes. This is an interesting study that addresses a topic where very little human data exist. I am supportive of this article and offer the following comments and suggestions:

1. Using the same NHANES population, Jain et al (2017) show that plasma fluoride levels were significantly higher among those who smoke compared with those who did not smoke. The authors may wish to consider exposure to smoke (first or second hand) as a covariate because smoking may impact the measurement of fluoride in plasma.

Thank you for this suggestion. Unfortunately, few participants (i.e. 65 at most) in our sample responded to questions pertaining to firsthand smoke exposure in the NHANES interview. Additionally, no participants in our sample indicated that they had been exposed to secondhand smoke in their homes. Therefore, we could not adjust for these variables in our analyses.

However, most participants in our sample had serum cotinine measurements, which provide a biomarker of smoke exposure. Serum cotinine concentrations among participants were generally low (75th percentile = 0.58 ng/mL) reflecting low nicotine exposure among participants. Still, we conducted a sensitivity analysis including cotinine as a covariate in our models of plasma fluoride with sleep outcomes. We now present these results as Supplemental Table S4 and report them in the sensitivity analysis section on lines 236-241 of the revised tracked-change manuscript. Results did not change appreciably with the addition of serum cotinine as a covariate in the models.

2. The authors comment in the limitations section that the sleep outcomes questionnaire is subject to recall biases. Has the measure of sleep disturbance been validated? If not, the authors should note this in the methods.

We confirmed with the National Center for Health Statistics that the sleep questionnaire has not been validated. We now note this on lines 113-114 of the revised manuscript.

3. The mean water fluoride level of 0.39 mg/L for a country with three quarters of the population receiving fluoridated water seems very low. Why may this be? Was there oversampling from non-fluoridated regions?
We agree that this water fluoride level is low. Unfortunately, NHANES does not publicly release information on the sites or geographic locations in which participants were sampled from. Therefore, we are unable to determine whether there was oversampling from non-fluoridated regions.

Our prior publication on fluoride exposure and kidney and liver function utilized NHANES data from 2013-2016 and found that water fluoride levels were 0.48 mg/L; however, in 2015 the US-recommended water fluoride level was lowered from 0.7-1.2 mg/L to 0.7 mg/L. This may partially explain why water fluoride levels are lower in the current study using only NHANES 2015-2016 data.

4. The paper makes reference to disruption of pineal gland function as a possible mechanism for altered sleep patterns. Support for this idea comes from an unpublished dissertation (Luke 1997) showing reduced synthesis of melatonin in gerbils with high exposure to fluoride. This thesis also reported some sex effects (e.g. female gerbils with high fluoride exposure showed accelerated pubertal development whereas male gerbils with high fluoride exposure had a lighter testicular weight than males with low fluoride). Did the authors consider sex as a potential effect modifier?

In response to your comment, we tested fluoride*sex interactions in our models. We now note this in the statistical analysis section on Lines 180-181 and present these results on Lines 222-235. Intriguingly, we found that males with higher water fluoride concentrations had significantly lower odds of snoring (OR = 0.62, uncorrected p = 0.005); whereas water fluoride was not associated with snoring among females. We discuss these findings on lines 305-316 of the discussion.

We also note that with the addition of this new statistically significant finding, the Holm-Bonferroni corrected p values have changed such that the association between water fluoride and bedtime now reaches the threshold for statistical significance following Holm-Bonferroni correction.

5. I appreciate how the authors note that a diagnosis of ADHD can precede sleep disturbances. Hence, a potential mechanism for disrupted sleep could relate to having a disorder like ADHD (which has also been linked to fluoride exposure). On the other hand, sleep disturbances can also impair an individual's functioning and resemble ADHD. Thus, a diagnosis of ADHD could also arise secondary to sleep disturbances. (this is more of a comment that sleep disturbance can have a significant effect on behavioral function and therefore represents an important outcome).

Thank you for this comment. We agree that sleep disturbance may contribute to ADHD symptoms. We now note this on Line 303-304 of the discussion.

5. Limitations: The study sample ranged in age from 16-19 years. This is an age range where sleep may be disrupted for many other reasons (e.g. video games, studying, job, social
influences, etc.). Future studies examining the role of fluoride on sleep patterns should also expand the age range to determine whether the effects are consistent in younger and older individuals and to test the association with cumulative exposure to fluoride over a longer life time.

We agree that this is an important limitation to note and have added it to the limitation section of the discussion on Lines 342-346.

Reviewer #2:

Fluoride exposure and sleep patterns among older adolescents in the United States: A cross-sectional study of NHANES 2015-2016

This is a very interesting and timely population based study that analyzed the relationship between fluoride content in tap water and in plasma with sleep patterns and daytime sleepiness based on a subsample of participants of NHANES 2015-2016.

This is a novel and well supported study that was carefully analyzed and well written. I only have to comments

Along the paper, there is a tendency to interpret p-values in a drastic dichotomous way: p<0.05 results are interpreted as significant and p>0.05, as non-significant. I recommend reading the following paper and make a broader interpretation of p-values:

Thank you for your positive comments and for suggesting this excellent article. We acknowledge that we have chosen a conservative alpha adjustment in this study and interpreted findings accordingly. However, we also agree that there may be important trends in the data that provide meaningful information pertaining to the impact of fluoride exposure on sleep, particularly in an exploratory study such as this. Therefore, in the revised manuscript, we now report these trends.

* The authors present results on fluoride in water and they mentioned that results with plasma fluoride were non-significant, but they didn't present them at all. I think that the authors should comment about those results. How big was the p-value to interpret it as non-significant? How about the point estimates that summarize the direction of the association?

Thank you for this comment. We now report results from plasma fluoride analyses in supplemental Table S3. We also report the range of p values for these results on line 235 of the revised manuscript.

Associations of plasma fluoride with sleep apnea symptoms, snoring and trouble sleeping were in the same direction as associations between water fluoride and these outcomes. However, associations of plasma fluoride with bedtime, wake time and daytime sleepiness were in the opposite direction.