Author’s response to reviews

Title: Fluoride exposure and pubertal development in children living in Mexico City

Authors:

Yun Liu (yunliu@umich.edu)
Martha Téllez Rojo (mmtellez@correo.insp.mx)
Howard Hu (hhu5@uw.edu)
Brisa Sánchez (brisa@umich.edu)
E. Martinez-Mier (esmartin@iu.edu)
Niladri Basu (niladri.basu@mcgill.ca)
Adriana Mercado-García (adrianam@insp.mx)
Maritsa Solano-González (msolano@insp.mx)
Karen Peterson (karenep@umich.edu)

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Dr. Philippe Grandjean
Editor-in-Chief
Environmental Health

Dear Dr. Grandjean:

Thank you very much for the opportunity to revise our manuscript (Ms. Ref. No.: ENHE-D-18-00444: Fluoride exposure and pubertal development in children living in Mexico City). We appreciate your careful and constructive comments before sending the manuscript for review. We have made revisions to the manuscript accordingly.

Comments from the editors:
1. First, the abstract should appropriately reflect the major details of the study, and especially the methods section is incomplete, as it does not mention the study design and the setting.

Response: we agree with this comment. Information on the study design and setting has now been included in the Abstract (lines 33-37), as follows:

“Background: Previous ecological studies have provided evidence for an earlier age at menarche in relation to childhood fluoride exposure in girls; however, no epidemiological studies have examined the association between fluoride exposure and pubertal development in boys and girls using individual-level biomarkers of fluoride. Capitalizing on an ongoing Mexican birth cohort study, we examined the association between concurrent urinary fluoride levels and physical markers of pubertal development in children.

Methods: We conducted a cross-sectional study of 157 boys and 176 girls at age 10-17 years living in Mexico City. We used ion-selective electrode-based diffusion methods to assess fluoride levels in urine, adjusting for urinary specific gravity. Pubertal stages were evaluated by a trained physician. Associations of fluoride with pubertal stages and age at menarche were studied using ordinal regression and Cox proportional-hazard regression, respectively.”

2. The validity of pubertal stage assessment needs to be carefully considered, as information on e.g. menstruation may be biased, and the age of menarche may be inaccurate. These concerns need to be considered, also in the discussion (also in regard to previous studies).

Response: thank you for the comments. We have now added information to address these comments, as follows:

In Methods (lines 120-121): “Tanner staging with a range from stage 1 indicating pre-puberty to stage 5 indicating fully mature was used to assess pubertal onset of all participants. Tanner staging of breast and pubic hair growth in girls (Marshall and Tanner 1969) as well as Tanner staging of genitalia and pubic hair growth in boys (Marshall and Tanner 1970) were assessed by a trained pediatrician using standardized protocols. The assessment of pubertal stages has been validated by comparing with a panel of serum hormones related to puberty development in ELEMENT children (Chavarro et al. 2017).”

In regard to previous studies (in Discussion lines 228-230): “In addition to the ecological design, these studies are limited by reliance on self-reported age at menarche, which can be susceptible to recall bias.”

In Discussion (lines 268-272): “As noted above, the use of self-reported age at menarche can be subject to recall bias. However, previous studies have shown that the actual menarcheal age was highly correlated with self-reported menarcheal age within 5 years of follow-up among peripubertal girls (Koprowski et al. 2001), within 7 years (Lundblad and Jacobsen 2017) among women and up to 33 years starting at age 7-9 years (Must et al. 2002).”
3. The wisdom of excluding girls who apparently had not had menarche needs to be reconsidered, and a supplementary analysis that includes these subjects is recommended, e.g. in terms of a survival analysis.

Response: thank you for these comments. We were not explicit about the models we used in the analysis which do include girls with and without menarche. We have now added a detailed statement describing the survival models used to analyze age at menarche in the Statistical analysis section (lines 151 to 156):

“We used survival techniques (time-to event) to examine the association between fluoride concentration and age at menarche in girls, which adequately account for censored data (Kleinbaum et al. 2012). Hazards ratios (HRs) and 95% confidence intervals (CIs) were estimated using Cox proportional-hazard regression models, which has been widely used to analyze age at menarche. Time to menarche was based on the self-reported age of menarche (years) or right-censored observations using the age at the interview.”

4. Finally, we advise you to pay attention to the author instructions in regard to format.

Response: thank you for this comment. We have revised the format to more closely address journal guidelines. Please let us know if further changes are needed.

Thank you very much for your consideration of our work.

Sincerely,

Martha Maria Téllez-Rojo, Ph.D.

Senior Researcher
Instituto Nacional de Salud Pública