Reviewer’s report

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

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Reviewer: Christine Till

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Comments to the Author on revised manuscript

This study examines the association between exposure to fluoride and pubertal maturation outcomes among 157 boys and 176 girls aged 10-17 yrs enrolled in the ELEMENT study and residing in Mexico City. The study measured urinary fluoride concentration at the same visit as the pubertal stage evaluation. Results show later markers of pubertal development among boys, but not girls. This is an interesting study that addresses a topic where very little human data exist. I am generally supportive of this article and offer the following comments and suggestions mainly addressed at strengthening the discussion and providing appropriate coverage of the limitations of the study.

1. Introduction: the authors note that Farkas et al (1983) report an earlier age at menarche in relation to fluoride exposure. However, this article states that there were no differences in menarcheal age among school-aged girls from two settlements that differed by drinking water fluoride concentration. This correction should be made throughout the paper.

2. The ELEMENT study is a longitudinal study consisting of several different cohorts that differed by aim of study. According to an earlier study conducted on this cohort (by Bashash et al, 2017), children provided urine samples at the same visit that assessed IQ when children were between 6-12 years. If these same children from this earlier visit were included in the 2015-2017 follow-up visit (where sexual maturation was assessed), it might be interesting to see if the early exposure predicts the later outcome (puberty), given that we would expect the exposure to occur at an earlier time period to affect the maturation of sexual organs. Confirmatory evidence between fluoride exposure and later pubertal onset using a prospective design would help strengthen the results of the current study (and the exposure measurement would be improved if there was more than one spot sample, especially given that fluoride levels in urine may be lowered during pubertal growth when bone mass is increasing - see next point).

3. Can the authors comment on whether the urinary fluoride levels are within the expected range? It seems that the urinary fluoride levels reported in the 10-17 yr old sample (mean=0.58 mg/L) is lower than the average urinary fluoride level that was reported for the 6-12 yr old sample (0.84 mg/L) measured in the same cohort, though no reference is made to this earlier study that was based on the same cohort. The authors do compare the mean urinary fluoride concentration of the 10-17 year olds with several other studies conducted in youth.
4. The discussion makes reference to disruption of pineal gland function as a possible mechanism for altered pubertal development. Support for this idea comes from an unpublished dissertation (Luke 1997) showing reduced synthesis of melatonin in gerbils with high exposure to fluoride up until the time of sexual maturation. This thesis also reported a sex effect in that 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 (1.10 ± 0.11 versus vs. 1.32 ± 0.18 g, respectively (p < 0.002). The discussion in the current study could be enhanced by making reference to this male effect found in the gerbils.

5. The discussion about melatonin is hard to follow. One sentence states that reduced melatonin synthesis can accelerate pubertal development. However, in a subsequent sentence, it states that melatonin stimulates sex hormones, which in turn accelerate pubertal development. I struggled to understand whether the authors are saying that high or low melatonin synthesis is associated with accelerated pubertal development. While I appreciate that the mechanisms underlying initiation of puberty are complex and very little is known about how fluoride may interact with these mechanisms, I think this section on potential mechanisms can be made clearer.

6. A wide range of fluoride dosage is described for the fluoridated Newburgh (0.01-0.2 mg/kg/day) versus non-fluoridated Kingston (0.001-0.02 mg/kg/day) 1956 study. Do these concentrations take into account all sources of fluoride? (0.2 mg/kg/day is very high!) It would be easier to appreciate the concentration of fluoride in drinking water if the water fluoride concentrations are reported (i.e. 1.0-1.2 mg/L in Newburgh).

7. The discussion could be strengthened by some commentary on the implications of delayed puberty. We tend to think of precocious puberty as being associated with negative outcomes, such as increased risk of antisocial behaviors, substance use, and depression. What are some implications of later maturation in boys?

8. Limitations:
   a. The study sample ranged in age from 10 to 17 years; about 25% of the sample was <12 years, which is at the cusp of the puberty maturation. Indeed, about 22% of the females in the study had initiated menarche at time of follow up. Without continued follow-up of the younger youth (<12 yrs) who may not have yet reached pubertal onset, it would be difficult to assess the extent of delay in this group. It is not clear to me how the authors dealt with the children who had not yet reached menarche in their models.
   b. Exposure to other neurotoxins, such as lead, could also be associated with later pubertal onset (e.g. Williams et al, 2010). Could the authors include blood lead levels in their statistical models as a covariate? (or at least acknowledge this as a limitation).

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