Author’s response to reviews

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

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Author’s response to reviews:

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Dear Dr. Grandjean:

Thank you very much for the opportunity to revise our manuscript (Ms. Ref. No.: ENHE-D-18-00444R1: Fluoride exposure and pubertal development in children living in Mexico City). We thank each of the reviewers for their careful and constructive comments that undoubtedly have improved the quality of the manuscript.

We have made revisions to the manuscript accordingly. Please find our responses to each of the comments.

Comments from the editors and reviewers:

Your manuscript 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.

Reviewer #1: 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.

Response:

Thank you very much for pointing this out. In fact, there was a slight difference in the age at menarche (0.13 months) among these 2 towns reported by the authors on p. 126 [1]. Since the difference was small, the authors stated that there were no essential or substantial changes in the age at menarche; they thus considered the observed differences as no effects. We understand the reviewer’s concerns regarding the interpretation of this study, so we have edited the following sentences to improve our interpretation of this paper:

On p.4, line 62-65 in Introduction:

“In addition to the effects of fluoride on bone and teeth, growing evidence is showing potential impact of fluoride exposure on the alterations in reproductive hormones, fertility, and possibly timing of sexual maturity [3]. However, studies of the association between fluoride exposure and sexual maturity are rare. One animal study reported that fluoride exposure can cause earlier sex maturation in female Mongolian gerbils [4]. An ecological study of girls in New York observed an earlier age at menarche in relation to fluoride exposure [5], while another ecological investigation of Hungarian girls reported no associations [6].”

On p.11, line 221-224 in Discussion:

“An ecological study of 804 Hungarian girls at age 10-19 years observed no substantial differences in the age at menarche between girls living in the high-fluoride town Kunszentmárton (with life-long exposure to fluoridated drinking water at 1.09 mg/L) and girls
living in the low-fluoride town Kiskunmaja (0.17 mg/L) [6]. On the contrary, an animal study of 24 female Mongolian gerbils revealed earlier sex maturation (i.e. earlier vaginal opening and the development of ventral gland) occurred in high fluoride treatment group (3.7 mg/kg/day) versus low fluoride treatment group (0.7 mg/kg/day) [4]. An earlier ecological study of 405 girls aged 7-18 years who had been exposed to fluoridated water up to 10 years showed that the average age at menarche was 12 years among girls in fluoridated Newburgh, New York (fluoridated drinking water 1.2 mg/L), versus 12 years 5 months among girls in Kingston, where water was not fluoridated (essentially fluoride-free) [5].”

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).

Response:

Thank you for the comments. We included 192 of the 234 participants from Bashash’s study [2] in our study. We did examine these associations as suggested by the reviewer and found no significant results (Supplemental Table 3 and 4), except that girls at age 10-17 years with childhood urinary fluoride (age 6-12 years) in the 3rd tertile had a later age at menarche compared with girls in the 1st tertile (HR=0.37, 95% CI: 0.16-0.86). It should be noted that the sample size for children who had data on urinary fluoride and creatinine (age 6-12 years) and pubertal development was much smaller than our study population (N=138 vs. 333), which may have reduced the power to detect significant associations, especially as we modeled ordinal outcomes (5 stages) vs. continuous outcomes assessed in Bashash’s study. We would like to point out that we did examine the prospective association between prenatal exposure to fluoride during pregnancy and pubertal development in boys and girls in this paper (Supplemental Table 1 and 2, as described on p.11, line 205-213). Similarly, since only a small subset of participants had data on prenatal fluoride, as measured by urinary fluoride in any trimester of pregnancy with the adjustment of creatinine in urine (N=201), we were underpowered to detect statistically significant results.

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 years old with several other studies conducted in youth.

Response:

This is a good point. Yes, we believe the urinary fluoride levels are within the expected range. First, as the reviewer stated previously, fluoride levels can be lowered during pubertal growth when bone mass is increasing. Thus, it is reasonable to observe a lower concentration of fluoride among children at older ages. Since a much higher percentage of children at age 10-17 years vs. age 6-12 years had gone through puberty, we would thus expect our participants in this study had lower urinary fluoride levels. Additionally, our study actually reported the geometric mean and 95% CI of urinary fluoride because fluoride levels were skewed in our study population. Direct comparison between mean and geometric mean is not appropriate for non-normally distributed variables. Based on our experience, the geometric mean was generally lower than mean values in our study population.

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.

Response:

Thank you for these thoughtful comments. However, it should be noted that we observed negative associations among both boys (statistically significant) and girls (not significant). We had added the information regarding the findings in male gerbils in the Discussion (p.13, line 255-257).

“Although decreased melatonin levels due to fluoride exposure could potentially increase testosterone as described above [4], it has been suggested that fluoride may directly impair the structure and function of Leydig cells and disrupt the activities of the hypothalamic–pituitary–thyroid (HPT) axis, and hence the release of testosterone can be reduced [23, 24]. In addition, Luke’s study showed that the testicular weight in gerbils at 16 weeks was lower in high fluoride treatment group versus low fluoride treatment group [4]. It is possible that the decreased levels of testosterone and inhibin-B may in turn postpone puberty, which may explain the negative associations observed in our study.

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.

Response:

Thank you for pointing this out. We are sorry for the confusion. We meant that decreased melatonin was associated with increased sex hormones, which subsequently accelerate puberty. We have revised the confusing sentences, as follows:

On p.12, line 237-239 in Discussion:

“It has been proposed by Luke that earlier pubertal onset may be a result of reduced release of melatonin from pineal gland in response to fluoride exposure [4]. Decreased melatonin has been shown to increase gonadotropins and subsequently stimulate sex hormones and eventually accelerate pubertal development [21], but the effect of fluoride on the melatonin production has not been well characterized.”

On p.13, line 251-252 in Discussion:

“Although decreased melatonin levels due to fluoride exposure could potentially increase testosterone as described above [4], it has been suggested that fluoride may directly impair the structure and function of Leydig cells and disrupt the activities of the hypothalamic–pituitary–thyroid (HPT) axis, and hence the release of testosterone can be reduced [23, 24].”

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).

Response:

Thank you for pointing this out. We have revised the sentences (p.12, line 227-231), as follows:

“An earlier ecological study of 405 girls aged 7-18 years who had been exposed to fluoridated water up to 10 years showed that the average age at menarche was 12 years among girls in fluoridated Newburgh, New York (fluoridated drinking water at 1.2 mg/L), versus 12 years 5 months among girls in Kingston, where water was not fluoridated (essentially fluoride-free) [5].”
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?

Response:

Thank you for this helpful feedback. We have added the following text to address this comment in Discussion (p.13, line 259-262):

“According to a recent review [25], late puberty has been associated with low self-esteem, disruptive behavior disorder and substance use in young adults, depression and anxiety in later adulthood. Late puberty may also be associated with an increased risk of bone fracture and coronary heart disease in adults [25-27].”

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.

Response:

We appreciate the concerns; however, we are not sure if we fully understand the reviewer’s points. For the children aged 10-17 years included in this study, the majority of them had already reached the onset of puberty. Indeed, most of the children have reached an advanced stage of puberty. For example, about 80% of these girls had initiated menses. Only less than 10% of these girls had not initiated puberty in terms of pubic hair or breast growth. For pubic hair and breast, we used ordinal regression to model 5 stages of pubertal development among all participants including prepubertal children (stage 1).

As described under Statistical Analysis, the survival analysis, a time-to-event (i.e. time to menarche) method, was performed to assess the age at menarche, also using data from children who had not yet initiated menses. To elaborate, this technique has been widely used in published studies that assessed associations between age at menarche (risk of menarche) and a variety of factors in a population of participants who had and had not have menarche [3-7]. Time to menarche was based on the age of menarche (years) or the age at the interview for girls who were prepubertal (censoring). Both time and censoring index are essential variables in survival analysis. The “survival time” is the time duration to the achievement of menarche among girls that had started menses. Since all female participants at age 10-17 years in our study are at the “risk” of having menses, the counterpart “censoring time” is the age of girls who had not menstruated at the time of the study visit. In the models, the censoring variable for girls who had
initiated menstruation was set as “1” and “0” for those who had not [7]. The results would have been biased without considering children who had not initiated menstruation. The detailed information regarding survival analysis including formulae can be found elsewhere [8].

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).

Response:

Thank you for these insightful comments. Williams et al. [9] observed an inverse association between blood lead levels and pubertal onset (not pubertal stages in this study) in a group of boys at younger age than ours. Previously, we found that early lead exposure was associated with later pubertal development only in girls [3]. However, we did not observe any associations between peripubertal blood lead levels and pubertal development in children aged 10-17 years in this sample. Taken together, we decided not to adjust for lead but acknowledge that peripubertal lead was not considered as a covariate in this study due to the lack of association with pubertal development. We have added the following text to reflect this comment under Statistical Analysis (p.7, line 134-139):

“Lead has also been shown to be associated with pubertal development. In fact, we previously reported that prenatal and early childhood exposure to lead were associated with later pubertal development in girls [14]. However, in this study, we did not include peripubertal lead levels at age 10-17 years in the models because we did not observe a significant association between peripubertal lead levels and any markers of pubertal development.”

Thank you very much for your consideration of our work.

Sincerely,

Martha Maria Téllez-Rojo, Ph.D.
Senior Researcher
Instituto Nacional de Salud Pública

Reference:


in children at 4 and 6-12 years of age in Mexico. Environ Health Perspect 2017, 125(9):097017.


8. [https://stats.idre.ucla.edu/sas/seminars/sas-survival/]