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Domain-specific effects of prenatal fluoride exposure on child IQ at 4, 5, and 6–12 years in the ELEMENT cohort

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Abstract

Objective: Prenatal exposure to fluoride has been associated with adverse neurodevelopmental outcomes. However, the neuropsychological profile of fluoride's developmental neurotoxicity at low levels and the stability of this relationship across childhood has not been characterized. We investigated the longitudinal and domain specific effect of prenatal fluoride exposure on IQ among children ages 4, 5, and 6–12 years in the Early Life Exposures in Mexico to Environmental Toxicants (ELEMENT) cohort.

Methods: We measured the average of maternal urinary fluoride at each trimester of pregnancy adjusted for creatinine (MUF_{CRE}). Children were administered the McCarthy Scales of Children's Abilities at ages 4 (N = 386) and 5 (N = 308), and the Wechsler Abbreviated Scale of Intelligence at age 6–12 (N = 278). We used generalized estimating equation (GEE) models to estimate the population averaged effect of MUF_{CRE} concentration on longitudinal General Cognitive Index (GCI)/Full-Scale IQ (FSIQ), Verbal IQ (VIQ), and Performance IQ (PIQ) scores (N = 348). We tested for possible interactions between MUF_{CRE} and child sex as well as for MUF_{CRE} and time point on children's IQ. All models controlled for relevant available covariates.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2022.112993.

Results: The mean/median MUF_{CRE} concentration was 0.90/0.83 mg/L (SD = 0.39; IQR, 0.64–1.11 mg/L). A 0.5 mg/L increase in MUF_{CRE} predicted an average 2.12-point decrease in GCI/FSIQ (95% CI: −3.49, −0.75) and 2.63-point decrease in PIQ (95% CI: −3.87, −1.40). MUF_{CRE} was marginally associated with VIQ across time (B = −1.29, 95% CI: −2.60, 0.01). No interactions between MUF_{CRE} and child sex or MUF_{CRE} and time were observed.

Conclusion: The negative association between prenatal fluoride exposure and longitudinal IQ was driven by decrements in non-verbal intelligence (i.e. PIQ), suggesting that visual-spatial and perceptual reasoning abilities may be more impacted by prenatal fluoride exposure as compared to verbal abilities.

Keywords

Fluoride; Pregnancy; Longitudinal; IQ; Verbal intelligence; Non-verbal intelligence; Neurodevelopment

1. Introduction

Fluoride is added to drinking water and salt for the prevention of dental caries (CDC, 2016). Other sources of fluoride include fluoridated dental products and supplements, certain foods that absorb naturally occurring fluoride, such as green and black tea, and foods that are sprayed with fluoride-containing pesticides (i.e., grapes; Nutrient Data Laboratory, 2015; Zohoori et al., 2013). Recent studies conducted in the United States (Abduweli Uyghurturk et al., 2020), Canada (Till et al., 2018), and Mexico (Thomas et al., 2016; Castiblanco-Rubio et al., 2021) have reported positive associations between fluoride from dietary sources, including drinking water and salt, and urinary fluoride levels in pregnant women. Because of its ubiquity and its ability to pass through the placenta and blood-brain barrier to reach the fetal brain (Agency for Toxic Substances and Disease Registry, 2003), the safety of fluoride exposure in pregnancy has received much attention, both in endemic fluorosis areas (Jiménez et al., 2017) and communities that have fluoridation programs (Green et al., 2019; Bashash et al., 2017).

While it is not disputed that fluoride is a developmental neurotoxicant at high exposure levels, there are relatively few studies that have assessed fluoride's potential neurotoxicity at levels found in fluoridated areas (i.e., 0.7 mg/L), particularly for pregnant women and young infants. In 2021, the National Toxicology Program (National Toxicology Program, 2020) conducted a systematic review on the impact of fluoride on neurodevelopmental outcomes. The NTP identified two high-quality prospective birth cohort studies that were conducted in Mexico City where salt is fluoridated at 250 ppm (Bashash et al., 2017) and in Canada where drinking water is fluoridated at 0.7 mg/L (Green et al., 2019). Both cohort studies found a 4-to 6-point lower Full-Scale IQ score in children per 1 mg/L increase in maternal urinary fluoride level; in the Canadian cohort the effect of maternal urinary fluoride on IQ was only found in boys while the effect of drinking water fluoride level on IQ was found in both boys and girls.

Most human developmental toxicology studies focus on global or composite test scores, such as Full-Scale IQ, which are derived from a diverse set of tasks (Kamphaus, 2019).

While global outcomes are considered highly significant from a public health (Lanphear, 2015) and economic standpoint (Gould, 2009), a low composite score does not convey specific information about the child's intellectual and cognitive profile. When there are strengths and weaknesses in a cognitive profile, the use of a composite score may have high sensitivity, but at the cost of low specificity (Fiorello et al., 2007). Partitioning Full-Scale IQ into domain specific intellectual abilities, such as verbal and nonverbal skills, may reveal particular cognitive domains that are more sensitive to neurotoxic exposures or may be differentially affected over time (Bellinger et al., 2016). For example, studies have demonstrated that prenatal and early-life lead exposure is more strongly associated with non-verbal intelligence compared to verbal intelligence between the ages of 2–7 (Bellinger et al., 1991; Desrochers-Couture et al., 2018; Dietrich et al., 1991, 1993; Factor-Litvak et al., 1999; Jusko et al., 2008; Wasserman et al., 1997). Similarly, prenatal and early-life fluoride exposure has been associated with greater deficits in non-verbal abilities than verbal abilities in preschool years (Till et al., 2020; Farmus et al., 2021; Cantoral et al., 2021); however, other studies examining early-life exposure to fluoride did not find observe this profile (Ibarluzea et al., 2021) or did not report verbal and non-verbal intelligence (Bashash et al., 2017). Whether non-verbal intelligence is associated with early life exposure to fluoride over the course of child development has not been examined.

In the present study, we examined the longitudinal and domain specific effects (i.e., verbal and nonverbal intelligence) of prenatal fluoride exposure on IQ in mother-child dyads from the Early Life Exposures in Mexico to Environmental Toxicants (ELEMENT) cohort. Since our prior publication on this cohort (Bashash et al., 2017), we received additional maternal urinary fluoride and creatinine data enabling us to examine children's IQ at three separate time points (age 4, 5, and 6–12 years) and using a larger sample size at each time point relative to our prior work. We also examined the potential for sex-specific effects based on findings that boys may be more susceptible to prenatal exposure than girls (Cantoral et al., 2021; Comfort and Re, 2017; Green et al., 2019, 2020; Torres-Rojas and Jones, 2018).

2. Methods

2.1. Study sample

The ELEMENT project enrolled mother-child pairs from three hospitals in Mexico City serving low to middle income families. Participants were recruited as part of four longitudinal birth cohort studies and are described in a recent cohort profile paper (see Perng et al., 2019). Of the four cohorts, cohorts 2 A and 3 had prenatal information and archived maternal urine samples collected during pregnancy. Cohort 2 A included 327 women recruited between 1997 and 1999 for an observational study of prenatal lead exposure and neurodevelopment outcomes in children (Hu et al., 2006). Cohort 3 included 670 women recruited between 2001 and 2003 for a randomized trial of the effect of calcium supplementation during pregnancy on maternal blood levels (Ettinger et al., 2009). Women were included in cohorts 2 A and 3 if they were planning to conceive or were pregnant at less than 14 weeks gestation and intended to reside in Mexico City for at least five years. Women were excluded if they reported a history of psychiatric disorders or substance use, a high-risk pregnancy, and other medical conditions (see Bashash et al., 2017). For

the purpose of the current study, women from cohorts 2 A and 3 were included if they were at least 18 years of age, had at least one biobanked urine sample collected during pregnancy available for fluoride analysis, a urinary creatinine concentration, and if their child underwent IQ testing at the ages of 4, 5, or 6–12 years.

The institutional review boards of the National Institute of Public Health of Mexico, University of Toronto, University of Michigan, Indiana University, and Harvard T.H. Chan School of Public Health and participating clinics approved the study procedures. Participants were informed of study procedures prior to signing an informed consent required for participation in the study.

2.2. Measures

2.2.1. Maternal urinary fluoride (MUF) concentration—Spot (second morning void) urine samples were collected during one or more trimesters of pregnancy. The samples were collected into fluoride-free containers and immediately frozen at the field site. Samples were then shipped and stored at −20 °C at the Harvard School of Public Health (HSPH), and then at −80 °C at the University of Michigan School of Public Health (UMSPH). All urine samples were analyzed at the Indiana University School of Dentistry using a modification of the hexamethyldisiloxane (Sigma Chemical Co., USA) microdiffusion method with the ion-selective electrode (Martinez-Mier et al., 2011).

To account for variations in urinary dilution, each trimester MUF value (mg/L) was adjusted for urinary creatinine (prior to calculating the overall average MUF concentration) using this formula (Thomas et al., 2016; Till et al., 2018):

$$MUF_{CRE(mg/L)} = (MUF_i/CRE_i) * CRE_{avg}$$

where $MUF_{CRE\ (mg/L)}$ is the creatinine adjusted fluoride concentration, MUF_i is the observed fluoride concentration, CRE_i is the observed creatinine concentration for that individual, and CRE_{avg} is the average creatinine concentration of the sample available at each trimester.

The average creatinine concentration (CRE_{avg}) value used at each trimester were derived from the sample of participants in cohorts 2 A and 3 with MUF data at trimester 1, 2, and 3: 100.13, 83.34, and 71.42 (mg/L), respectively. After calculating MUFCRE, extreme outliers (values greater than 3.5 standard deviations from the mean) were dropped (consistent with Bashash et al., 2017). An average of all available maternal urinary fluoride adjusted for creatinine concentrations (MUF_{CRE}) during pregnancy (maximum 3 samples and minimum one sample) was computed and used as the exposure measure.

2.2.2. Assessment of intelligence—Trained psychologists administered the McCarthy Scales of Children's Abilities (MSCA; McCarthy, 1991) to children aged 4 and 5, translated into Spanish. The MSCA includes 18 subtests which yield scores on five domains: perceptual-performance, verbal, quantitative, memory, and a motor index. For the purposes of this study, we included children assessed on two of the five domains given our

primary interest in examining the differential effects of fluoride on verbal and performance intelligence; specifically, children were assessed on the verbal scale (VIQ; a measure of verbal reasoning and comprehension) and the perceptual-performance scale (PIQ; a measure of nonverbal reasoning and perceptual information processing), which are each made up of seven subtests. Children were also assessed on the General Cognitive Index (GCI), which is the standardized composite score derived from the verbal, perceptual-performance, and quantitative scales produced by the MSCA.

Trained psychologists administered the Spanish version of Wechsler Abbreviated Scale of Intelligence (WASI; Wechsler, 1999) to children aged 6–12 years. The WASI includes four subtests; Vocabulary and Similarities are combined to provide estimates for Verbal (VIQ; a measure of verbal reasoning and comprehension), Block Design and Matrix Reasoning are combined to provide estimates for Performance (PIQ; a measure of nonverbal reasoning and spatial processing), and Full-Scale intelligence (FSIQ; a measure of global intellectual functioning) is the composite score of all 4 subtests.

Each child was evaluated by one of three psychologists who was unaware to the child's prenatal fluoride exposure and supervised by an experienced developmental psychologist (L.S.). The inter-examiner reliability of the psychologists on the MSCA was evaluated by having all three psychologists participate in assessments on a set of 30 individuals. For these 30 participants, one examiner would administer and score the MSCA; the other two psychologists observed the assessment and scored the test independent from the person who was administering the test. Each psychologist was observed for 10 of the 30 participants. The intraclass correlation coefficient ($r > 0.90$) was evaluated by calculating the correlation in GCI scores (standardized for age and sex). Inter-examiner reliability was not examined on the WASI test. All raw scores were standardized (mean = 100; SD = 15) for age.

2.2.3. Covariates—We used the same covariates from a previous publication based on factors associated with fluoride and children's intellectual abilities (Bashash et al., 2017). These included maternal education (coded as number of years), maternal age at delivery, marital status at delivery (coded as married: yes/no), maternal smoking (coded as ever smoked: ever/never), gestational age (in weeks), weight at birth (in kilograms), birth order (coded as first child: yes/no), child age (in years and months) at each outcome measurement (i.e., MSCA at age 4, MSCA at age 5, and WASI between age 6–12), and cohort (coded as: Cohort 2 A, Cohort 3 + Ca supplement, and Cohort 3 + placebo). Given that we adjusted for maternal education (a proxy for IQ), and to preserve a larger sample size due to missing data, we did not include maternal IQ as a covariate in our main models, however, we included it as a covariate in sensitivity analyses. A continuous measure of quality of home environment using the Home Observation for Measurement of the Environment (HOME) - Revised Edition (Caldwell and Bradley, 1984) translated into Spanish was available on a subset of the cohort and therefore included as a covariate in sensitivity analyses (see Bashash et al., 2017 for more details). Furthermore, maternal one-month post-partum patella bone lead, and tibia bone lead were available on a subset of the cohort and were included in sensitivity analyses, to determine whether their inclusion significantly altered the results (Gomaa et al., 2002).

2.2.4. Statistical analyses—Descriptive statistics were computed for exposure and outcome variables, as well as model covariates. Chi-square tests for categorical covariates and t-tests for continuous covariates were used to test for sampling differences amongst our included sample and the excluded sample with data on MUF_{CRE}. Pearson correlation coefficients were used to determine the correlation between IQ scores at each time point (age 4, 5, and 6–12).

To estimate the average effect of MUF_{CRE} exposure on children's IQ scores across time, we used generalized estimating equation (GEE) population averaged models for panel data with an autoregressive correlation structure. The panel data were ordered by timepoint, such that time one represented IQ testing at age 4, time two represented IQ testing at age 5, and time three represented IQ testing between the ages of 6–12. Individuals were included in the model if they had data for at least two time points; thus, using the first-order autoregressive structure enabled us to take into account the correlation between IQ scores even if observations were unequally spaced in time.

In supplemental analyses, we ran age-stratified multiple linear regression analyses to estimate the individual associations between MUF_{CRE} exposure and children's IQ scores at each time point (i.e., age 4, 5, and 6–12). Regression diagnostics did not reveal any model specification issues and confirmed that absence of collinearity (all variance inflation factors <4.5) in any of the models. Model residuals were approximately normally distributed, and in sensitivity analyses, extreme outliers depicted in Q-Q plots did not substantially change the results. When we plotted model residuals against model fitted values, there were no indications that the assumption of linearity or heteroscedasticity was violated.

To assess whether sex might be an effect modifier, we re-estimated all models with interactions between child sex and MUF_{CRE} concentration. We also tested the stability of the effect of MUF_{CRE} on IQ scores by including assessment time point by MUFcre interaction in all GEE models.

We used a statistical significance level of $\alpha = 0.05$, two-tailed for all tests. We report coefficients corresponding to a 0.5 mg/L increase in MUF_{CRE} which represents the approximate difference between the 25th and 75th percentile of MUF_{CRE} in our sample. All analyses were conducted using STATA version 17.0 (STATA corporation).

2.2.5. Sensitivity analyses—As previously mentioned, sensitivity analyses were conducted with maternal IQ, HOME scores, maternal one-month post-partum patella bone lead, and tibia bone lead added to the model, to determine whether their inclusion significantly altered the results. Consistent with Bashash et al. (2017), participants in Cohort 3 randomized to the calcium intervention were excluded given the potential impact of calcium on fluoride (Mulualem et al., 2021). Models were also examined excluding IQ scores less than 70 to ensure that very low IQ scores were not influential outliers. Models were further examined including the number/timing of urine samples provided (i.e., trimester 1, 2, 3, 1 and 2, 1 and 3, 2 and 3, or 1 and 2 and 3) as a covariate. Moreover, children's quantitative scores on the MSCA were examined. These were not included in the

primary analyses as there is no equivalent measure on the WASI to examine longitudinal changes in our GEE analyses.

3. Results

3.1. Sample characteristics

Out of the 997 mothers from cohorts 2 A and 3, 971 were at least 18 years of age. Of the 971, 585 had urinary fluoride and urinary creatinine measured for at least one trimester (6 were excluded for having MUF_{CRE} concentrations greater than 3.5 standard deviations from the mean). Of the 585 mothers with MUF_{CRE} data, 391 had MSCA data at age 4, 314 had MSCA data at age 5, and 282 had WASI data at age 6–12. The final sample included 348 mother-child dyads with complete covariate, MUF_{CRE}, and outcome data for at least two time points (see Fig. 1); from hereon, this sample is referred to as the primary sample.

Table 1 describes the sociodemographic characteristics of the mother-child dyads included in the current analysis. Mothers were approximately 27 years old at delivety and had on average 11 years of education. The majority of mothers were married (~70%) and about one-third were nulliparous (~34%). Roughly half of the participants reported ever smoking (~49%).

The current study sample consisted of about 36% of women from cohort 2 A, 35% from cohort 3-Ca, and 29% from cohort 3-Placebo. The mean birth weight of the children was within normal range (~3.13 kg) and the mean gestational age was within a full-term pregnancy (~39 weeks). Children's age at outcome testing ranged from 3 years and 8 months to 11 years and 9 months. Approximately half of the sample was male (~48%). Demographic characteristics of the 348 mother-child dyads with MUF_{CRE} and IQ data did not significantly differ from the subset of participants without complete IQ data on any of the demographic characteristics other than the percentage of people from each cohort (n = 210; Supplemental Table 1)

3.2. MUF_{CRE} data

The mean/median MUF_{CRE} concentration in our primary sample was 0.90/0.83 mg/L (SD = 0.39; range, 0.14–3.01 mg/L). Of the 348 mother-child dyads in our primary sample, 55 had MUF_{CRE} data from each of the three trimesters; 141 had data from two of the three trimesters (n = 102, T1 and T2; n = 29, T1 and T3; and n = 10, T2 and T3); and 152 had data from one of the three trimesters (n = 72, T1; n = 64, T2; and n = 16, T3).

3.3. IQ data

The mean scores of, and the correlation between, the MSCA and WASI are shown in Table 2. GCI/FSIQ, PIQ, and VIQ scores across ages 4, 5 and 6–12 years were moderately to highly correlated, with correlations decreasing across time (r values ranging from 0.49 to 0.76).

3.4. Effects of MUF_{CRE} on IQ

3.4.1. GEE population-averaged models—We observed statistically significant negative associations between MUF_{CRE} concentration and GCI/FSIQ and PIQ scores (Table 3). Specifically, after covariate adjustment, every 0.5 mg/L increase in MUF_{CRE} concentration was significantly associated with an average 2.12-point decrease in GCI/FSIQ (95% CI: −3.49, −0.75, $p = .002$) and 2.63-point decrease in PIQ (95% CI: −3.87, −1.40, $p < .001$) across age. The association of MUF_{CRE} concentration with VIQ across age was negative but only of borderline statistical significance (B = −1.29, 95% CI: −2.60, 0.01, $p = .053$). No interactions were observed between MUF_{CRE} and time in any of the models. Similarly, no interactions were observed between MUF_{CRE} and child sex (i.e., all p values > .10). Fig. 2 shows the longitudinal relationship between MUF_{CRE} and IQ outcomes.

3.4.2. Linear regression analyses—In Supplemental Table 2, we found a significant negative relationship between MUF_{CRE} concentration and FSIQ and PIQ at ages 4 and 5. Specifically, at age 4, we observed a 2.12-point decrease in FSIQ (95% CI: −3.83, −0.41, $p = .015$) and a 3.08-point decrease in PIQ (95% CI: −4.69, −1.47, $p < .001$) for every 0.5 mg/L increase in MUF_{CRE} concentration. At age 5, we observed a 1.97-point decrease in FSIQ (95% CI: −3.64, −0.30, $p = .021$) and a 2.46-point decrease in PIQ (95% CI: −4.04, −0.87, $p = .003$) for every 0.5 mg/L increase in MUF_{CRE} concentration. At both ages 4 and 5, MUF_{CRE} concentration was not significantly associated with VIQ. At ages 6–12 years, we observed a significant negative association between MUF_{CRE} concentration and all three IQ outcomes. Specifically, for every 0.5 mg/L increase in MUF_{CRE} concentration, we observed a 2.01-point decrease in FSIQ (95% CI: −3.66, −0.46, $p = .012$), a 1.80-point decrease in PIQ (95% CI: −3.39, −0.21, $p = .027$), and a 1.93-point decrease in VIQ (95% CI: −3.67, −0.18, $p = .031$). No interactions were observed between MUF_{CRE} and child sex in any of the models (i.e., all p values > .10).

3.5. Sensitivity analyses

The inclusion of number/timing of urine samples, maternal IQ, HOME scores, patella bone lead, or tibia bone lead in our GEE population-averaged models predicting FSIQ/GCI and PIQ, did not substantially alter the results. Similarly, the exclusion of participants with IQ scores less than 70 or in cohort 3 randomized to the calcium supplementation did not significantly affect our GEE population-averaged models predicting FSIQ/GCI and PIQ. The inclusion of maternal IQ and patella lead resulted in slightly more negative coefficients for our GEE population-averaged models predicting VIQ (see Supplemental Table 3). Models examining the effects of fluoride on the quantitative scale of the MSCA at ages 4 and 5 were non-significant (data not shown).

4. Discussion

We examined the association between prenatal fluoride exposure and IQ scores in children of mothers included in the Early Life Exposures in Mexico to Environmental Toxicants (ELEMENT) study. Consistent with past research conducted on a smaller number of mother-child dyads from this cohort (Bashash et al., 2017), an increment of 0.5 mg/L in maternal urinary fluoride concentration was associated with a 2-point decrement in children's Full-

Scale IQ scores. Results remained consistent when we averaged IQ scores across ages 4, 5, and 6–12 or looked at fluoride-IQ associations separately at each age. We then examined the domain specific effects for the association between prenatal fluoride exposure and children's intelligence. Using a repeated-measures approach, results showed that higher maternal urinary fluoride (MUF_{CRE} exposure was significantly associated with deficits in non-verbal intelligence (PIQ) and marginally associated with deficits in verbal intelligence (VIQ). These findings indicate that the association between prenatal fluoride exposure and IQ is stable over time, and that visual-spatial and perceptual reasoning abilities may be more impacted by fluoride exposure as compared to verbal abilities.

While we found that prenatal fluoride exposure was more negatively associated with PIQ than VIQ using a repeated-measures approach, our age-stratified analyses only indicated this pattern at ages 4 and 5. Between ages 6–12, prenatal fluoride exposure was significantly associated with decreases in *both* PIQ and VIQ, perhaps reflecting the use of an abbreviated measure (i.e. WASI) to assess intelligence as opposed to the full-length MSCA used with pre-school-aged children. Our findings of lower non-verbal intelligence in preschool years are in line with previous studies conducted in Canada and Mexico. Specifically, a Canadian prospective cohort study found that every 0.5 mg/L increase in prenatal fluoride exposure was significantly associated with a 4-point decrement in PIQ in boys but not similarly associated with VIQ in boys at age 3–4 (Farmus et al., 2021). A recent prospective study conducted in Mexico City on the PROGRESS cohort found that higher dietary fluoride intake during pregnancy was associated with poorer non-verbal abilities in boys at age 2 (Cantoral et al., 2021). In contrast, a study conducted in Spain found that higher maternal urinary fluoride was positively associated with IQ in boys at age 4 (Ibarluzea et al., 2021). However, the positive association between maternal urinary fluoride and higher verbal and performance IQ was driven by those living in non-fluoridated communities and further, was attenuated when adjusting for other neurotoxicants.

Despite consistencies with the Canadian and Mexican study with the specificity of our findings on non-verbal intelligence, we did not similarly find boys to be more vulnerable than girls in the current cohort. This discrepancy may reflect differences in contextual factors, such as socioeconomic status that interact with sex-specific genetic expressions or a lack of statistical power to detect effect modification with precision (Bellinger, 2000). Given these mixed findings, child sex should continue to be investigated in relation to fluoride neurotoxicity.

One reason for the stronger effects of fluoride on non-verbal intelligence, especially at younger ages, may relate to the influence of modifiable environmental factors. Within the context of neurotoxic exposures, social and parenting factors have been found to account for 40% or more of the variance in children's neurocognitive outcomes (Weiss, 2000), emphasizing the importance of the home environment. A more enriched home environment, involving parental responsiveness, involvement, and acceptance, as well as social, academic, and language stimulation, predicts greater verbal intelligence in young children (Luster and Dubow, 1992). Thus, our findings showing a marginal negative association between fluoride exposure and verbal abilities at earlier ages may reflect a potential buffering effect of the home environment. Future research is needed to explore whether the home environment may

differentially influence offspring outcomes and whether other outcomes, such as executive functioning and self-regulation, may also be affected (Morawska et al., 2019; Shonkoff et al., 2016) in the context of chemical exposures.

Another reason why non-verbal skills may be more vulnerable to prenatal fluoride exposure may relate to the mechanism of fluoride neurotoxicity. There is evidence from animal (Bobek et al., 1976; Cinar and Selcuk, 2005; Wang et al., 2009) and human studies (Chaitanya et al., 2018) that disruption to thyroid function may be involved. Thyroid hormones play an important role in brain development (Bernal, 2005), especially during early fetal development when the fetus is entirely dependent on maternal thyroid hormone but also from mid-gestation until birth when the fetus is partially dependent on maternal thyroid hormone (Morreale de Escobar, 2001; Thorpe-Beeston et al., 1991). Several studies demonstrate that low maternal free T4 is associated with decreased intelligence in children (Haddow et al., 1999; Henrichs et al., 2010; Julvez et al., 2013; Korevaar et al., 2016), and a recent meta-analysis of thyroid function and child IQ in 9036 mother-child dyads from Spain, the Netherlands, and the United Kingdom, found that decreased free T4 in early pregnancy was associated with a significant decrease in non-verbal intelligence (decrease of 3.9 points) but not in verbal intelligence (Levie et al., 2018). Taken together, fluoride exposure across pregnancy may cause reductions in maternal thyroid hormone, which in turn may result in more pronounced effects on non-verbal intelligence. Future research should explore free T4 as a mediator between prenatal fluoride exposure and non-verbal intelligence in children.

Lastly, there is neurochemical evidence from some (Ferreira et al., 2021; Niu et al., 2018; Qian et al., 2013), but not all (McPherson et al., 2018), animal studies that fluoride alters synaptic structures of the hippocampus. Further, fluoride has been found to be related to spatial learning and memory impairment in mice (Jiang et al., 2014) and human studies have demonstrated a direct role of the hippocampus in higher-order visual-spatial perception (Lee et al., 2012). Therefore, non-verbal skills may be more susceptible to prenatal fluoride exposure due to fluoride's impact on the hippocampus.

5. Limitations

A limitation of our study is that fluoride exposure was estimated through maternal non-fasting spot urine samples. Urinary fluoride has a short half-life (approximately 5 h), and measurement of fluoride may be diluted by lack of control for behaviours that could contribute to acute changes in fluoride levels, such as consumption of fluoride-free bottled water prior to urine sampling. We minimized these limitations by collecting serial urine samples across more than one trimester of pregnancy (i.e., 1–3 urine samples) and adjusting for urinary dilution; however, only 196 of 348 (56.3%) of women included in the longitudinal sample provided 2 or 3 urine samples and the timing of urine collection was not standardized across the sample. Further, the correlations of MUF between trimesters were weak (r values ranging from 0.15 to 0.27). Future research should examine prenatal fluoride exposure using biomarkers that capture more chronic exposure to fluoride, such as fingernails or toenails, 24-h urine samples, or measurement of fluoride in shed deciduous teeth (Arora and Austin, 2013). Furthermore, the performance and verbal subscales of

the MSCA and WASI are made up of different subtests, which may capture different abilities, and are normed on different populations. For better interpretation, future studies should assess longitudinal outcomes using more similar tests across age such as the Wechsler Preschool & Primary Scale of Intelligence and the Wechsler Intelligence Scale for Children (Wahlstrom et al., 2018; Wechsler, 1989). A final limitation is that we are missing important covariate data for a large number of participants (for example, HOME scores and maternal lead), and as such, these variables could not be included in the main analyses. Important strengths of the study included the prospective and longitudinal design and blinded assessment of intelligence using standardized measures.

6. Conclusion

In conclusion, prenatal exposure to fluoride is associated with sustained impacts on IQ. Non-verbal abilities may be more susceptible to impairment from prenatal fluoride exposure as compared to verbal abilities. These results were found among mother-child pairs living in a region of Mexico in which fluoride is added to salt. These findings contribute to the growing body of evidence on fluoride's neurotoxicity, and indicate a need to develop recommendations for pregnant women. Future research should continue to investigate the mechanisms of action of low-level fluoride exposure with an emphasis on differences between non-verbal and verbal intelligence, which is important for risk assessment.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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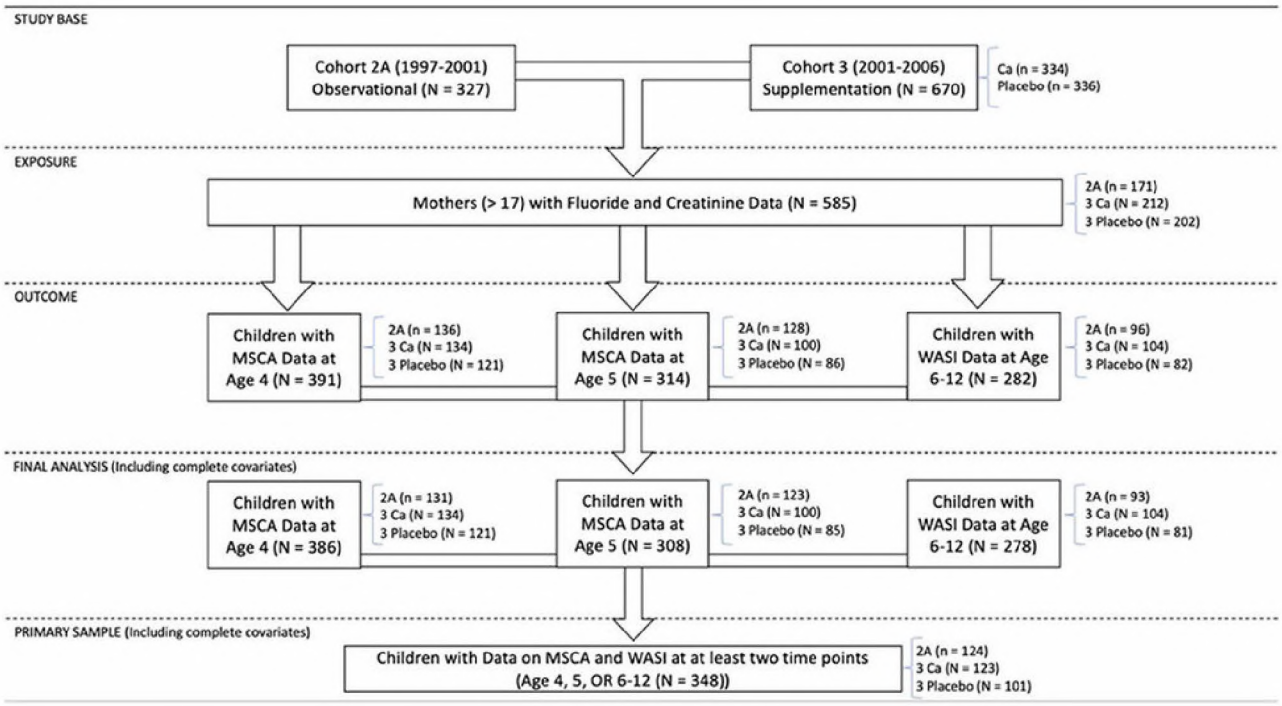


Fig. 1.
Study sample inclusion flow chart.

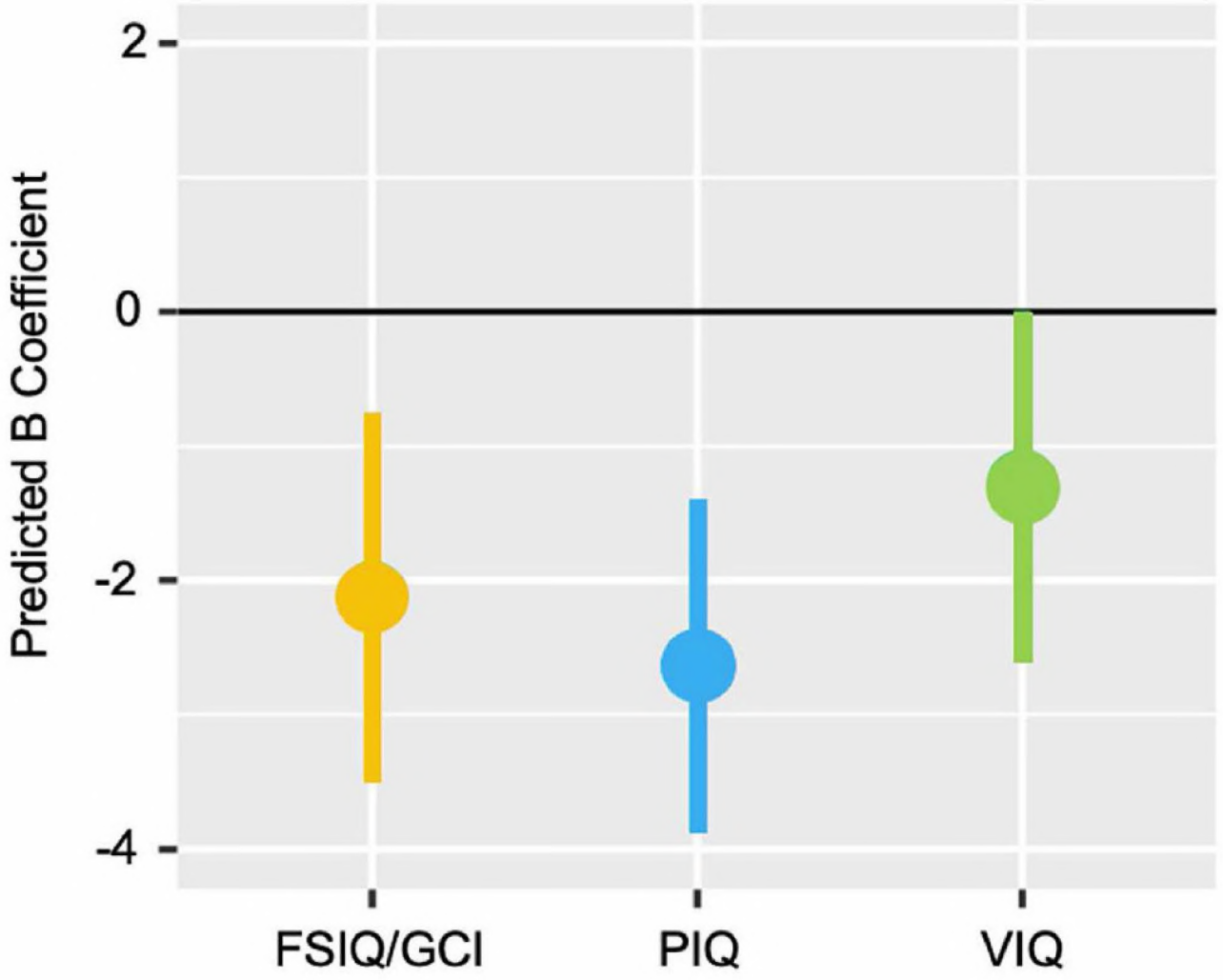


Fig. 2.
Visual representation of the adjusted GEE coefficients (and 95% CI) for the longitudinal association between MUF_{CRE} and IQ.

Table 1

Sociodemographic characteristics of the mother-child pairs included in the present study and at each time point (M ± SD for continuous variables, n (%) for categorical variables).

Characteristic	Samples			
	Primary Sample	Age 4	Age 5	Age 6-12
	N = 348	N = 386	N = 308	N = 278
Maternal Characteristics				
Married (yes)	246 (70.69)	273 (70.73)	216 (70.13)	200 (71.94)
Age at delivery[a] (yrs)	26.77 (18–44)	26.77 (18–44)	26.48 (18–43)	27.04 (18–44)
Education (yrs)	10.77 ± 2.82	10.77 ± 2.83	10.61 ± 2.87	10.91 ± 2.87
Ever smoked	171 (49.14)	189 (48.96)	158 (51.30)	138 (49.64)
First Child	120 (34.48)	134 (34.72)	103 (33.44)	93 (33.45)
Cohort				
2 A	124 (35.63)	131 (33.94)	123 (39.94)	93 (33.45)
3 Calcium	123 (35.34)	134 (34.72)	100 (32.47)	104 (37.41)
3 Placebo	101 (29.02)	121 (31.35)	85 (27.60)	81 (29.14)
MUF_{CRE} (mg/L)[b]	0.90 (0.64–1.11)	0.91 (0.64–1.12)	0.91 (0.64–1.13)	0.89 (0.61–1.10)
Child Characteristics				
Birth Weight (kg)	3.13 ± 0.51	3.13 ± 0.50	3.13 ± 0.51	3.13 ± 0.52
Gestational Age (wks)	38.62 ± 1.85	38.69 ± 1.81	38.61 ± 1.84	38.65 ± 1.76
Child age[a] (yrs)	–	4.04 (3.81–4.31)	5.06 (4.83–5.24)	8.40 (6.3–11.9)
Males	167 (47.99)	183 (47.41)	151 (49.03)	132 (47.48)

Abbreviation: MUF_{CRE}, maternal urinary fluoride adjusted for creatinine collected during pregnancy.

Reported as the.

[a]Mean (range)

[b].Mean (IQR).

Table 2

MSCA and WASI scores (Mean ± SD) and Pearson correlations among the scores at each time point.

FSIQ/GCI	N	M ± SD	Pearson Correlations		
			Age 4	Age 5	Age 6-12
Age 4	386	96.58 ± 13.96	–		
Age 5	308	96.62 ± 12.52	0.76 **a	–	
Age 6-12	278	96.20 ± 11.12	0.58 **b	0.64 **c	–
PIQ	**N**	**M ± SD**	**Age 4**	**Age 5**	**Age 6-12**
Age 4	386	102.51 ± 13.31	–		
Age 5	308	101.95 ± 11.80	0.63 **a	–	
Age 6-12	278	95.54 ± 10.99	0.49 **b	0.53 **c	–
VIQ	**N**	**M ± SD**	**Age 4**	**Age 5**	**Age 6-12**
Age 4	386	97.49 ± 11.86	–		
Age 5	308	97.03 ± 12.62	0.71 **a	–	
Age 6-12	278	97.63 ± 11.85	0.54 **b	0.54 **c	–

Abbreviations: FSIQ = Full-Scale IQ; GCI = General Cognitive Index; PIQ = Performance Intelligence; VIQ = Verbal Intelligence; MSCA = McCarthy Scales of Children's Abilities; WASI = Wechsler Abbreviated Scales of Intelligence.

** $p < .01$.

[a] N = 297.

[b] N = 262.

[c] N = 219.

Table 3

Adjusted GEE models for the association between MUF_{CRE} and IQ longitudinally (N = 348).

	FSIQ/GCI		PIQ		VIQ	
	B	95% CI	B	95%CI	B	95%CI
MUF_{CRE}[a]	−2.12**	−3.49, −0.75	−2.63**	−3.87, −1.40	−1.29	−2.60, 0.01

Abbreviations: MUF_{CRE} = Maternal Urinary Fluoride Adjusted for Creatinine; GCI = General Cognitive Index; FSIQ = Full-Scale Intelligence; PIQ = Performance Intelligence; VIQ = Verbal Intelligence.

** *p* value is < .010.

[a] All models adjusted for gestational age, weight at birth, sex, parity (being the first child), age at outcome measurement, time of testing, smoking history (ever smoked during the pregnancy vs. non-smoker), marital status (married vs. others), maternal age at delivery, maternal education, and cohort/calcium treatment.

Supplemental Tables and Figures

Supplemental Table 1.

Comparison between our primary sample and the excluded sample with MUF_{CRE} data but without IQ data (M ± SD for continuous variables, n(%) for categorical variables)

Characteristic	Primary Included Sample	Excluded Sample	p-value
	N = 348	N = 210	
Maternal Characteristics			
Married	246 (70.69)	146 (69.52)	.770
Age[a]	26.77 (18-44)	26.90 (18-42)	.781
Education (yrs)	10.77 ± 2.82	10.81 ± 3.01	.253
Ever smoked	171 (49.14)	102 (48.57)	.897
First Child	120 (34.48)	72 (34.29)	.962
Cohort			
2A	124 (35.63)	38 (18.10)	<.001
3 Calcium	123 (35.34)	78 (44.76)	
3 Placebo	101 (29.02)	94 (37.14)	
MUF_{CRE} (mg/L)[b]	0.90 (0.64-1.11)	0.88 (0.64-1.03)	.558
Child Characteristics			
Birth Weight (kg)	3.13 ± 0.51	3.14 ± 0.48	1.00
Gestational Age (wks)	38.62 ± 1.85	38.76 ± 1.59	.362
Males	167 (47.99)	110 (52.38)	.315

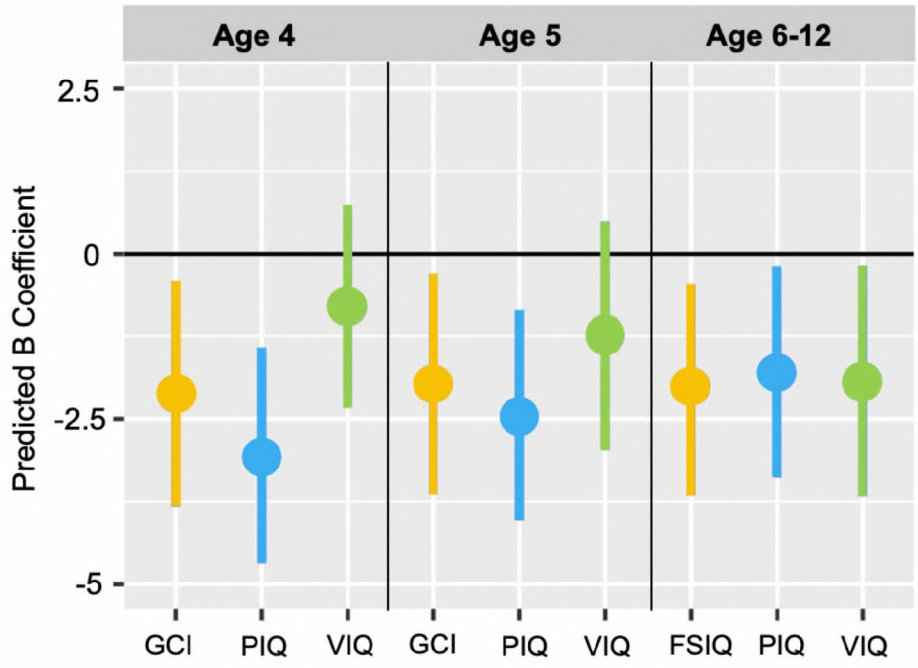
Reported as the [a]mean(range), [b]mean(IQR)

Supplemental Table 2.

Adjusted multiple linear regression models for the association between MUF_{CRE} and IQ stratified by age.

MUF_{CRE}	N	GCI/FSIQ		PIQ		VIQ	
		B	95% CI	B	95%CI	B	95%CI
Age 4[a]	386	-2.12*	-3.83, -0.41	-3.08**	-4.69, -1.47	-0.81	-2.30, 0.69
Age 5[a]	308	-1.97*	-3.64, -0.30	-2.46**	-4.04, -0.87	-1.24	-2.97, 0.49
Age 6-12[a]	278	-2.01*	-3.66, -0.46	-1.80*	-3.39, -0.21	-1.93*	-3.67, -0.18

Abbreviations: MUF_{CRE} = Maternal Urinary Fluoride Adjusted for Creatinine; GCI = General Cognitive Index; FSIQ = Full-Scale Intelligence; PIQ = Performance Intelligence; VIQ = Verbal Intelligence
** *p* value is < .010, * *p* value is < .050.
All models are reported for every 0.5 mg/L increase of MUF_{CRE}.
[a]All models adjusted for gestational age, weight at birth, sex, parity (being the first child), age at outcome measurement, smoking history (ever smoked during the pregnancy vs. non-smoker), marital status (married vs. others), maternal age at delivery, maternal education, and cohort/calcium treatment.



Supplemental Figure 1. Visual representation of the adjusted multiple linear regression coefficients (and 95% CI) for the association between MUF_{CRE} and IQ stratified by age.

Supplemental Table 3.

Adjusted GEE models for the association between MUF_{CRE} and IQ

		FSIQ/GCI		PIQ		VIQ	
	n	B	95% CI	B	95% CI	B	95% CI
Model A[a]	348	**-2.10**	**-3.47, -0.73**	**-2.61**	**-3.85, -1.38**	-1.28	-2.58, 0.03
Model A + num samples	348	**-2.12**	**-3.49, -0.75**	**-2.63**	**-3.86, -1.39**	-1.30	-2.60, 0.01
Model A – IQ < 70	340	**-1.67**	**-2.93, -0.41**	**-2.61**	**-3.81, -1.42**[a]	-1.05	-2.31, 0.21[b]
Model A – Cohort 3 Ca	225	**-1.98**	**-3.70, -0.27**	**-3.13**	**-4.67, -1.58**	-0.69	-2.31, 0.94
Model A – Mat IQ	319	**-2.40**	**-3.79, -1.01**	**-2.78**	**-4.04, -1.52**	**-1.55**	**-2.86, -0.24**
Model A + Mat IQ	319	**-2.09**	**-3.44, -0.73**	**-2.46**	**-3.68, -1.24**	**-1.33**	**-2.62, -0.04**
Model A – HOME	189	**-2.33**	**-4.46, -0.20**	**-3.67**	**-5.52, -1.82**	-0.71	-2.72, 1.30
Model A + HOME	189	**-2.11**	**-4.06, -0.16**	**-3.44**	**-5.15, -1.72**	-0.54	-2.43, 1.35
Model A – Patella Lead	280	**-2.42**	**-3.98, -0.86**	**-2.66**	**-4.05, -1.27**	**-1.62**	**-3.12, -0.11**
Model A + Patella Lead	280	**-2.41**	**-3.98, -0.85**	**-2.65**	**-4.04, -1.27**	**-1.62**	**-3.13, -0.11**
Model A – Tibia Lead	237	**-2.75**	**-4.61, -0.89**	**-2.81**	**-4.46, -1.16**	**-2.09**	**-3.88, -0.31**
Model A + Tibia Lead	237	**-2.23**	**-4.09,- 0.38**	**-2.41**	**-4.07, -0.76**	-1.65	-3.44, 0.14
Model A – Tibia and Patella Lead	225	**-2.73**	**-4.71, -0.76**	**-2.75**	**-4.50, -1.00**	**-2.09**	**-3.99, -0.19**
Model A + Tibia and Patella Lead	225	**-2.20**	**-4.18, -0.22**	**-2.32**	**-4.08, -0.56**	-1.63	-3.55, 0.28

Abbreviations: MUF_{CRE} = Maternal Urinary Fluoride Adjusted for Creatinine; GCI = General Cognitive Index; FSIQ = Full-Scale Intelligence; PIQ = Performance Intelligence; VIQ = Verbal Intelligence

Bolded values represent those with a *p*-value < .050

All models are reported for every 0.5 mg/L increase of MUF_{CRE}.

[a]Coefficients from GEE models adjusted for gestational age, weight at birth, sex, parity (being the first child), age at outcome measurement, time of testing, smoking history (ever smoked during the pregnancy vs. non-smoker), marital status (married vs. others), maternal age at delivery, maternal education, and cohort/calcium treatment. Model A + num samples, model A including the number/timing of urine samples provided as a covariate. Model A – IQ < 70, model A excluding cases with FSIQ/GCI, PIQ, or VIQ scores less than 70. Model A – Cohort 3 Ca, model A excluding the subset of cases who received calcium supplementation. Model A – Mat IQ, model A for the subset of cases who have data on maternal IQ. Model A + Mat IQ, model A for the subset of cases who have data on maternal IQ, adjusted for maternal IQ. Model A – HOME, model A for the subset of cases who have data on Home Observation for the Measurement of the Environment (HOME) scores. Model A+HOME, model A for subset of cases with HOME score, adjusted for HOME score. Model A– Patella Lead, model A for subset of cases who have data on maternal patella lead. Model A + Patella Lead, model A for subset of cases with data on maternal patella lead, adjusted for maternal patella lead. Model A– Tibia Lead, model A for subset of cases who have data on maternal tibia lead. Model A + Tibia Lead, model A for subset of cases with data on maternal tibia lead, adjusted for maternal tibia lead. Model A– Tibia and Patella Lead, model A for subset of cases who have data on maternal tibia and patella lead. Model A + Tibia and Patella Lead, model A for subset of cases with data on maternal tibia and patella lead, adjusted for maternal tibia and patella lead.

[b]N = 346