Appendix C
THE NIH-FUNDED STUDIES

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Prenatal Fluoride Exposure and Cognitive Outcomes in Children at 4 and 6–12 Years of Age in Mexico

Morteza Bashash,[1] Deena Thomas,[2] Howard Hu,[1] E. Angeles Martinez-Mier,[3] Brisa N. Sanchez,[2] Niladri Basu,[4] Karen E. Peterson,[2,5,6] Adrienne S. Ettinger,[2] Robert Wright,[7] Zhenzhen Zhang,[2] Yun Liu,[2] Lourdes Schnaas,[8] Adriana Mercado-García,[9] Martha María Téllez-Rojo,[9] and Mauricio Hernández-Avila[9]

[1]Dalla Lana School of Public Health, University of Toronto, Toronto, Ontario, Canada
[2]University of Michigan School of Public Health, Ann Arbor, Michigan, USA
[3]Indiana University School of Dentistry, Indiana University-Purdue University Indianapolis, Indianapolis, Indiana, USA
[4]Faculty of Agricultural and Environmental Sciences, McGill University, Montreal, Quebec, Canada
[5]Center for Human Growth and Development, University of Michigan, Ann Arbor, Michigan, USA
[6]Harvard T.H. Chan School of Public Health, Boston, Massachusetts, USA
[7]Icahn School of Medicine at Mount Sinai, New York, New York, USA
[8]Instituto Nacional de Perinatología, Mexico City, Mexico
[9]Instituto Nacional de Salud Pública, Cuernavaca, Morelos, Mexico

Background: Some evidence suggests that fluoride may be neurotoxic to children. Few of the epidemiologic studies have been longitudinal, had individual measures of fluoride exposure, addressed the impact of prenatal exposures or involved more than 100 participants.

Objective: Our aim was to estimate the association of prenatal exposure to fluoride with offspring neurocognitive development.

Methods: We studied participants from the Early Life Exposures in Mexico to Environmental Toxicants (ELEMENT) project. An ion-selective electrode technique was used to measure fluoride in archived urine samples taken from mothers during pregnancy and from their children when 6–12 y old, adjusted for urinary creatinine and specific gravity, respectively. Child intelligence was measured by the General Cognitive Index (GCI) of the McCarthy Scales of Children's Abilities at age 4 and full scale intelligence quotient (IQ) from the Wechsler Abbreviated Scale of Intelligence (WASI) at age 6–12.

Results: We had complete data on 299 mother–child pairs, of whom 287 and 211 had data for the GCI and IQ analyses, respectively. Mean (SD) values for urinary fluoride in all of the mothers ($n = 299$) and children with available urine samples ($n = 211$) were 0.90 (0.35) mg/L and 0.82 (0.38) mg/L, respectively. In multivariate models we found that an increase in maternal urine fluoride of 0.5 mg/L (approximately the IQR) predicted 3.15 (95% CI: −5.42, −0.87) and 2.50 (95% CI −4.12, −0.59) lower offspring GCI and IQ scores, respectively.

Conclusions: In this study, higher prenatal fluoride exposure, in the general range of exposures reported for other general population samples of pregnant women and nonpregnant adults, was associated with lower scores on tests of cognitive function in the offspring at age 4 and 6–12 y. https://doi.org/10.1289/EHP655

Introduction

Community water, salt, milk, and dental products have been fluoridated in varying degrees for more than 60 y to prevent dental caries, while fluoride supplementation has been recommended to prevent bone fractures (Jones et al. 2005). In addition, people may be exposed to fluoride through the consumption of naturally contaminated drinking water, dietary sources, dental products, and other sources (Doull et al. 2006). Whereas fluoride is added to drinking water [in the United States at levels of 0.7–1.2 mg/L (Doull et al. 2006)] to promote health, populations with exceptionally high exposures, often from naturally contaminated drinking water, are at risk of adverse health effects, including fluorosis.

In the United States, the U.S. Environmental Protection Agency (EPA) is responsible for establishing maximum permissible concentrations of contaminants, including fluoride, in public drinking-water systems. These standards are guidelines for restricting the amount of fluoride contamination in drinking water, not standards for intentional drinking-water fluoridation. In 2006 the U.S. EPA asked the U.S. National Research Council (NRC) to reevaluate the existing U.S. EPA standards for fluoride contamination, including the maximum contaminant level goal (MCLG, a concentration at which no adverse health effects are expected) of 4 mg/L, to determine if the standards were adequate to protect public health (Doull et al. 2006). The committee concluded that the MCLG of 4 mg/L should be lowered because it puts children at risk of developing severe enamel fluorosis, and may be too high to prevent bone fractures caused by fluorosis (Doull et al. 2006). The Committee also noted some experimental and epidemiologic evidence suggesting that fluoride may be neurotoxic (Doull et al. 2006).

The National Toxicology Program (NTP) recently reviewed animal studies on the effects of fluoride on neurobehavioral outcomes and concluded that there was a moderate level of evidence for adverse effects of exposures during adulthood, a low level of evidence for effects of developmental exposures on learning and memory, and a need for additional research, particularly on the developmental effects of exposures consistent with those resulting from water fluoridation in the United States (Doull et al. 2006; NTP 2016). Human studies have shown a direct relationship between the serum fluoride concentrations of maternal venous blood and cord blood, indicating that the placenta is not a barrier to the passage of fluoride to the fetus (Shen and Taves, 1974). Fluoride was shown to accumulate in rat brain tissues after chronic exposures to high levels, and investigators have speculated that accumulation in the hippocampus might explain effects on learning and memory (Mullenix et al. 1995). An experimental study on mice has shown that fluoride exposure may have adverse effects on neurodevelopment, manifesting as both cognitive and behavioral abnormalities later in life (Liu et al. 2014).

Please send correspondence to M. Bashash, Dalla Lana School of Public Health, 6th floor, 155 College St., Toronto, Ontario M5R3M7 Canada. Telephone: +1-416-978-6512. Email: m.bashash@utoronto.ca

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Most epidemiologic studies demonstrating associations between fluoride exposure and lower neuropsychological indicators have been conducted in populations living in regions with endemic fluorosis that are exposed to high levels of fluoride in contaminated drinking water. The epidemiologic evidence is limited, however, with most studies using an ecologic design to estimate childhood exposures based on neighborhood measurements of fluoride (e.g., drinking water levels) rather than personal exposure measures. Moreover, almost all existing studies of childhood outcomes are cross-sectional in nature, rendering them weak contributors towards causal inference.

The main objective of this study was to assess the potential impact of prenatal exposures to fluoride on cognitive function and test hypotheses related to impacts on overall cognitive function. We hypothesized that fluoride concentrations in maternal urine samples collected during pregnancy, a proxy measure of prenatal fluoride exposure, would be inversely associated with cognitive performance in the offspring children. Overall, to our knowledge, this is one of the first and largest longitudinal epidemiologic studies to exist that either address the association of early life exposure to fluoride to childhood intelligence or study the association of fluoride and cognition using individual biomarker of fluoride exposure.

Methods

This is a longitudinal birth cohort study of measurements of fluoride in the urine of pregnant mothers and their offspring (as indicators of individual prenatal and postnatal exposures to fluoride, respectively) and their association with measures of offspring cognitive performance at 4 and 6–12 y old. 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.

Participants

Mother–child pairs in this study were participants from the successively enrolled longitudinal birth cohort studies in Mexico City that comprise the Early Life Exposures in Mexico to Environmental Toxicants (ELEMENT) project. Of the four ELEMENT cohorts [that have been described elsewhere (Afeiche et al. 2011)], Cohort 1 and Cohort 2B recruited participants at birth and did not have archived maternal-pregnancy urine samples required for this analysis; they were thus excluded. Mothers for Cohort 2A ($n = 327$) and 3 ($n = 670$) were all recruited from the same three hospitals in Mexico City that serve low-to-moderate income populations. Cohort 2A was an observational study of prenatal lead exposure and neurodevelopmental outcomes in children (Hu et al. 2006). Women who were planning to become pregnant or were pregnant were recruited during May 1997–July 1999 and were considered eligible if they consented to participate; were ≤ 14 wk of gestation at the time of recruitment; planned to stay in the Mexico City study area for at least 5 y; did not report a history of psychiatric disorders, high-risk pregnancies, gestational diabetes; did not report current use of daily alcohol, illegal drugs, and continuous prescription drugs; and were not diagnosed with preeclampsia, renal disease, circulatory diseases, hypertension, and seizures during the index pregnancy.

Cohort 3 mothers were pregnant women (≤ 14 wk of gestation) recruited from 2001 to 2003 for a randomized trial of the effect of calcium supplementation during pregnancy on maternal blood lead levels (Ettinger et al. 2009). Eligibility criteria were the same as for Cohort 2A, and 670 agreed to participate.

Exposure Assessment

By virtue of living in Mexico, individuals participating in the study have been exposed to fluoridated salt (at 250 ppm) (Secretaría-de-Salud 1995, 1996) and to varying degrees of naturally occurring fluoride in drinking water. Previous reports, based on samples taken from different urban and rural areas, indicate that natural water fluoride levels in Mexico City may range from 0.15 to 1.38 mg/L (Juárez-López et al. 2007; Martínez-Mier et al. 2005). Mean fluoride content for Mexico City's water supply is not available because fluoride is not reported as part of water quality control programs in Mexico.

Mother–child pairs with at least one archived urine sample from pregnancy and measures of neurocognitive function in the offspring were included in this study. In terms of when the archived samples were collected, the pregnant mothers were invited for assessments with the collection of samples during trimester 1 (13.6 ± 2.1 wk for Cohort 3 and 13.7 ± 3.5 wk for Cohort 2A), trimester 2 (25.1 ± 2.3 wk for Cohort 3 and 24.4 ± 2.9 wk for Cohort 2A), and trimester 3 (33.9 ± 2.2 wk for Cohort 3 and 35.0 ± 1.8 wk for Cohort 2A).

A spot (second morning void) urine sample was targeted for collection during each trimester of pregnancy of ELEMENT mothers as well as the offspring children at the time of their measurements of intelligence at 6–12 y old. The samples were collected into fluoride-free containers and immediately frozen at the field site and shipped and stored at -20°C at the Harvard T. H. Chan School of Public Health (HSPH), and then at -80°C at the University of Michigan School of Public Health (UMSPH).

A procedure for urine analysis of fluoride described elsewhere (Martínez-Mier et al. 2011) was adapted and modified for this study. The fluoride content of the urine samples was measured using ion-selective electrode-based assays. First, 3 M sulfuric acid saturated with hexamethyldisiloxane (HMDS) was added to the sample to allow fluoride to diffuse from the urine for 20–24 hr. The diffused fluoride was allowed to collect in 0.05 M of sodium hydroxide on the interior of the petri dish cover. Once the diffusion was complete, 0.25 M of acetic acid was added to the sodium hydroxide to neutralize the solution and then analyzed directly using a fluoride ion-selective electrode (Thermo Scientific Orion, Cat#13-642-265) and pH/ISE meter (Thermo Scientific Orion, Cat#21-15-001). All electrode readings (in millivolts) were calculated from a standard curve. Analyses were performed in a Class 100/1,000 clean room. Quality control measures included daily instrument calibration, procedural blanks, replicate runs, and the use of certified reference materials (Institut National de Santé Publique du Québec, Cat #s 0910 and 1007; NIST3183, Fluoride Anion Standard). Urinary fluoride concentrations were measured at the UMSPH and the Indiana University Oral Health Research Institute (OHRI) as previously described (Thomas et al. 2016). A validation study comparing measures taken by the two labs in the same samples revealed a between-lab correlation of 0.92 (Thomas et al. 2016).

There were a total of 1,484 prenatal samples measured at the UMSPH lab. All of these samples were measured in duplicate. Of these, 305 (20%) of them did not meet the quality control criteria for ion-selective electrode-based methods (i.e., RSD $<20\%$ for samples with F level <0.2 ppm or RSD $<10\%$ when F level >0.2 ppm) (Martinez-Mier et al. 2011). Of these 305, 108 had a second aliquot available and were successfully measured at the OHRI lab in Indiana (sufficient urine volume was not available for the remaining 197 samples). The OHRI lab in Indiana also measured an additional 289 samples. Of the 397

total samples measured at the OHRI lab in Indiana, 139 (35%) were measured in duplicate, for which >95% complied with the quality control criteria above; thus, all 139 values were retained. The remaining 258 (65%) were not measured in duplicate because of limitations in available urine volume, but were included in the study given the excellent quality control at the OHRI lab. In total, we ended up with 1,576 prenatal urine samples with acceptable measures of fluoride.

Of these 1,576 urine samples, 887 also had data on urinary creatinine and were associated with mother–offspring pairs who had data on the covariates of interest and GCI or IQ in the offspring. The urinary creatinine data were used to correct for variations in urine dilution at the time of measurement (Baez et al. 2014). Creatinine-adjusted urinary fluoride concentrations were obtained for each maternally derived sample by dividing the fluoride concentration (MUF) in the sample by the sample's creatinine concentration (MUC), and multiplying by the average creatinine concentration of samples available at each trimester ($MUC_{average}$) using the formula: $(MUF/MUC) \times MUC_{average}$. The values of average creatinine concentration used for the $MUC_{average}$ at each trimester were derived from the larger pool of trimester-1, -2, and -3 samples from Cohorts 2A and 3 examined in our previous report on maternal fluoride biomarker levels (Thomas et al. 2016): 100.81, 81.60, and 72.41 (mg/L), respectively. For each woman, an average of all her available creatinine-adjusted urinary fluoride concentrations during pregnancy (maximum three samples and minimum one sample) was computed and used as the exposure measure (MUF_{cr}). For children, as creatinine measurements were not available, urinary fluoride values (CUF) were corrected for specific gravity (SG) using the formula $CUFsg = CUF(1.02-1)/(SG-1)$ (Usuda et al. 2007).

After calculating MUF_{cr} for the 887 urine samples noted above, 10 values of MUF_{cr} were identified as extreme outliers (>3.5 SDs) and were dropped, leaving 877 measures of MUF_{cr}. These 877 measures of MUF_{cr} stemmed from 512 unique mothers. Of these 512, 71 participants had measurements from each of the three trimesters; 224 had measurements from two of the three trimesters (74, T1 and T2; 131, T1 and T3; and 19, T2 and T3); and 217 had measurements from only one of the trimesters (159, T1; 34, T2; and 24, T3).

Measurement of Outcomes

At age 4 y, neurocognitive outcomes were measured using a standardized version of McCarthy Scales of Children's Abilities (MSCA) translated into Spanish (McCarthy 1991). MSCA evaluates verbal, perceptual-performance, quantitative, memory, and motor abilities of preschool-aged children, and it has previously been successfully used in translated versions (Braun et al. 2012; Julvez et al. 2007; Kordas et al. 2011; Puertas et al. 2010). For this analysis, we focused on the General Cognitive Index (GCI), which is the standardized composite score produced by the MSCA (McCarthy 1991). For children 6–12 y old a Spanish-version of the Wechsler Abbreviated Scale of Intelligence (WASI) (Wechsler 1999) was administered. WASI includes four subtests (Vocabulary, Similarities, Block Design, and Matrix Reasoning), which provide estimates of Verbal, Performance, and Full-Scale IQ (Wechsler 1999). Both tests were administered by a team of three psychologists who were trained and supervised by an experienced developmental psychologist (L.S.). This team of three psychologists applied all of the McCarthy tests as well as the WASI-FSIQ tests. At the time of follow-up visits (age 4 and 6–12 y), each child was evaluated by one of the psychologists who was blind to the children's fluoride exposure. The inter-examiner reliability of the psychologists was evaluated by having all three psychologists participate in assessments on a set of 30 individuals. For these 30, the inter-examiner reliability of the psychologists was evaluated by calculating the correlation in GCI scores by two of the psychologists with the scores of a third psychologist whom they observed applying the test in all three possible combinations with 10 participants for each observers–examiner pair (i.e., psychologist A (applicant) was observed by psychologist B and psychologist C; psychologist B (applicant) was observed by psychologist A and psychologist C; and psychologist C (applicant) was observed by psychologist A and psychologist B). The mean observer–examiner correlation was 0.99. All raw scores were standardized for age and sex (McCarthy 1991). Inter-examiner reliability was not examined on the WASI test.

Measurement of Covariates

Data were collected from each subject by questionnaire on maternal age (and date of birth), education, and marital status at the first pregnancy visit; on birth order, birth weight, and gestational age at delivery; and on maternal smoking at every prenatal and postnatal visit. Gestational age was estimated by registered nurses. Maternal IQ was estimated using selected subtests of the Wechsler Adult Intelligence Scale (WAIS)-Spanish (Information, Comprehension, Similarities, and Block Design), which was standardized for Mexican adults (Renteria et al. 2008; Wechsler et al. 1981). Maternal IQ was measured at the study visit 6 mo after birth or at the 12-mo visit if the earlier visit was not completed.

The quality of the children's individual home environments was assessed using an age-appropriate version of the HOME score. However, the measure was not available for all observations because it was only added to on-going cohort evaluation protocols beginning in April 2003, when a version of the HOME score instrument that is age-appropriate for children 0–5 y old was adopted, following which a version of the HOME score instrument that is age-appropriate for children ≥6 y old was adopted in September 2009 (Caldwell and Bradley 2003). Thus, we adjusted for HOME score using the measures for 0- to 5-y-old children in the subset of children who had this data in our analyses of GCI, and we adjusted for HOME score using the measures for >6-y-old children in the subset of children who had this data in our analyses of IQ.

Statistical Analyses

Univariate distributions and descriptive statistics were obtained for all exposure variables, outcome variables, and model covariates. For each variable, observations were classified as outliers if they were outside the bounds of the mean ± 3.5 SDs. Primary analyses were conducted with exposure and outcome outliers excluded. Statistical tests of bivariate associations were conducted using chi-square tests for categorical variables and analysis of variance (ANOVA) to compare the means of the outcomes or exposure within groups defined according to the distribution of each covariate. Spearman correlation coefficients were used to measure the correlation between MUF_{cr} and CUF_{sg}. Regression models were used to assess the adjusted associations between prenatal fluoride and each neurocognitive outcome separately. Generalized additive models (GAMs) were used to visualize the adjusted association between fluoride exposure and measures of intelligence [SAS statistical software (version 9.4; SAS Institute Inc.)]. Because the pattern appeared curvilinear, and because GAMs do not yield exact *p*-values for deviations from linearity, we used a Wald *p*-value of a quadratic term of fluoride exposure to test the null hypothesis that a quadratic model fit the data better

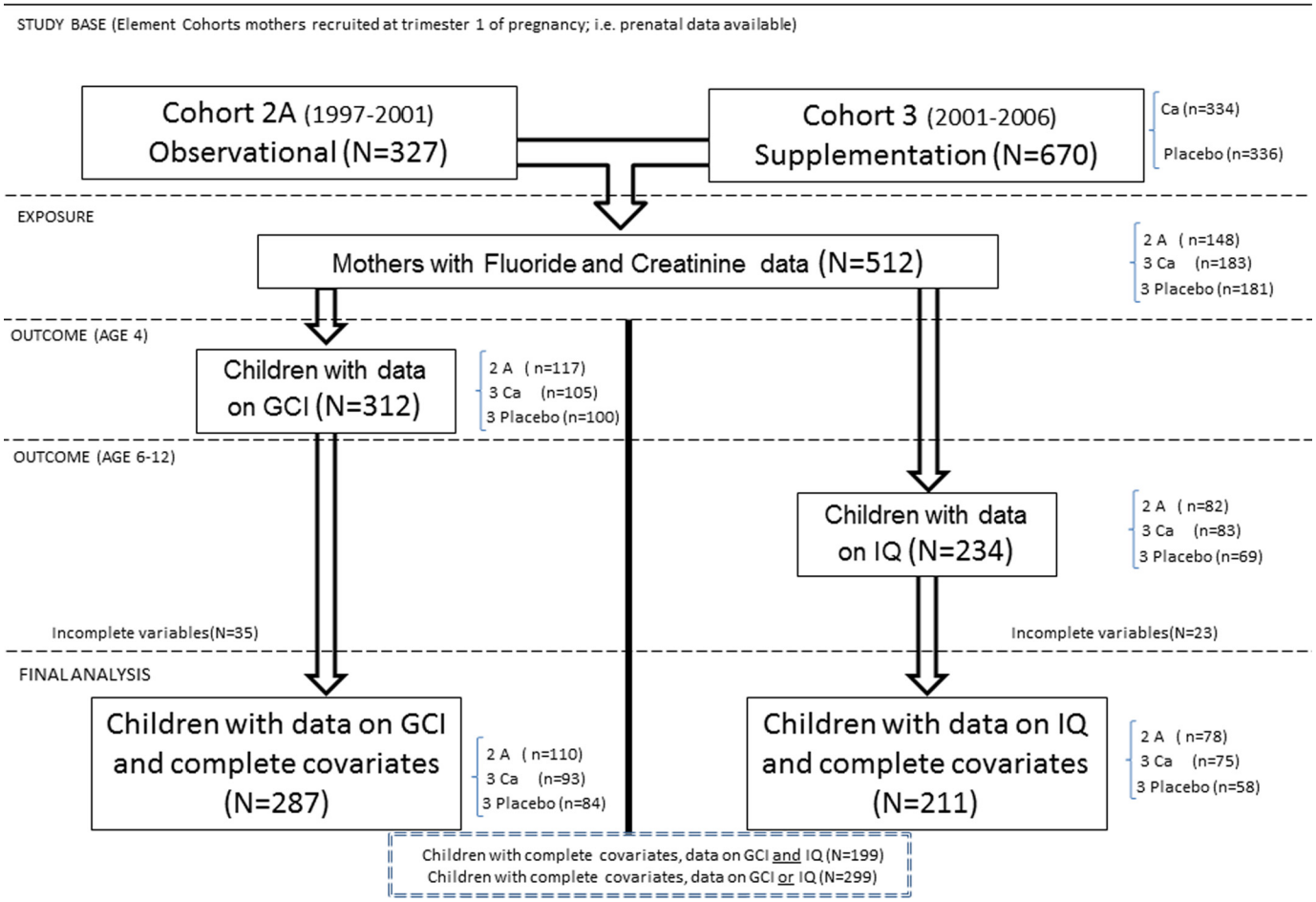

Figure 1. Flowchart describing source of mother–offspring subject pairs, fluoride and cognition study. Cohort 2A was designed as an observational birth cohort of lead toxicodynamics during pregnancy, with mothers recruited early during pregnancy from 1997 to 2001. Cohort 3 was designed as a randomized double-blind placebo-controlled trial of calcium supplements, with mothers recruited early during pregnancy from 2001 to 2006. "Ca" denotes subjects who were randomized to the calcium supplement; "placebo" denotes subjects who were randomized to the placebo. GCI is the McCarthy Scales General Cognitive Index (administered at age 4 y). IQ is the Wechsler Abbreviated Intelligence Scales Intelligence Quotient (administered at age 6–12 y and age-adjusted).

than the model assuming a linear relationship, and thus obtained a *p*-value for deviation from linearity of the fluoride–outcome associations. Residual diagnostics were used to examine other model assumptions and identify any additional potentially influential observations. Visual inspection of default studentized residual versus leverage plot from SAS PROC REG did not identify potential influential observations. Visual inspection of the histogram of the residuals did not indicate lack of normality; however, a fanning pattern in the residual versus predicted value plot indicated lack of constant variance (data not shown). Hence, robust standard errors were obtained using the "empirical" option in SAS PROC GENMOD.

Our overall strategy for selecting covariates for adjustment was to identify those that are well known to have potential associations with either fluoride exposure or cognitive outcomes and/or are typically adjusted for as potential confounders in analyses of environmental toxicants and cognition. All models were adjusted for gestational age at birth (in weeks), birthweight (kilograms), birth order (first born yes vs. no), sex, and child's age at the time of the neurocognitive test (in years). All models were also adjusted for maternal characteristics including marital status (married vs. others), smoking history (ever-smoker vs. never-smoker), age at delivery, IQ, and education (itself also a proxy for socioeconomic status). Finally, all models adjusted for potential cohort effects by including indicator variables denoting from which cohort (Cohort 2A, Cohort 3 + Ca supplement, and Cohort 3 -placebo) the participants came. We used 0.5 mg/L, which was close to the interquartile range of MUF_{cr} for the analyses of both GCI (IQR = 0.45) and IQ (IQR = 0.48), as a standard measure of incremental exposure. SAS statistical software (version 9.4; SAS Institute Inc.) was used for all data analyses described.

Sensitivity Analyses

Models were further adjusted for variables that relate to relatively well-known potential confounders (but for which we were missing a significant amount of data) and variables that were less-well known but possible confounders. The HOME scores were subject to sensitivity analyses because, as noted in the "Methods" section, they were not added to the subject evaluation protocols until 2003, resulting in a significantly smaller subsample of participants with this data. Models of the association between prenatal fluoride exposure (MUF_{cr}) and IQ at 6–12 y old were also adjusted for the child's urine fluoride concentration at 6–12 y of

Table 1. Comparisons across cohorts with respect to the distributions of biomarkers of exposure to prenatal fluoride (MUF_{cr}), prenatal lead (maternal bone Pb), prenatal mercury (maternal blood Hg), and contemporaneous childhood fluoride (CUF_{sg}); and cognitive outcomes (GCI and IQ).

							Percentiles				
Analysis	Measurement	Cohort	*N*	Mean	SD	Min	25	50	75	Max	*p*-Value[a]
GCI Analysis	GCI	Cohort 3-Ca	84	96.88	14.07	50	88	96	107	124	0.997
		Cohort 3-placebo	93	96.80	13.14	50	89	96	105	125	
		Cohort 2A	110	96.95	15.46	56	88	98	110	125	
		Total[b]	287	96.88	14.28	50	88	96	107	125	
	MUF_{cr} (mg/L)	Cohort 3-Ca	84	0.92	0.41	0.28	0.60	0.84	1.14	2.36	0.57
		Cohort 3-placebo	93	0.87	0.34	0.23	0.62	0.82	1.10	2.01	
		Cohort 2A	110	0.92	0.33	0.23	0.68	0.86	1.11	2.14	
		Total[b]	287	0.90	0.36	0.23	0.65	0.84	1.11	2.36	
	Maternal bone Pb (μg/g)	Cohort 3-Ca	62	7.30	7.37	0.05	0.75	4.40	12.93	26.22	<0.01
		Cohort 3-placebo	43	9.21	7.31	0.11	1.50	8.60	13.97	27.37	
		Cohort 2A	62	13.60	11.36	0.15	5.35	10.52	19.46	47.07	
		Total[c]	167	10.13	9.41	0.05	2.37	8.22	15.37	47.07	
	Maternal blood Hg (μg/L)	Cohort 3-Ca	38	3.32	1.40	0.73	2.40	3.00	4.15	7.06	0.12
		Cohort 3-placebo	28	2.80	1.33	1.27	1.89	2.53	3.40	7.22	
		Cohort 2A	75	4.53	5.61	0.77	2.30	3.24	4.37	35.91	
		Total[c]	141	3.86	4.25	0.73	2.20	3.08	4.15	35.91	
IQ Analysis	IQ	Cohort 3-Ca	58	94.91	9.86	76	87	96	100	120	0.69
		Cohort 3-placebo	75	96.29	9.63	75	89	97	102	124	
		Cohort 2A	78	96.47	13.20	67	87	96	107	131	
		Total[d]	211	95.98	11.11	67	88	96	107	131	
	MUF_{cr} (mg/L)	Cohort 3-Ca	58	0.89	0.38	0.29	0.57	0.84	1.10	1.85	0.86
		Cohort 3-placebo	75	0.87	0.35	0.23	0.61	0.82	1.11	2.01	
		Cohort 2A	78	0.90	0.34	0.23	0.67	0.85	1.09	2.14	
		Total[d]	211	0.89	0.36	0.23	0.64	0.82	1.07	2.14	
	Maternal bone Pb (μg/g)	Cohort 3-Ca	67	6.97	7.20	0.05	0.76	4.36	11.73	26.22	<0.01
		Cohort 3-placebo	48	9.07	7.42	0.11	1.00	8.49	14.41	27.37	
		Cohort 2A	62	13.60	11.36	0.15	5.35	10.52	19.46	47.07	
		Total[e]	177	9.86	9.33	0.05	2.29	7.95	15.22	47.07	
	Maternal blood Hg (μg/L)	Cohort 3-Ca	43	3.25	1.41	0.51	2.43	2.87	4.02	7.06	0.067
		Cohort 3-placebo	31	2.66	1.36	0.78	1.81	2.40	3.26	7.22	
		Cohort 2A	75	4.53	5.61	0.77	2.30	3.24	4.37	35.91	
		Total[e]	149	3.77	4.16	0.51	2.19	2.90	4.11	35.91	
	CUF_{sg} (mg/L)	Cohort 3-Ca	71	0.84	0.4	0.31	0.53	0.78	1.12	2.8	0.29
		Cohort 3-placebo	53	0.85	0.38	0.35	0.57	0.75	1.14	1.85	
		Cohort 2A	65	0.76	0.34	0.18	0.51	0.7	0.89	1.76	
		Total[e]	189	0.82	0.38	0.18	0.54	0.73	1.01	2.8	
All available measurements	GCI	Cohort 3-Ca	133	97.32	13.67	50	88	96	107	124	0.57
		Cohort 3-placebo	149	95.99	13.07	50	88	96	106	125	
		Cohort 2A	150	97.57	14.63	56	88	99	109	131	
		Total[f]	432	96.95	13.80	50	88	96	107	131	
	IQ	Cohort 3-Ca	91	95.92	10.15	76	88	95	103	120	0.92
		Cohort 3-placebo	114	96.56	9.84	75	89	96	102	124	
		Cohort 2A	111	96.25	12.67	67	87	95	105	131	
		Total[f]	316	96.27	10.97	67	88	96	103	131	
	MUF_{cr} (mg/L)	Cohort 3-Ca	181	0.89	0.36	0.28	0.64	0.83	1.09	2.36	0.11
		Cohort 3-placebo	183	0.84	0.31	0.02	0.61	0.81	1.02	2.01	
		Cohort 2A	148	0.91	0.35	0.23	0.67	0.86	1.10	2.15	
		Total[f]	512	0.88	0.34	0.02	0.64	0.82	1.07	2.36	
	Maternal bone Pb (μg/g)	Cohort 3-Ca	97	7.07	7.26	0.01	0.83	4.36	11.78	26.22	<0.01
		Cohort 3-placebo	74	9.15	8.38	0.11	0.85	8.62	13.41	40.8	
		Cohort 2A	86	13.77	11.30	0.15	5.49	10.52	20.58	47.07	
		Total[f]	257	9.91	9.51	0.01	2.01	7.64	15.31	47.07	
	Maternal blood Hg (μg/L)	Cohort 3-Ca	55	3.03	1.41	0.51	2.12	2.77	3.62	7.06	0.09
		Cohort 3-placebo	48	2.87	2.09	0.34	1.82	2.37	3.34	13.47	
		Cohort 2A	104	4.06	4.88	0.77	2.14	3.10	4.16	35.91	
		Total[f]	207	3.51	3.70	0.34	2.07	2.80	3.79	35.91	
	CUF_{sg} (mg/L)	Cohort 3-Ca	104	0.84	0.39	0.31	0.56	0.75	1.07	2.80	0.227
		Cohort 3-placebo	84	0.90	0.46	0.35	0.58	0.75	1.09	2.89	
		Cohort 2A	96	0.79	0.34	0.18	0.53	0.73	0.92	2.11	
		Total[f]	284	0.84	0.40	0.18	0.57	0.74	1.00	2.89	

[a]Analysis of variance across cohorts.
[b]Total number of subjects included in GCI main analysis.
[c]Total number of subjects included in GCI sensitivity analysis.
[d]Total number of subjects included in IQ main analysis.
[e]Total number of subjects included in IQ sensitivity analysis.
[f]Total number of subjects with available measurements, combining Cohort 2A and Cohort 3.

Table 2. Analysis comparing subjects with and without data of interest [n (%) or mean ± SD] with respect to characteristics of mothers and children and sensitivity analysis covariates.

Characteristic	GCI analysis		IQ analysis	
	Included	Excluded	Included	Excluded
Total number[a]	287	710	211	786
Sex				
Female	160 (56%)	244 (47%)	116 (55%)	288 (48%)
Male	127 (44%)	275 (53%)	95 (45%)	307 (52%)
Birth order				
First child	96 (33%)	184 (35%)	93 (32%)	279 (36%)
≥2nd child	191 (67%)	335 (65%)	118 (68%)	507 (65%)
Birth weight (kg)	3.11 ± 0.45	3.11 ± 0.44	3.11 ± 0.46	3.11 ± 0.43
Gestational age (wk)	38.66 ± 1.84	38.58 ± 1.68	38.56 ± 1.80	38.63 ± 1.72
Age at outcome assessment (y)	4.04 ± 0.05	4.05 ± 0.05	8.50 ± 1.31	8.83 ± 1.64
Maternal age at delivery (y)	26.78 ± 5.53	26.49 ± 5.37	27.16 ± 5.61	26.41 ± 5.36
Maternal education (y)[b]	10.63 ± 2.76	10.75 ± 3.08	10.80 ± 2.85	10.69 ± 3.03
Maternal IQ[c]	88.63 ± 12.17	89.27 ± 14.6	89.01 ± 12.45	88.27 ± 13.00
Marital status[d]	3.11 ± 0.45	3.11 ± 0.44	3.11 ± 0.46	3.11 ± 0.43
Married	201 (70%)	493 (70%)	149 (71%)	544 (69%)
Other	86 (30%)	216 (30%)	62 (29%)	240 (31%)
Maternal smoking[e]				
Ever	141 (49%)	335 (51%)	102 (48%)	374 (51%)
Never	146 (51%)	325 (49%)	109 (52%)	362 (49%)
Cohort				
Cohort 3-Ca	93 (32%)	241 (34%)	76 (36%)	259 (33%)
Cohort 3-placebo	84 (29%)	252 (36%)	59 (28%)	278 (35%)
Cohort 2A	110 (38%)	217 (31%)	78 (37%)	249 (32%)
Sensitivity Analyses				
HOME score[f]	$N^{\dagger} = 138$	$N^{\ddagger} = 87$	$N^{\dagger} = 124$	$N^{\ddagger} = 55$
	35.24 ± 6.31	33.23 ± 6.55	35.54 ± 7.46	35.8 ± 7.44
SES[g]	$N^{\dagger} = 188$	$N^{\ddagger} = 110$	$N^{\dagger} = 199$	$N^{\ddagger} = 98$
	6.35 ± 2.43	6.94 ± 2.72	6.36 ± 2.41	6.98 ± 2.79
Maternal Bone Pb (μg/g)[h]	$N^{\dagger} = 167$	$N^{\ddagger} = 91$	$N^{\dagger} = 177$	$N^{\ddagger} = 80$
	9.26 ± 10.55	8.97 ± 10.32	9.02 ± 10.43	9.48 ± 10.55
Maternal Blood Hg (μg/L)[i]	$N^{\dagger} = 141$	$N^{\ddagger} = 67$	$N^{\dagger} = 149$	$N^{\ddagger} = 58$
	3.86 ± 4.25	2.76 ± 1.95	3.77 ± 4.16	2.83 ± 2.01
CUF_{sg}[j] (mg/L)			$N^{\dagger} = 124$	$N^{\ddagger} = 55$
			35.54 ± 7.46	35.8 ± 7.44

[a]The total number of subjects ($n = 997$) are all mother–offspring pairs who participated in the original Cohort 2A and Cohort 3 studies.
[b]Maternal education at the time of the child's birth.
[c]Maternal IQ measured at 6 mo after child's birth.
[d]Mother's marital status at the time of the child's birth.
[e]History of any maternal smoking.
[f]HOME score measured using the separate age-appropriate instruments pertaining to children of ≤5 y old; and children >5 y old.
[g]Family socioeconomic status (SES) measured by questionnaire of family possessions at follow-up.
[h]Maternal patella bone lead measured by KXRF after birth.
[i]Maternal average blood mercury during pregnancy.
[j]Children's specific gravity–corrected urinary fluoride measured at the time of each child's IQ test (6–12 y old).
$N^{\dagger}$ Number of subjects with measurements of MUF_{cr}, cognitive outcome, main covariates, and sensitivity covariates (they are included in the sensitivity model).
$N^{\ddagger}$ Number of subjects with measurements of sensitivity covariates, but missing data on exposure, outcomes, or main covariates (they are excluded from the sensitivity model).

age (CUF_{sg}), a measure that was collected in a significantly smaller subset of individuals, to evaluate the potential role of contemporaneous exposure. Associations between prenatal fluoride exposure (MUF_{cr}) and GCI at 4 y old could not be adjusted for contemporaneous fluoride exposure because urine samples were not collected from children when the MSCA (from which the GCI is derived) was administered. Maternal bone lead measured by a 109-Cd K-X-ray fluorescence (KXRF) instrument at 1 mo postpartum, a proxy for lead exposure from mobilized maternal bone lead stores during pregnancy (Hu et al. 2006), was included in the model to test for the possible confounding effect of lead exposure during pregnancy. We focused on the subset of women who had patella bone lead values because these were found to be most influential on our previous prospective study of offspring cognition (Gomaa et al. 2002). Average maternal mercury level during pregnancy was also tested for being a potential confounder (Grandjean and Herz 2011). Mercury was measured as total mercury content in the subsample of women who had samples of archived whole blood samples taken during pregnancy with sufficient volume to be analyzed using a Direct Mercury Analyzer 80 (DMA-80, Milestone Inc., Shelton, CT, USA) as previously described (Basu et al. 2014).

To address the potential confounding effect of socioeconomic status (SES) we conducted sensitivity analyses that adjusted our model for SES (family possession score). The socioeconomic questionnaire asked about the availability of certain items and assets in the home. Point values were assigned to each item, and SES was calculated based on the sum of the points across all items (Huang et al. 2016). Given that the calcium intervention theoretically could have modified the impact of fluoride, in examining our results, we repeated the analyses with and without the Cohort 3 participants who were randomized to the calcium intervention to omit any potential confounding effect of this intervention. Another sensitivity test was performed to examine the potential effect of the psychologist who performed the WASI test by including tester in the regression model. The information about psychologists who performed the WASI was available for 75% of participants, as recording this data was

Table 3. Distributions of maternal creatinine-adjusted urinary fluoride (MUF_{cr}) and offspring cognitive scores across categories of main covariates.

	GCI Analysis					IQ Analysis				
Covariate	*n*	MUF_{cr}[a]	*p*-Value	GCI (Age 4)	*p*-Value	*n*	MUF_{cr}[a]	*p*-Value	IQ (Age 6–12)	*p*-Value
Mothers										
Age										
≥25 y	123	0.88 ± 0.36	0.45	96.22 ± 14.12	0.50	88	0.89 ± 0.37	0.98	95.75 ± 11.64	0.80
<25 y	164	0.92 ± 0.36		97.37 ± 14.43		123	0.89 ± 0.35		96.15 ± 10.76	
Education										
<12 y	153	0.91 ± 0.4	0.92	94.22 ± 14.23	0.001	111	0.87 ± 0.37	0.53	93.09 ± 10.54	<0.001
12 y	97	0.89 ± 0.34		98.56 ± 14.46		70	0.93 ± 0.35		98.29 ± 10.72	
>12 y	37	0.89 ± 0.42		103.49 ± 11.21		30	0.85 ± 0.31		101.3 ± 11.16	
Marital status										
Married	201	0.90 ± 0.37	0.81	96.40 ± 14.46	0.39	62	0.90 ± 0.35	0.79	96.55 ± 11.06	0.63
Other	86	0.91 ± 0.33		98.00 ± 13.88		149	0.88 ± 0.36		95.74 ± 11.16	
Smoking										
Ever smoker	141	0.90 ± 0.36	0.80	97.77 ± 13.9	0.30	102	0.90 ± 0.36	0.56	97.21 ± 10.7	0.12
Nonsmoker	146	0.91 ± 0.35		96.01 ± 14.63		109	0.87 ± 0.35		94.83 ± 11.41	
HOME score [b]										
Mid-low ≤30	49	0.88 ± 0.37	0.47	90.73 ± 13.36	<0.001	32	0.87 ± 0.36	0.85	89.88 ± 8.45	0.011
High >30	137	0.92 ± 0.38		99.29 ± 14.61		92	0.88 ± 0.38		99.05 ± 11.65	
Maternal IQ										
Mid-low ≤85	116	0.95 ± 0.35	0.09	93.16 ± 15.04	<0.001	86	0.92 ± 0.36	0.23	91.26 ± 9.72	<0.001
High >85	171	0.87 ± 0.36		99.4 ± 13.21		125	0.86 ± 0.35		99.23 ± 10.87	
Children										
Sex										
Boy	127	0.94 ± 0.36	0.09	93.93 ± 13.98	0.002	95	0.96 ± 0.38	0.008	96.82 ± 12.02	0.32
Girl	160	0.87 ± 0.36		99.22 ± 14.12		116	0.83 ± 0.32		95.29 ± 10.31	
Birthweight										
≥3.5 kg	241	0.91 ± 0.36	0.57	96.52 ± 14.36	0.33	201	0.89 ± 0.36	0.88	95.66 ± 11.29	0.58
<3.5 kg	46	0.87 ± 0.35		98.76 ± 13.88		10	0.88 ± 0.34		97.38 ± 9.42	
Gestational age										
≤39 wk	192	0.90 ± 0.35	0.90	96.66 ± 14.23	716	146	0.89 ± 0.36	0.712	95.71 ± 11.62	0.65
>39 wk	95	0.90 ± 0.37		97.32 ± 14.46		65	0.88 ± 0.34		96.58 ± 9.91	
First child										
Yes	96	0.91 ± 0.38	0.75	99.97 ± 12.87	0.009	68	0.88 ± 0.36	0.91	97.00 ± 11.00	0.36
No	191	0.90 ± 0.35		95.32 ± 14.73		143	0.89 ± 0.36		95.50 ± 11.17	
CUF_{sg} [c]										
≥0.80 mg/L						112	0.86 ± 0.32	0.49	96.80 ± 11.16	0.37
<0.80 mg/L						77	0.90 ± 0.38		95.37 ± 10.31	

[a]Maternal creatinine-adjusted urinary fluoride (mg/L).
[b]Home Observation for the Measurement of the Environment (HOME) score, measured using the separate age-appropriate instruments pertaining to children of ≤5 y old; and children >5 y old.
[c]Child contemporaneous specific gravity–adjusted urinary fluoride (available at the time of each child's IQ test).

added later to the study protocol. We also re-ran models with exposure outliers included as a sensitivity step. Finally, we ran models that focused on the cross-sectional relationship between children's exposure to fluoride (reflected by CUF_{sg}) and IQ score, unadjusted; adjusting for the main covariates of interest; and adjusting for prenatal exposure (MUF_{cr}) as well as the covariates of interest.

Results

Flow of Participants

Of the 997 total mothers from two cohorts evaluated, 971 were eligible after removing mothers <18 y old. Of these 971, 825 had enough urine sample volume to measure fluoride in at least one trimester urine sample, and of these 825 participants, 515 participants had urine samples with previously measured creatinine values, enabling calculation of creatinine-adjusted urinary fluoride (MUF_{cr}) concentrations. Of these 515, 3 participants were excluded based on the 10 extreme outlier values identified for MUF_{cr} (see the "Methods" section, "Exposure Assessment" subsection) and not having any other MUF_{cr} values to remain in the analysis. Thus, we had a total of 512 participants (mothers) with at least one value of MUF_{cr} for our analyses (Figure 1).

Of these 512 mothers, 312 had offspring with outcome data at age 4 (i.e., GCI), and 234 had offspring with outcome data at age 6–12 (i.e., IQ). Of these, complete data on all the covariates of main interest (as specified in the "Methods" section) were available on 287 mother–child pairs for the GCI analysis and 211 mother–child pairs for the IQ analysis. A total of 299 mother–child pairs had data on either GCI or IQ, and 199 mother–child pairs had data on both GCI and IQ (Figure 1).

Number of Exposure Measures per Subject

In terms of repeated measures of MUF_{cr} across trimesters, of the 287 participants with data on GCI outcomes; 25 participants had MUF_{cr} data for all three trimesters (11 from Cohort 2A and 14 from Cohort 3), 121 participants had MUF_{cr} data from two trimesters (48 from Cohort 2A and 73 from Cohort 3), and 141 participants had MUF_{cr} data from one trimester (51 from Cohort 2A and 90 from Cohort 3). Of the 211 participants with data on IQ outcomes, 10 participants had MUF_{cr} data for all three trimesters (6 from Cohort 2A and 4 from Cohort 3), 82 participants had data from two trimesters (32 from Cohort 2A and 50 from Cohort 3), and 119 participants had data from one trimester (40 from Cohort 2A and 79 from Cohort 3).

Comparisons across the Cohorts

In terms of the mother–child pairs who had data on all covariates as well as data on either GCI or IQ ($n = 299$), the mean (SD)

Table 4. Multivariate regression models: unadjusted and adjusted differences in GCI and IQ per 0.5 mg/L higher maternal creatinine-adjusted urinary fluoride (MUF_{cr}).

	GCI			IQ		
Estimate	*n*	β (95%CI)	*p*-Value	*n*	β ± S.E (95%CI)	*p*-Value
Unadjusted	287	−3.76 (−6.32, −1.19)	<0.01	211	−2.37 (−4.45, −0.29)	0.03
model A[a]	287	−3.15 (−5.42, −0.87)	0.01	211	−2.50 (−4.12, −0.59)	0.01
Model A − HOME	138	−3.63 (−6.48, −0.78)	<0.01	124	−2.36 (−4.48, −0.24)	0.03
Model A + HOME	138	−3.76 (−7.08, −0.45)	0.03	124	−2.49 (−4.65, −0.33)	0.02
Model A − CUF_{sg}				189	−1.79 (−3.80, 0.22)	0.08
Model A + CUF_{sg}				189	−1.73 (−3.75, 0.29)	0.09
Model A − SES	188	−4.55 (−7.23, −1.88)	0.01	199	−2.10 (−4.02, −0.18)	0.03
Model A + SES	188	−4.45 (−7.08, −1.81)	0.01	199	−2.10 (−4.06, −0.15)	0.04
Model A − Pb	167	−5.57 (−8.48, −2.66)	<0.01	177	−3.21 (−5.17, −1.24)	<0.01
Model A + Pb	167	−5.63 (−8.53, −2.72)	<0.01	177	−3.22 (−5.18, −1.25)	<0.01
Model A − Hg	141	−7.13 (−10.26, −4.01)	<0.01	149	−4.59 (−7.00, −2.17)	<0.01
Model A + Hg	141	−7.03 (−10.19, −3.88)	<0.01	149	−4.58 (−6.99, −2.16)	<0.01
Model A − Ca	194	−3.67 (−6.57, −0.77)	0.01	136	−3.23 (−5.88, −0.57)	0.02

[a]Coefficients from linear regression models adjusted for gestational age, weight at birth, sex, parity (being the first child), age at outcome measurement, and maternal characteristics including smoking history (ever smoked during the pregnancy vs. nonsmoker), marital status (married vs. others), age at delivery, IQ, education, and cohort (Cohort 3-Ca, Cohort 3-placebo and Cohort 2A). Model A–HOME, model A for subset of cases who have data on Home Observation for the Measurement of the Environment (HOME) scores (but the model did not include HOME score). Model A + HOME, model A for subset of cases with HOME score, adjusted for HOME score. Model A − CUF_{sg}, model A for subset of cases who have data on child contemporaneous specific gravity–adjusted urinary fluoride CUF_{sg} (but the model did not include CUF_{sg}). Model A + CUF_{sg}, model A for subset of cases with CUF_{sg}, adjusted for CUF_{sg}. Model A−SES, model A for subset of cases who have data on socioeconomic status (family possession measured by questionnaire of family possessions) (but the model did not include SES). Model A + SES, model A for subset of cases with SES data, adjusted for SES. Model A–Pb, model A for subset of cases who have data on maternal bone lead (but the model did not include maternal bone lead). Model A + Pb, model A for subset of cases with data on maternal bone lead, adjusted for maternal bone lead. Model A−Hg, model A for subset of cases who have data on maternal blood mercury (but the model did not include maternal blood mercury). Model A + Hg, model A for subset of cases who have data on maternal blood mercury, adjusted for maternal blood mercury. Model A − Ca, model A for subset of cases who did not receive the Ca supplement (they received the placebo).

values of creatinine–corrected urinary fluoride for the mothers was 0.90 (0.36) mg/L. The distributions of the urinary fluoride, outcomes (GCI and IQ), and additional exposure variables examined in our sensitivity analyses (maternal bone lead, maternal blood mercury, and children's contemporaneous urinary fluoride) across the three cohort strata (Cohort 3-Calcium, Cohort 3-placebo, and Cohort 2A) and all strata combined are shown in Table 1 for the mother–child pairs who had data for the GCI outcome ($n = 287$) and the IQ outcome ($n = 211$). The distributions showed little variation across the cohort strata except for bone lead and possibly blood mercury, for which, in comparison with Cohort 3, Cohort 2A clearly had higher mean bone lead levels ($p < 0.001$) and possibly higher blood mercury levels ($p = 0.067$). The mean (SD) values of specific gravity–corrected urinary fluoride for the children who had these measures (only available for those children who had IQ; $n = 189$) were 0.82 (0.38) mg/L.

In terms of the comparability of the participants across Cohort 2A and Cohort 3 with respect to our covariates, the distribution of the covariates was very similar with the exception of age of the offspring when IQ was measured, for which the mean ages were 7.6 and 10.0 y, respectively; and birth weight in the GCI analysis, for which Cohort 3 participants were slightly heavier than Cohort 2 participants (see Table S1).

GCI versus IQ Scores

There was a significant correlation between GCI at 4 y and IQ at 6–12 y old (Spearman $r = 0.55$; $p < 0.01$). There was no significant correlation between prenatal MUF_{cr} and offspring CUF_{sg} (Spearman $r = 0.54$, $p = 0.44$).

Comparisons of Participants in Relation to Missing Data

In comparing the participants who were included for the GCI and IQ analyses with the participants who were not included (based on data missing on GCI, IQ or other covariates), the distribution of covariates were similar except for sex, for which the proportion of females was somewhat higher in the included versus excluded group for both the GCI and IQ analyses (Table 2).

In terms of the sensitivity analyses, for each sensitivity variable of interest, we compared participants who had data on our exposures, outcomes, covariates, and the sensitivity variable of interest (and were thus included in the sensitivity analysis) versus participants who had data on the sensitivity variable of interest but were missing data on the exposure, outcomes, and/or covariates of interest (and were thus excluded from the sensitivity analysis; Table 2). It can be seen that for each sensitivity analysis, most of the participants with data on the sensitivity variable of interest also had data on the exposures, outcomes, and covariates and were therefore included in the sensitivity analysis. In addition, the distributions appeared to be similar comparing those included with those excluded in each sensitivity analysis (means were within 10% of each other), with the exception of maternal blood Hg, for which the mean levels for those included were 28.5% and 24.9% higher than the mean levels for those excluded in the GCI and IQ analyses, respectively.

Comparisons of GCI and IQ across Covariates

Table 3 shows mean and SD values for MUF_{cr} and offspring cognitive scores across categories of the covariates. In the participants with GCI data, the offspring cognitive scores were higher among mothers with higher levels of education, measured IQ, and HOME scores for both analyses; and scores were higher among first children and girls. In the IQ analysis a statistically significant difference was observed in MUF_{cr} as a function of child sex. No significant differences in MUF_{cr} values across levels of other covariates were observed. A modest difference (not statistically significant), was observed in MUF_{cr} as a function of maternal IQ ($p = 0.09$), and MUF_{cr} as a function of child sex ($p = 0.09$). Among other co-variates there were significant differences in age ($p < 0.01$) in both analyses.

Regression Models of GCI

Before adjustment, a 0.5 mg/L increase in MUF_{cr} was negatively associated with GCI at 4 y old [mean score −3.76; 95% confidence interval (CI): −6.32, −1.19] (Table 4). The association was somewhat attenuated after adjusting for the main covariates

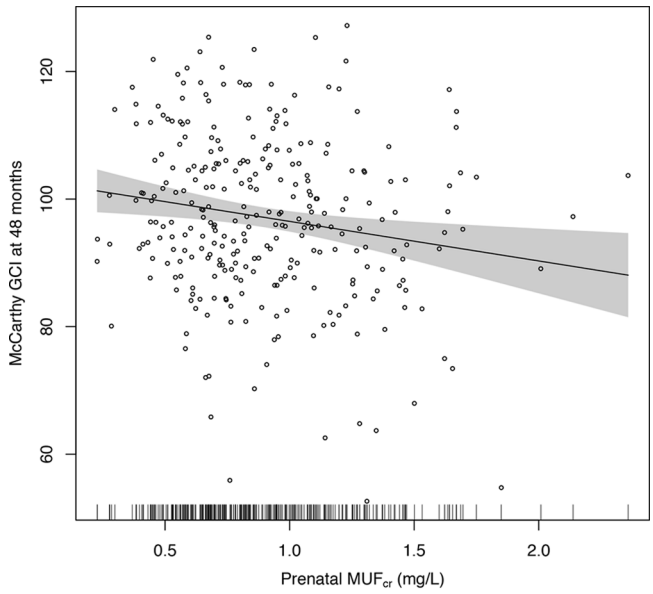

Figure 2. Adjusted association of maternal creatinine-adjusted urinary fluoride (MUF_{cr}) and General Cognitive Index (GCI) scores in children at age 4 y. Adjusted for gestational age, weight at birth, sex, parity (being the first child), age at outcome measurement, and maternal characteristics including smoking history (ever smoked vs. nonsmoker), marital status (married vs. others), age at delivery, IQ, education, and cohort (Cohort 3-Ca, Cohort 3-placebo and Cohort 2A). Shaded area is 95% confidence interval. Short vertical bars on the *x*-axis reflect the density of the urinary fluoride measures. Individual data points are individual observations, $n = 287$.

(model A, −3.15; 95% CI: −5.42, −0.87). The smooth plot of the association between GCI and maternal prenatal urinary fluoride from an adjusted GAM model suggested a linear relation over the exposure distribution (Figure 2).

Regression Models of IQ

A 0.5 mg/L increase in prenatal fluoride was also negatively associated with IQ at age 6–12 y based on both unadjusted (−2.37; 95% CI: −4.45, −0.29) and adjusted models (−2.50; 95% CI: −4.12, −0.59) (Table 4). However, estimates from the adjusted GAM model suggest a nonlinear relation, with no clear association between IQ scores and values below approximately 0.8 mg/L, and a negative association above this value (Figure 3A). There was a nonsignificant improvement in the fit of the model when a quadratic term was added to the linear model ($p = 0.10$).

Sensitivity Analyses

In sensitivity analyses, adjustment for HOME score increased the magnitude of the association between MUF_{cr} and GCI, though the difference was less pronounced when associations with and without adjustment for HOME score were both estimated after restricting the model to the subset of 138 children with HOME score data (Table 4). The association of IQ scores with MUF_{cr} did not substantially change after adding HOME score to the model (Table 4).

The association between MUF_{cr} and IQ was attenuated slightly after adjusting for contemporaneous children's urinary fluoride (CUF_{sg}) and comparing estimates with [−1.73 (95% CI: −3.75, 0.29)] and without [−1.94 (95% CI: −4.15, 0.26)] adjustment for CUF_{sg} among the 189 children with this data (Table 4). In addition, the evidence of nonlinearity was more pronounced, with no clear evidence of an association for MUF_{cr} <1.0 mg/L

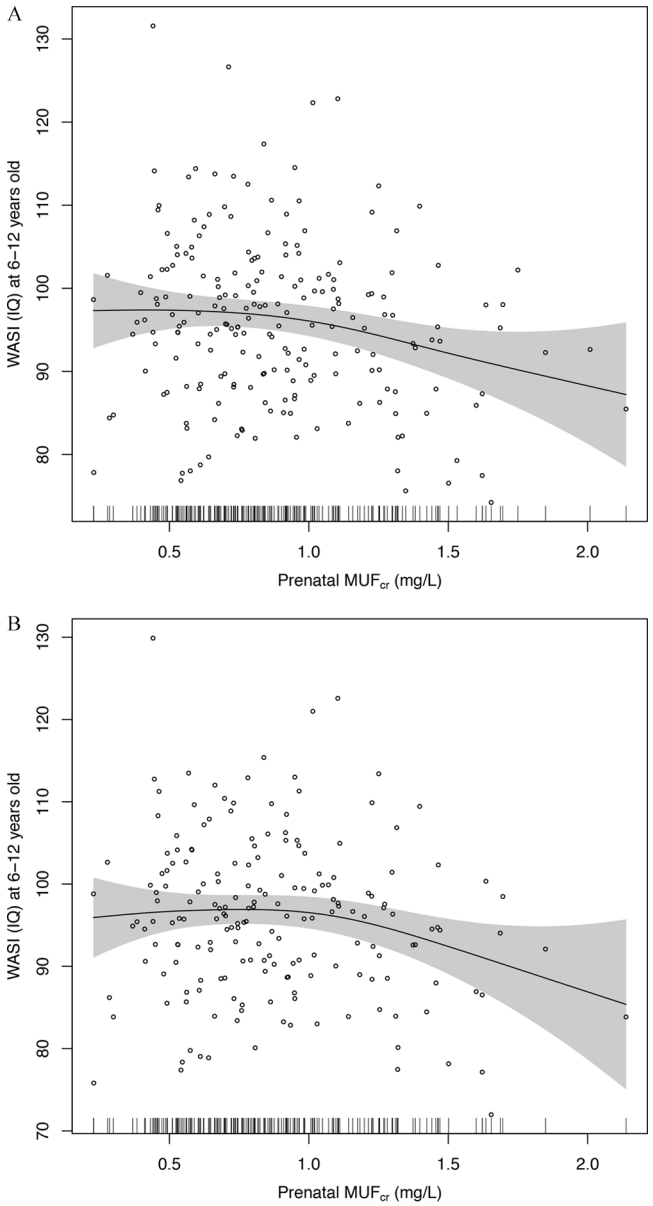

Figure 3. (A) Adjusted association of maternal creatinine-adjusted urinary fluoride (MUF_{cr}) and children's IQ at age 6–12 y. Adjusted for gestational age, weight at birth, sex, parity (being the first child), age at outcome measurement, and maternal characteristics including smoking history (ever smoked vs. nonsmoker), marital status (married vs. others), age at delivery, IQ, education, and cohort (Cohort 3-Ca, Cohort 3-placebo and Cohort 2A). Short vertical bars on the *x*-axis reflect the density of the urinary fluoride measures. Individual data points are individual observation, $n = 211$. (B) Association of maternal creatinine-adjusted urinary fluoride ($MUFU_{cr}$) and children's IQ at age 6–12 y, adjusted for specific gravity–adjusted child urinary fluoride (CUF_{sg}). Adjusted for gestational age, weight at birth, sex, parity (being the first child), age and CUF_{sg} at outcome measurement, and maternal characteristics including smoking history (ever smoked vs. nonsmoker), marital status (married vs. others), age at delivery, IQ, education. and cohort (Cohort 3-Ca, Cohort 3-placebo and Cohort 2A). Shaded area is 95% confidence interval. Short vertical bars on the *x*-axis reflect the density of the urinary fluoride measures. Individual data points are individual observation, $n = 189$.

based on the GAM model (Figure 3B), and a significant improvement in model fit when a quadratic term was added to the linear regression model ($p = 0.01$).

When we restricted models to subsets of children with available data for each additional covariate, there was little difference

between adjusted and unadjusted associations between MUF_{cr} and GCI or IQ when socioeconomic status (family possession), maternal bone lead, and blood mercury, were added to models (Table 4). However, the effect estimates associated with MUF_{cr} for these analyses appear to be higher in the subsets with available data for these variables.

Adding tester (psychologist who performed WASI) in the model did not substantially change the results (data not shown). In the sensitivity analyses in which we excluded Cohort 3 participants who received the calcium supplement, we continued to observe a negative association between MUF_{cr} and GCI [0.5 mg/L increase in MUF_{cr} associated with 3.67 lower GCI (95% CI: −6.57, −0.77), $n = 194$]; and between MUF_{cr} and IQ [0.5 mg/L increase in MUF_{cr} associated with 3.23-lower IQ (95% CI: −5.88, −0.57), $n = 136$].

In sensitivity analyses in which we re-ran models that included the 10 outliers with respect to fluoride exposure (for each of seven participants already in our models, an additional value of MUF_{cr} [from a different trimester]; for three participants, a value of MUF_{cr} that then allowed the participants to be added to our models), the results did not change in any meaningful way (data not shown). There were no outliers with respect to cognitive outcomes.

Independent Influence of Child Fluoride Exposure

Finally, in models that focused on the cross-sectional relationship between children's exposure to fluoride (reflected by their specific gravity–adjusted urinary fluoride levels) and IQ score and that contained the main covariates of interest, there was not a clear, statistically significant association between contemporaneous children's urinary fluoride (CUF_{sg}) and IQ either unadjusted or adjusting for MUF_{cr}. A 0.5 mg/L increase in CUF_{sg} was associated with a 0.89 lower IQ (95% CI: −2.63, 0.85) when not adjusting for MUF_{cr}; and 0.77-lower IQ (95% CI: −2.53, 0.99), adjusting for MUF_{cr} ($n = 189$).

Discussion

In our study population of Mexican women and children, which accounted for two of the three cohorts included in the ELEMENT study, higher prenatal exposure to fluoride (as indicated by average creatinine-adjusted maternal urinary fluoride concentrations during pregnancy) was associated with lower GCI scores in children at approximately 4 y old, and with lower Full-Scale IQ scores at 6–12 y old. Estimates from adjusted linear regression models suggest that mean GCI and IQ scores were about 3 and 2.5 points lower in association with a 0.5 mg/L increase in prenatal exposure, respectively. The associations with GCI appeared to be linear across the range of prenatal exposures, but there was some evidence that associations with IQ may have been limited to exposures above 0.8 mg/L. In general, the negative associations persisted in sensitivity analyses with further adjustment for other potential confounders, though the results of sensitivity analyses were based on subsets of the population with available data.

Overall, our results are somewhat consistent with the ecological studies suggesting children who live in areas with high fluoride exposure (ranging from 0.88 to 11.0 mg/L fluoride in water, when reported) have lower IQ scores than those who live in low-exposure or control areas (ranging from 0.20 to 1.0 mg/L fluoride in water) (Choi et al. 2012) and with results of a pilot study of 51 children (mean age 7 y) from southern Sichuan, China, that reported that children with moderate or severe dental fluorosis (60% of the study population) had lower WISC-IV digit span scores than other children (Choi et al. 2015). A distinction is that our study, which was longitudinal with repeated measures of exposure beginning in the prenatal period, found associations with respect to prenatal fluoride exposures.

To our knowledge, the only other study that is similar to ours was only recently published. Valdez Jiménez et al. (2017) studied the association of prenatal maternal urinary fluoride levels (not corrected for dilution) and scores on the Bayley Scales of Infant Development II among 65 children evaluated at age 3–15 mo (average of 8 mo). The mothers in their study had urinary fluoride levels of which the means at each of the three trimesters of pregnancy (1.9, 2.0, 2.7 mg/L) were higher than the mean MUF_{cr} in our participants (0.88 mg/L) (Valdez Jiménez et al. 2017). These levels of exposure were found to be associated with statistically significantly lower scores on the Bayley Scales' Mental Development Index (MDI) score after adjusting for gestational age, age of child, a marginality index, and type of drinking water (Valdez Jiménez et al. 2017). By comparison, our study had much longer periods of follow-up and larger sample sizes, controlled for a much larger set of covariates and sensitivity variables, and used creatinine–corrected urinary fluoride measures (which, by adjusting for urinary dilution effects, provides a more reliable measure of internal fluoride exposure).

With respect to understanding the generalizability of our findings to other populations, there are very few studies that measured prenatal fluoride levels among women derived from population-based samples. Gedalia et al. (1959) measured urinary fluoride in multiple samples collected from each of 117 healthy pregnant women living in Jerusalem, where fluoride in the water was approximate 0.50 mg/L, and reported mean levels per person that ranged from 0.29 to 0.53 mg/L. However, these analysis were not conducted utilizing modern analytical techniques. In a study of 31 pregnant women living in Poland, Opydo-Szymaczek and Borysewicz-Lewicka (2005) measured urinary fluoride in healthy pregnant women patients of a maternity hospital in Poland, where fluoride in the water ranged from 0.4 to 0.8 mg/L, and found a mean level of 0.65 mg/L for women in their 28th week of pregnancy, 0.84 mg/L in their 33rd week, and 1.30 mg/L in healthy non-pregnant women of similar age. This would suggest that the mothers in our study, who had a mean MUF_{cr} value of 0.90 mg/L, had fluoride exposures slightly higher than prior-mentioned populations.

In terms of comparing our findings with other studies of fluoride (using urinary fluoride as a biomarkers of exposure) and intelligence (i.e., those not involving prenatal exposures), of the 27 epidemiologic studies on fluoride and IQ reviewed by Choi et al. in their 2012 meta-analysis, only 2 had measures of urinary fluoride. Both were of urinary fluoride measures in children (not pregnant mothers), and neither corrected for dilution (either by correcting for urinary creatinine or specific gravity). Of these two, in comparison with the urinary fluoride levels of both our mothers (0.88 mg/L) and our children (0.82 mg/L), the mean levels of children's urinary fluoride were higher in the non-fluorosis (1.02 mg/L) and high-fluorosis (2.69 mg/L) groups found by Li et al. (1995) as well as the control (1.5 mg/L) and high-fluorosis (5.1 mg/L) groups described by Wang et al. (2007).

Among the limitations of our study are that we measured fluoride in spot (second morning void) urine samples instead of 24-hr urine collections. However, others have noted a close relationship between the fluoride concentrations of early morning samples and 24-hr specimens (Watanabe et al. 1994; Zohouri et al. 2006). Another limitation relates to the potential differences in the distribution of covariates over our study cohorts, raising the issue of potential bias. In the analyses we conducted across cohorts, we saw that, in comparison with Cohort 3, Cohort 2A clearly had

higher mean bone lead levels ($p < 0.001$) and possibly higher blood mercury levels ($p = 0.067$). However, we saw no other differences and the differences in these measures have a clear likely explanation: Cohort 2A had bone lead levels measured in 1997–2001 and Cohort 3 had bone lead levels measured in 2001–2005. Given that environmental lead and mercury exposures were steadily decreasing during this time interval (due to the phase-out of lead from gasoline), this difference likely relates to an exposure–time–cohort effect. We do not anticipate that this phenomenon would have introduced a bias in our analyses of fluoride and cognition controlling for bone lead.

Another limitation relates to the missing data that pertain to our covariate and sensitivity variables. In the comparisons of participants in relation to missing data (Table 2A,B), the proportion of females was somewhat higher in the included versus excluded group for both the GCI and IQ analyses, and the mean levels of maternal blood Hg for those included were 28.5% and 24.9% higher than the mean levels for those excluded in the GCI and IQ analyses, respectively. We also note that the coefficients for the associations between fluoride on cognition varied substantially in some of the sensitivity analyses, particularly with respect to the subgroups of participants who have data on SES, lead exposure, and mercury exposure (of which, for the latter, the effect estimates almost doubled). We do not have a ready explanation for this phenomenon, given that there is no obvious way that each of the selection factors governing which mothers had these measurements (discussed above) could have influenced the fluoride–cognition relationship. Nevertheless, it is not possible to entirely rule out residual confounding or in the population as a whole (that might have been detected had we had full data on larger sample sizes) or bias (should the subpopulations that had the data for analysis have a different fluoride–cognition relationship than those participants who were excluded from the analyses).

Other limitations include the lack of information about iodine in salt, which could modify associations between fluoride and cognition; the lack of data on fluoride content in water given that determination of fluoride content is not reported as part of the water quality monitoring programs in Mexico; and the lack of information on other environmental neurotoxicants such as arsenic. We are not aware of evidence suggesting our populations are exposed to significant levels of arsenic or other known neurotoxicants; nevertheless, we cannot rule out the potential for uncontrolled confounding due to other factors, including diet, that may affect urinary fluoride excretion and that may be related to cognition.

Another potential limitation is that we adjusted maternal urinary fluoride levels based on urinary creatinine, whereas we adjusted children's urinary fluoride levels based on urinary specific gravity; however, these two methods are almost equivalent in their ability to account for urinary dilution. We also had no data to assess the inter-examiner reliability of the testers administering the WASI test; however, the excellent reliability of these same testers in administering the McCarthy tests provides some reassurance that the WASI tests were conducted in a consistent manner.

Finally, our ability to extrapolate our results to how exposures may impact on the general population is limited given the lack of data on fluoride pharmacokinetics during pregnancy. There are no reference values for urinary fluoride in pregnant women in the United States. The Centers for Disease Control and Prevention has not included fluoride as one of the population exposures measured in urine or blood samples in its nationally representative sampling. The WHO suggests a reference value of 1 mg/L for healthy adults when monitoring renal fluoride excretion in community preventive programs (Marthaler 1999). As part of the NRC's review of the fluoride drinking-water standard, it was noted that healthy adults exposed to optimally fluoridated water had urinary fluoride concentrations ranging from 0.62 to 1.5 mg/L.

Conclusion

In this study, higher levels of maternal urinary fluoride during pregnancy (a proxy for prenatal fluoride exposure) that are in the range of levels of exposure in other general population samples of pregnant women as well as nonpregnant adults were associated with lower scores on tests of cognitive function in the offspring at 4 and 6–12 y old.

Community water and salt fluoridation, and fluoride toothpaste use, substantially reduces the prevalence and incidence of dental caries (Jones et al. 2005) and is acknowledged as a public health success story (Easley 1995). Our findings must be confirmed in other study populations, and additional research is needed to determine how the urine fluoride concentrations measured in our study population are related to fluoride exposures resulting from both intentional supplementation and environmental contamination. However, our findings, combined with evidence from existing animal and human studies, reinforce the need for additional research on potential adverse effects of fluoride, particularly in pregnant women and children, and to ensure that the benefits of population-level fluoride supplementation outweigh any potential risks.

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Supplemental Material

Prenatal Fluoride Exposure and Cognitive Outcomes in Children at 4 and 6-12 Years of Age in Mexico

Morteza Bashash, Deena Thomas, Howard Hu, E. Angeles Martinez-Mier, Brisa N. Sanchez, Niladri Basu, Karen E. Peterson, Adrienne S. Ettinger, Robert Wright, Zhenzhen Zhang, Yun Liu, Lourdes Schnaas, Adriana Mercado-García, Martha María Téllez-Rojo, Mauricio Hernández-Avila

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Table S1. Characteristics of children and their mothers within cohorts for GCI and IQ outcome

		GCI			IQ		
CHILDREN		**N**	**Mean ± SD**	**p**	**N**	**Mean ± SD**	**p**
Sex (Female)	**Cohort 3- Placebo**	**84**	**59.50%**	**0.66**	**58**	**58.60%**	**0.75**
	Cohort 3- Ca	**93**	**52.70%**		**75**	**52.00%**	
	Cohort 2A	**110**	**55.50%**		**78**	**55.10%**	
Parity (first child)	**Cohort 3- Placebo**	**84**	**32.10%**	**0.94**	**58**	**29.30%**	**0.67**
	Cohort 3- Ca	**93**	**33.30%**		**75**		
	Cohort 2A	**110**	**34.50%**		**78**	**35.90%**	
Birthweight (Kilograms)	**Cohort 3- Placebo**	**84**	**3.13 ± 0.39**	**0.02**	**58**	**3.12 ± 0.39**	**0.07**
	Cohort 3- Ca	**93**	**3.19 ± 0.42**		**75**	**3.2 ± 0.46**	
	Cohort 2A	**110**	**3.02 ± 0.49**		**78**	**3.03 ± 0.5**	
Gestational age at birth (weeks)	**Cohort 3- Placebo**	**84**	**38.87 ± 1.76**	**0.4**	**58**	**38.47 ± 1.94**	**0.81**
	Cohort 3- Ca	**93**	**38.63 ± 1.22**		**75**	**38.67 ± 1.31**	
	Cohort 2A	**110**	**38.51 ± 2.29**		**78**	**38.54 ± 2.09**	
Age at measurement (years)	**Cohort 3- Placebo**	**84**	**4.04 ± 0.05**	**<0.01**	**58**	**7.6 ± 0.47**	**<0.01**
	Cohort 3- Ca	**93**	**4.05 ± 0.05**		**75**	**7.6 ± 0.51**	
	Cohort 2A	**110**	**4.02 ± 0.04**		**78**	**10.04 ± 0.68**	
MOTHERS		**N**	**Mean ± SD**	**p**	**N**	**Mean ± SD**	**p**
Age at delivery (years)	**Cohort 3- Placebo**	**84**	**26.74 ± 5.05**	**0.76**	**58**	**26.67 ± 5.2**	**0.28**
	Cohort 3- Ca	**93**	**27.12 ± 6.07**		**75**	**27.99 ± 6.19**	
	Cohort 2A	**110**	**26.54 ± 5.44**		**78**	**26.73 ± 5.28**	
Education (years)	**Cohort 3- Placebo**	**84**	**10.45 ± 2.63**	**0.73**	**58**	**10.55 ± 2.69**	**0.69**
	Cohort 3- Ca	**93**	**10.78 ± 2.69**		**75**	**10.99 ± 2.99**	
	Cohort 2A	**110**	**10.63 ± 2.93**		**78**	**10.81 ± 2.85**	
IQ	**Cohort 3- Placebo**	**84**	**87.39 ± 12.63**	**0.55**	**58**	**88.97 ± 12.77**	**0.96**
	Cohort 3- Ca	**93**	**89.15 ± 11.4**		**75**	**89.32 ± 12.48**	
	Cohort 2A	**110**	**89.13 ± 12.49**		**78**	**88.76 ± 12.33**	
Marital Status (married)	**Cohort 3- Placebo**	**84**	**70.20%**	**0.95**	**58**	**74.10%**	**0.74**
	Cohort 3- Ca	**93**	**71.00%**		**75**	**70.70%**	
	Cohort 2A	**110**	**69.10%**		**78**	**67.90%**	
Smoking (ever smoked)	**Cohort 3- Placebo**	**84**	**47.60%**	**0.39**	**58**	**53.40%**	**0.55**
	Cohort 3- Ca	**93**	**54.80%**		**75**	**46.70%**	
	Cohort 2A	**110**	**45.50%**		**78**	**55.10%**	

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Prenatal fluoride exposure and attention deficit hyperactivity disorder (ADHD) symptoms in children at 6–12 years of age in Mexico City

Morteza Bashash[a,*], Maelle Marchand[a], Howard Hu[a,1], Christine Till[b], E. Angeles Martinez-Mier[c], Brisa N. Sanchez[d], Niladri Basu[e], Karen E. Peterson[d,f,g], Rivka Green[b], Lourdes Schnaas[h], Adriana Mercado-García[i], Mauricio Hernández-Avila[i], Martha María Téllez-Rojo[i]

[a] Dalla Lana School of Public Health, University of Toronto, Toronto, ON, Canada
[b] Faculty of Health - Department of Psychology, York University, ON, Canada
[c] Indiana University School of Dentistry, Indianapolis, IN, United States of America
[d] University of Michigan School of Public Health, Ann Arbor, MI, United States of America
[e] Faculty of Agricultural and Environmental Sciences, McGill University, Montreal, QC, Canada
[f] Center for Human Growth and Development, University of Michigan, Ann Arbor, MI, United States of America
[g] Harvard W.T. Chan School of Public Health, Boston, MA, United States of America
[h] Instituto Nacional de Perinatología, Mexico City, Mexico
[i] Instituto Nacional de Salud Pública, Cuernavaca, Morelos, Mexico

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ABSTRACT

Background: Epidemiologic and animal-based studies have raised concern over the potential impact of fluoride exposure on neurobehavioral development as manifested by lower IQ and deficits in attention. To date, no prospective epidemiologic studies have examined the effects of prenatal fluoride exposure on behavioral outcomes using fluoride biomarkers and sensitive measures of attention.

Objective: We aimed to examine the association between prenatal fluoride exposure and symptoms associated with attention-deficit/hyperactivity disorder (ADHD).

Method: 213 Mexican mother-children pairs of the Early Life Exposures to Environmental Toxicants (ELEMENT) birth cohort study had available maternal urinary samples during pregnancy and child assessments of ADHD-like behaviors at age 6–12. We measured urinary fluoride levels adjusted for creatinine (MUF_{cr}) in spot urine samples collected during pregnancy. The Conners' Rating Scales-Revised (CRS-R) was completed by mothers, and the Conners' Continuous Performance Test (CPT-II) was administered to the children.

Results: Mean MUF_{cr} was 0.85 mg/L (SD = 0.33) and the Interquartile Range (IQR) was 0.46 mg/L. In multivariable adjusted models using gamma regression, a 0.5 mg/L higher MUF_{cr} (approximately one IQR higher) corresponded with significantly higher scores on the CRS-R for DSM-IV Inattention (2.84 points, 95% CI: 0.84, 4.84) and DSM-IV ADHD Total Index (2.38 points, 95% CI: 0.42, 4.34), as well as the following symptom scales: Cognitive Problems and Inattention (2.54 points, 95% CI: 0.44, 4.63) and ADHD Index (2.47 points; 95% CI: 0.43, 4.50). The shape of the associations suggested a possible celling effect of the exposure. No significant associations were found with outcomes on the CPT-II or on symptom scales assessing hyperactivity.

Conclusion: Higher levels of fluoride exposure during pregnancy were associated with global measures of ADHD and more symptoms of inattention as measured by the CRS-R in the offspring.

Abbreviations: ADHD, attention-deficit hyperactivity disorder; cm^3, cubic centimeters; CNS, Central Nervous System; CPT-II, Conners' Continuous Performance Test – Second Edition; CRS-R, Conners' Rating Scale – Revised; CUF_{sg}, specific gravity adjusted child urinary fluoride; DSM-IV, Diagnostic and Statistical Manual of Mental Disorders – Fourth Edition; ELEMENT, Early Life Exposures in Mexico to Environmental Toxicants; EPA, U.S. Environmental Protection Agency; HOME, Home Observation for Measurement of the Environment; L, liter; mg, milligram; MUF_{cr}, creatinine adjusted maternal urinary fluoride; SD, Standard Deviation; SE, Standard Error

* Corresponding author at: Dalla Lana School of Public Health, 6th floor, 155 College Street, Toronto, ON M5R3M7, Canada.
E-mail addresses: m.bashash@utoronto.ca (M. Bashash), howard.hu@utoronto.ca (H. Hu).
[1] Reprint requests: Dalla Lana School of Public Health, 6th floor, 155 College Street, Toronto, ON M5R3M7, Canada.

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1. Introduction

Fluoride, the ionized form of the halogen element fluorine, exists widely in the environment and is the most electronegative and reactive among all elements (ATSDR, 2010). Its well-known cariostatic effect led to the addition of fluoride to water, salt, and milk in some countries. Other sources of fluoride include dental products, such as toothpastes, mouth rinses, and varnishes, supplements, processed foods made with fluoridated water, fluoride-containing pesticides, teas, and fluorinated pharmaceuticals. Systemic ingestion of fluoride through water and water-based beverages is the main source of fluoride intake, accounting for approximately 75% of dietary fluoride intake among adults living in communities that fluoridate their water supply in the United States (U.S. Environmental Protection Agency, 2010; USDA (U.S. Department of Agriculture), 2005). However, in Mexico City, individuals are primarily exposed to fluoride through fluoridated salt (mean concentration of fluoride in salt is 250 ± 50 ppm), and to varying degrees of naturally-occurring fluoride in water, which have been reported to range from 0.15 to 1.38 mg/L (Juárez-López et al., 2007; Martinez-Mier et al., 2005). Public water supplies are not fluoridated in Mexico and the mean fluoride content of the water supply is not publicly available.

Long-term exposure to fluoride is regarded by the World Health Organization as being beneficial, including both prevention of dental caries and treating osteoporosis, though excess intake can also cause potential health hazards, including dental and skeletal fluorosis. Fluoride is also shown to readily cross the placenta (Shen and Taves, 1974) and accumulate in fetal brain tissues (Narayanaswamy and Piler, 2009), thereby inducing toxicity (Dong et al., 1993; Jiang et al., 2014). Several animal (Chen et al., 2003; Mcpherson et al., 2018; Mullenix et al., 1995) and human studies (Bashash et al., 2017; Choi et al., 2012) have explored associations between early-life exposure to fluoride and decrements in cognitive function and attention-related behaviors. An ecologic study reported an association between state level prevalence of community water fluoridation and prevalence of ADHD among youth living in the United States (Malin and Till, 2015). Given the increased vulnerability of the developing fetus to environmental exposures (Lanphear, 2015), as well as the widespread distribution of fluoride in society, the potential impact of prenatal exposure to fluoride warrants further study.

Attention-deficit hyperactivity disorder (ADHD) is the most common neurodevelopmental disorder in school-aged children and adolescents, with a worldwide prevalence estimated at about 5% (Polanczyk et al., 2007). Symptoms of ADHD include difficulties with attention, impulsivity, and/or hyperactivity at a level that is severe enough to be associated with impairments in academic and social functioning (American Psychiatric et al., 2013). Although genetics have been shown to play an important role in an individual's susceptibility to ADHD, with estimates of heritability from twin studies in the range of 60–70% (Posthuma and Polderman, 2013), several environmental factors have also been implicated. Environmental risk factors for ADHD include prenatal tobacco and alcohol exposure, heavy metal and chemical exposures, including lead (Huang et al., 2016), mercury, organochlorines, air pollution (Fuertes et al., 2016; Perera et al., 2018; Sentís et al., 2017) and nutritional factors (Polańska et al., 2012).

The purpose of the current study was to prospectively assess the relationship between prenatal exposure to fluoride and parent-reported behaviors associated with ADHD among 6–12 year old children born to mothers living in Mexico City. We tested whether fluoride exposure associated with inattentive and/or hyperactive behaviors.

2. Methods

2.1. Study population

Participants included a subset of mother-child dyads enrolled in various longitudinal birth cohort studies of the Early Life Exposure in Mexico to Environmental Toxicants (ELEMENT) project (Aafeiche et al., 2011; Bashash et al., 2017). We included mother-child pairs from two of the four ELEMENT cohorts (cohorts 2A and 3) for which maternal urinary samples during trimesters of pregnancy were available. Participants in cohort 2A (Bashash et al., 2017) were recruited between 1997 and 1999 whereas participants in cohort 3 were recruited between 2001 and 2003. We included participants if they had at least one archived urine sample from pregnancy, were ≤14 weeks of gestation at the time of recruitment, and their children underwent behavioral testing between the ages of 6 and 12, as described elsewhere (Bashash et al., 2017). Participants were excluded if they reported a history of psychiatric disorder(s), if there were medical complications (i.e. high-risk pregnancy, gestational diabetes, pre-eclampsia, renal disease, circulatory diseases, hypertension, continuous use of prescription drugs, or seizures during the index pregnancy), or if there was known maternal alcohol or illegal drug use during pregnancy. The study procedures were approved by the Institutional Review Boards of the National Institute of Public Health of Mexico, University of Michigan, Indiana University, University of Toronto, and Harvard School of Public Health, as well as participating clinics. Written informed consent was obtained from all participating families prior to study evaluation.

2.2. Fluoride measurements

Concentration of fluoride measured in maternal urinary samples was used as biomarker of prenatal fluoride exposure. Urine has been described as a suitable biomarker for fluoride since it serves as the main pathway through which fluoride is eliminated from the body and excretion is proportional to the total fluoride intake, but modified by factors like diet and various systemic conditions, such as recent fluoride exposure and urinary pH, as well as variation in creatinine excretion by muscle mass, age, sex, and other factors (Barr et al., 2005; Aylward et al., 2015). Ideally, overnight fasting or 24-hour urine samples are considered to be the optimal dosimeter for measuring chronic fluoride exposure in order to limit diurnal variations and the influence of diet associated with spot samples (Petersen et al., 2014). Because 24-hour urinary samples were not available in our sample, we used spot samples that were corrected for urinary dilution using urinary creatinine, as described elsewhere (Petersen et al., 2014). Each woman in the current sample provided at least one spot (second morning void) urine sample (Thomas et al., 2016) during pregnancy (range: 10 to 38 weeks). We then calculated the average of all available creatinine-adjusted maternal urinary fluoride (MUF) concentrations (Bashash et al., 2017). Further information regarding participant recruitment, data collection methods, as well as methods for fluoride sample shipping, storage, and analysis can be found elsewhere (Bashash et al., 2017).

2.3. Attention outcomes

Behaviors associated with ADHD were assessed using the Spanish version of the Conners' Rating Scales-Revised (CRS-R) (Conners, 1997), which has been validated for the evaluation of ADHD (Ortiz-Luna and Acle-Tomasini, 2006). The CRS-R contains three ADHD scales that correspond with the Diagnostic and Statistical Manual of Mental Disorders – 4th edition (DSM-IV) criteria for ADHD: 1) DSM-IV Inattention Index, 2) DSM-IV Hyperactive-Impulsive Index, and 3) DSM-IV Total Index (inattentive and hyperactive-impulsive behaviors combined). It also examines seven types of behavior problems that were derived through factor analysis, including: Oppositional, Anxious-Shy, Cognitive Problem/Inattention, Hyperactivity, Perfectionism, Psychosomatic, and Social Problems. In addition, the CRS-R contains four index scores that were derived based on theory and prior research: Conners' ADHD Index; Conners' Global Index (CGI): Restless-Impulsive; CGI: Emotional Lability, and CGI. For the purpose of the current study, we examined the three DSM-IV ADHD scales as our primary outcomes because these scales are intended to screen for ADHD, and are commonly used to

study the association between diverse environmental contaminants and ADHD-behavior problems (Huang et al., 2016; Perera et al., 2018). We also examined outcomes from two behavior scales (Cognitive Problem/Inattention and Hyperactivity) and two index scores (Conners' ADHD Index and CGI: Restless-Impulsive), as done in our prior work with lead (Huang et al., 2016). The Conners' ADHD Index, in particular, has been shown to exhibit favorable specificity and sensitivity in ADHD assessment (Chang et al., 2016). In addition, we assessed sustained attention and inhibitory control using the Conners' Continuous Performance Test (CPT-II, 2nd Edition), a computer-administered signal detection paradigm (Conners, 2000). Using the CPT-II, we measured errors of omission and commission, and hit reaction time (response latency). Mothers completed the CRS-R at the same follow-up visits that the child completed the CPT-II. All measures were standardized for age- and sex. Higher T-scores (mean of 50, SD of 10) indicate poorer performance. All psychometric tests were applied under the supervision of an experienced psychologist (LS).

2.4. Measurement of covariates

Covariate data were individually obtained throughout the duration of the study. During the first pregnancy visit, questionnaires were used to collect information concerning maternal age, maternal education, history of smoking, and marital status. At delivery, information regarding birth weight, child sex, birth order, and gestational age (calculated by nurses) was obtained. Mothers also responded to a socioeconomic status questionnaire (Bashash et al., 2017) during the visit when the psychometric tests were administered. Individual items on this questionnaire assess the ability of households to meet the needs of its members in terms of housing, health, energy, technology, prevention and intellectual development; an overall score was derived by summing across each item. The Home Observation for Measurement of the Environment (HOME) Inventory, a semi-structured interview that measures quality and quantity of the caregiving environment, was administered in a subset of participants at approximately the same time as the visits for the neurobehavioral tests.

2.5. Data analysis

Univariate statistics, appropriate transformations, and graphical displays were obtained for all variables before bivariate analyses. Bivariate analysis of the data included Chi-square tests for categorical variables and analysis of variance (ANOVA) to compare the continuous outcomes or exposure within groups defined according to the distribution of each covariate. In initial fully adjusted linear regression models, the outcomes demonstrated highly skewed residuals (with the exception of CPT-II commission score and hit reaction time). Thus, to address the skewness of the residuals, gamma regression was used to examine the adjusted association between prenatal fluoride and each neurobehavioral outcome instead of log transformation, which may obscure model interpretation. In the gamma regression, we selected an identity link, so that the interpretation for the regression coefficients is the same as the linear regression (i.e., absolute difference in the mean of the outcome per unit change in predictor). All statistical analyses were performed in SAS software version 9.4. Covariates were selected a priori based on their theoretical relevance or observed associations with fluoride exposure, and/or the analyzed neurobehavioral outcomes. As such, models were adjusted for the following maternal characteristics: age at delivery (continuous, in years), years of education (continuous, in years), marital status (married vs. others), and smoking history (ever-smoker vs. non-smoker). Models were further adjusted for the following child characteristics: gestational age at birth (continuous, in weeks), age at neurobehavioral measurement (continuous, in years), sex (female vs. male), and birth order (first born vs. others), and socioeconomic status through a continuous measure based on reported possessions and household assets (Thomas et al., 2016). Lastly, models adjusted for potential cohort and Ca intervention effects through inclusion of a variable denoting from which study the participants originated (cohort 2 - A, an observational cohort), cohort 3 subjects who were randomized to the calcium supplement (cohort 3 - Ca^{+}), and cohort 3 subjects who were randomized to the placebo (cohort 3 - Placebo) (Bashash et al., 2017).

Other potential confounders that were examined in sensitivity analyses involving subsets of participants included the home environment (i.e. HOME score) (Thomas et al., 2016) assessed at the time of outcome measurement, child contemporaneous fluoride exposure measured by child urinary fluoride adjusted for specific gravity (CUF_{sg}) (Bashash et al., 2017), as well as maternal blood mercury and maternal bone lead levels (a proxy for prenatal lead exposure to the fetus) given that both are established neurodevelopmental toxicants (Bashash et al., 2017). This was done through identifying and including the subset of cases with data on each respective variable. In each subset, results were then compared between the model adjusting for that variable and the model not adjusting for that variable.

Model diagnostics were used to assess for violations of the model assumptions and for identification of remaining influential observations. Cook's D identified three exposure observations as potentially influential to the model results, and models were run with and without these observations (Supplementary Fig. S1). Generalized additive models (GAMs), estimated via cross validation in the R software, were used to visualize the adjusted association between fluoride exposure and measures of attention to examine potential non-linearity. Non-linearity of the fluoride-outcome association was tested through the inclusion of a quadratic term in the model, and found to be significant in four out of ten of the models (see Fig. 2 and Supplementary Fig. 1). We applied the Benjamini–Hochberg false discovery rate (FDR) procedure to address multiple testing corrections, using a false discovery rate of $Q = 0.05$ and $m = 10$ tests to determine significance (Benjamini and Hochberg, 1995).

3. Results

3.1. Population characteristics

Overall, 231 mothers with a minimum of one MUF_{cr} and a matching outcome (CRS-R or CPT-II) were identified for this project. However, complete demographic and outcome information were missing among 17 mother-child pairs, leaving 214 participants for our analyses, of whom 210 mother-child pairs had data for the CRS-R and CPT-II analyses (206 had data for both) (Fig. 1). Demographic information on participants including data on exposure, outcomes, and key covariates by cohort is shown in Table 1. Of the mothers included in our analysis, 154 (72%) were married at the time of recruitment, and 103 (48%) had reported a history of ever smoking. Among the children, 147 (69%) were not of firstborn status and 116 (54%) were female. Besides for younger age of participants in cohorts 3 relative to cohort 2-, there were no significant differences of maternal and children characteristics across cohorts.

3.2. Exposure and outcome assessment

Of the 214 participants included in the study, 175 participants had measurements of MUF_{cr} from trimester 1, 80 from trimester 2, and 62 from trimester 3; 14 (6.5%) participants had all three measures, 78 (36.4%) had two measures, and 122 (57.0%) had one. The overall mean level of MUF_{cr} averaged across all the trimesters was 0.85 mg/L, with an Interquartile Range (IQR) of 0.46 mg/L. There was no significant difference of MUF_{cr} across cohorts (Table 1A).

Overall, Mean $\pm$ SD scores across all of the CRS-R scales fell within the average range (i.e. mean $T = 50 \pm 10$) (Table 1B). Around 10% of participants fell in the clinically significant range (i.e. T-score ≥ 70 on the Index scores); specifically, clinically elevated scores were found

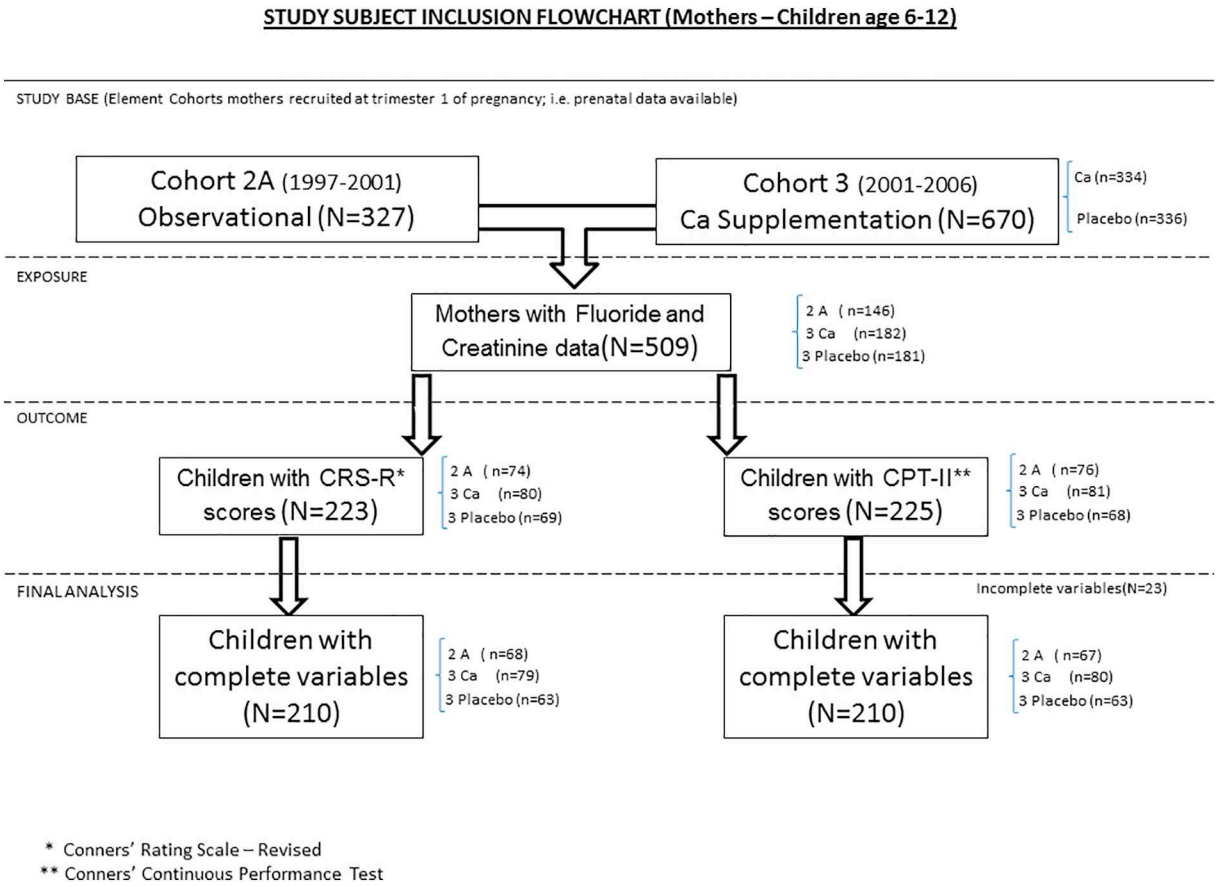

Fig. 1. Flowchart describing source of mother–offspring subject pairs, fluoride and cognition study. Cohort 2A was designed as an observational birth cohort from 1997 to 2001. Cohort 3 was designed as a randomized double-blind placebo-controlled trial of calcium supplements, with mothers recruited early during pregnancy from 2001 to 2006. "Ca" denotes subjects who were randomized to the calcium supplement; "placebo" denotes subjects who were randomized to the placebo. CRS-R denotes Conners' Rating Scale – Revised and CPTII is Conners' Continuous Performance Test.

among 22 (10.5%) children on the DSM-IV Hyperactivity-Impulsivity scale, 18 (8.6%) children on the DSM-IV ADHD Total scale, and 17 (8.1%) children on the DSM-IV Inattention scale.

Mean ± SD for CPT-II scores for Omission Errors (55.53 ± 13.67), Commission Errors (49.93 ± 9.22), and Hit Reaction Time (52.51 ± 10.53) also fell within the average range (Table 1B).

3.3. Models of neurobehavioral outcomes

MUF_{cr} was significantly associated with measures of the CRS-R in the adjusted model (Table 2). Higher concentrations of MUF_{cr} were associated with a higher likelihood of parent-endorsed symptoms on the following scales: DSM-IV Inattention, DSM-IV Total ADHD, Cognitive Problem/Inattention, and the ADHD Index. On average, a 0.5 mg/L higher MUF_{cr} corresponded with a 2.84 ($p < 0.01$) higher score on the DSM-IV Inattention Index; 2.38 ($p = 0.02$) higher score on the DSM-IV Total ADHD Index; a 2.54 ($p = 0.02$) higher score on the Cognitive Problem/Inattention Index; and 2.47 ($p = 0.02$) higher score on the ADHD index. The associations between MUF_{cr} and CRS-R scales remained significant after correction for the multiple testing. As shown in Fig. 2, a subset of the CRS-R outcomes exhibit a non-linear association with the relationship between MUF_{cr} and behavioral outcomes increasing at first, followed by a plateau. There were no significant associations between MUF_{cr} and any of the outcomes on the CPT-II (Table 2; and Supplementary Fig. S2).

3.4. Sensitivity analyses

Sensitivity analyses on adjusted models for the study population subset that also had HOME scores, CUF_{sg} (there was no correlation between MUF_{cr} and CUF_{sg}), maternal lead and mercury exposures did not appreciably change the results for the CRS-R scores (Supplementary Table 1). There was no significant interaction between sex and MUF_{cr} when we added an interaction term to the models.

4. Discussion

The current study aimed to characterize the longitudinal association between prenatal fluoride exposure and symptoms of ADHD in offspring as measured by parent-ratings on the CRS-R and a computerized test of sustained attention and inhibitory control (CPT-II). In our cohort, higher prenatal fluoride exposure, as measured by MUF_{cr}, corresponded to more ADHD-like symptoms on the CRS-R, particularly related to inattention as indicated by the strong association with the following two scales: Cognitive Problem/Inattention, and DSM-IV Inattention. In contrast, MUF_{cr} during pregnancy did not predict child performance on any of the hyperactivity measures (i.e. Restless-Impulsive; Hyperactivity; DSM-IV Hyperactivity-Impulsivity) nor the CPT-II outcomes.

In general, a 0.5 mg/L higher MUF_{cr} (approximately the IQR) corresponded to higher scores on the CRS-R for DSM-IV Inattention (2.84 points) and Cognitive Problems and Inattention (2.54 points). Consistency in these results across both of these outcome measures strengthens the conclusion that inattention appears to be associated with prenatal exposure to fluoride. These two scales contribute to the global ADHD Index and the DSM-IV Total scores, which were also associated with higher levels of prenatal fluoride exposure; a 0.5 mg/L increase in MUF_{cr} corresponded to a 2.38 higher point score on the DSM-IV ADHD Total Index and a 2.47 higher point score on the ADHD

Table 1
Cohort characteristics including key covariates, urinary fluoride levels, and outcome measurements. Statistical differences across the cohorts are reported upon in the final column.

	Cohort *	N	Mean (95% CI) %	p
A) PARTICIPANTS				
Child Sex (Girl)	2 A	37	52.90%	.831
	3 Ca	43	53.10%	
	3 Placebo	36	57.10%	
	Total	116	54.00%	
First Child	2 A	24	34.30%	.839
	3 Ca	25	30.90%	
	3 Placebo	18	28.60%	
	Total	67	31.00%	
Birth Weight (Kg)	2 A	70	3.05 (2.94, 3.16)	.103
	3 Ca	81	3.20 (3.10, 3.30)	
	3 Placebo	63	3.11 (3.01, 3.21)	
	Total	214	3.13 (3.07, 3.18)	
Gestational Age (Weeks)	2 A	70	38.60 (38.21, 38.99)	.647
	3 Ca	81	38.74 (38.46, 39.02)	
	3 Placebo	63	38.49 (38.01, 38.97)	
	Total	214	38.62 (38.41, 38.84)	
Age At Outcome Assessment (Year)	2 A	70	10.04 (9.89, 10.21)	*< .01*
	3 Ca	81	7.63 (7.52, 7.75)	
	3 Placebo	63	7.57 (7.44, 7.69)	
	Total	214	8.40 (8.23, 8.57)	
Marital Status (Married)	2 A	46	65.70%	.428
	3 Ca	60	74.10%	
	3 Placebo	48	76.20%	
	Total	154	72.00%	
Maternal Smoking (Ever Smoked)	2 A	32	46.40%	.726
	3 Ca	42	51.90%	
	3 Placebo	29	45.70%	
	Total	103	48.10%	
Maternal Education (Years)	2 A	70	10.73 (10.08, 11.37)	.580
	3 Ca	81	11.11 (10.47, 11.75)	
	3 Placebo	63	10.68 (10.03, 11.34)	
	Total	214	10.86 (10.49, 11.23)	
SES[1]	2 A	70	6.69 (6.10, 7.27)	.604
	3 Ca	81	6.32 (5.82, 6.82)	
	3 Placebo	63	6.33 (5.64, 7.02)	
	Total	214	6.44 (6.11, 6.78)	
B) EXPOSURE AND OUTCOMES				
MUF_{cr} [2]	2 A	70	0.87 (0.80, 0.95)	0.889
	3 Ca	81	0.85 (0.78, 0.92)	
	3 Placebo	63	0.85 (0.76, 0.95)	
	Total	214	0.85 (0.81, 0.90)	
CRS-R [3]				
Cognitive Problems + Inattention	2 A	68	55.16 (52.45, 57.87)	.884
	3 Ca	79	54.32 (51.85, 56.78)	
	3 Placebo	63	54.43 (51.78, 57.08)	
	Total	210	54.62 (53.14, 56.10)	
Restless-Impulsive	2 A	68	55.43 (52.56,58.30)	.490
	3 Ca	79	55.19 (52.32, 58.07)	
	3 Placebo	63	54.28 (52.11, 56.45)	
	Total	210	54.35 (52.94, 55.75)	
Hyperactivity	2 A	68	56.67 (53.62, 56.35)	.217
	3 Ca	79	54.52 (52.42, 56.62)	
	3 Placebo	63	53.70 (51.74, 55.66)	
	Total	210	54.97 (53.59, 56.35)	
ADHD [4] **Index**	2 A	68	54.53 (51.77, 56.29)	.865
	3 Ca	79	54.56 (52.29, 56.82)	
	3 Placebo	63	53.70 (51.25, 56.14)	
	Total	210	54.30 (52.88, 55.71)	
DSM-IV [5] **Inattention**	2 A	68	54.42 (51.60, 57.23)	.825
	3 Ca	79	53.92 (51.65, 56.22)	
	3 Placebo	63	53.29 (50.98, 55.60)	
	Total	210	53.89 (52.48, 55.30)	
DSM-IV Hyperactivity-Impulsivity	2 A	68	57.54 (54.77, 60.32)	.685
	3 Ca	79	56.80 (54.59, 59.01)	
	3 Placebo	63	56.00 (53.76, 58.24)	
	Total	210	56.80 (55.42, 58.18)	
DSM-IV ADHD Total	2 A	68	56.35 (53.72, 58.99)	.670
	3 Ca	79	55.63 (53.46, 57.81)	
	3 Placebo	63	54.79 (52.47, 57.12)	
	Total	210	55.61 (54.26, 56.97)	
CPT-II [6]				

(*continued on next page*)

Table 1 (*continued*)

	Cohort *	N	Mean (95% CI) %	p
Omission Errors	2 A	67	51.76 (48.49, 55.03)	*.024*
	3 Ca	80	57.56 (54.25, 60.87)	
	3 Placebo	63	56.80 (53.89, 59.70)	
	Total	210	55. 48 (53.62, 57.34)	
Commission Errors	2 A	67	46.99 (44.43, 49.55)	*.007*
	3 Ca	80	51.18 (49.17, 53.20)	
	3 Placebo	63	51.37 (49.59, 53.16)	
	Total	210	49.90 (48.65, 51.15)	
Hit Reaction Time	2 A	67	49.43 (47.00, 51.86)	*.016*
	3 Ca	80	53.77 (51.35, 56.18)	
	3 Placebo	63	54.05 (51.49, 56.60)	
	Total	210	52.46 (51.03, 53.90)	

*Cohort: 2 A; ELEMENT Cohort 2A, an observational birth cohort from 1997 to 2001. 3 Ca and 3 Placebo, ELEMENT Cohort 3, a randomized double-blind placebo-controlled trial of calcium supplements from 2001 to 2006. "Ca" denotes subjects who were randomized to the calcium supplement; "placebo" denotes subjects who were randomized to the placebo. 1) SES, socioeconomic status; 2) MUFcr, Creatinine adjusted maternal urinary fluoride; 3) CRS-R, Conners' Rating Scale – Revised; CPT-II; 4) ADHD, Attention Deficit Hyperactivity Disorder; 5) DSM-IV, Diagnostic and Statistical Manual of Mental Disorders – Fourth Edition; 6) CPT-II, Conners' Continuous Performance Test – Second Edition.

Table 2
Multivariate[a] gamma regression models of differences across CRS-R and CPT-II scores per 0.5 mg/L higher maternal creatinine-adjusted urinary fluoride (MUF_{cr}).

	β	95% CI		p
CRS-R scores (N = 210)				
Cognitive Problems + Inattention	2.54	0.44	4.63	*.0178*
Restless-Impulsive	1.92	−0.07	3.91	0.0586
Hyperactivity	1.05	−0.91	3.00	0.2953
ADHD Index	2.47	0.43	4.50	*0.0175*
DSM-IV Inattention	2.84	0.84	4.84	*0.0054*
DSM-IV Hyperactivity-Impulsivity	1.69	−0.33	3.70	0.1016
DSM-IV ADHD Total	2.38	0.42	4.34	*0.0176*
CPT-II scores (N = 210)				
Omission Errors	0.22	−2.30	2.74	0.8643
Commission Errors	−0.43	−2.38	1.51	0.6641
Hit Reaction Time	1.07	−1.19	3.32	0.3546

[a] Adjusted for gestational age, birth weight, sex, parity (being the first child), age at outcome measurement, and maternal characteristics including smoking history (ever smoked vs. non-smoker), marital status (married vs. others), education, socioeconomic status and cohort (Cohort 3 - Ca, Cohort 3 - placebo and Cohort 2A).

Index. Our observed association of MUF_{cr} and CRS-R seems to demonstrate a ceiling effect, suggesting that higher levels of urinary fluoride concentration did not substantially increase risk of ADHD-like symptoms.

Overall, our results are consistent with the ecological study by Malin and Till (2015). Their cross-sectional study used national health surveys conducted by the Centers for Disease Control and Prevention to estimate the prevalence of ADHD and water fluoridation for each state. Their findings showed that, after controlling for household income, states in which a greater proportion of people received community water fluoridation in 1992 tended to have a greater proportion of children and adolescents who received an ADHD diagnosis in 2003, 2007, and 2011. The prevalence of ADHD increased from 7.8% in 2003 to 11% in 2011. A distinction between this U.S. study and the current study is that our study was a longitudinal birth cohort with individual biomarkers of fluoride exposure obtained during pregnancy when the developing brain is thought to be at the highest risk for fluoride neurotoxicity (Bashash et al., 2017). Moreover, the current study controlled for relevant confounders that may be associated with ADHD, including smoking, maternal education, child sex, HOME score, and exposure to other contaminants. Further, our measure for ADHD was not physician-diagnosed but rather approximated through validated questionnaires and performance-based tests. Both studies examined populations that are exposed to "optimal" levels of fluoride either through water or salt fluoridation schemes. The exposure level in the Mexico City cohort is generally lower than the populations living in endemic fluorosis areas studied in China (Choi et al., 2012) where natural levels of fluoride are often higher (> 2 mg/L) than recommended levels for North American (0.7 mg/L). The mean concentration of MUF in our study is in the range of the Canadian cohort (in review), and a New Zealand cohort of 59 pregnant women (median MUF = 0.82 mg/L) (Skeaff and Te Morenga, 2017).

Our finding of an association between MUF_{cr} and symptoms of inattention are consistent with the growing body of evidence showing dose-response relationships between early-life exposure to fluoride and attention outcomes. Animal studies (Mullenix et al., 1995) reported fewer behavioral initiations and less time exhibiting exploration behaviors among male and female rats exposed to 100 or 125 ppm fluoride as weanlings (21 days postnatal) and among male rats whose mothers were injected with 0.13 mg/L of sodium fluoride on gestational days 17–19. These particular behavioral effects are suggestive of hypoactivity. In human studies, high exposure to fluoride, as reflected by the presence of moderate to severe dental fluorosis in primary teeth of children living in southern Sichuan, China, was associated with poor working memory, but not with other cognitive domains that were assessed (Choi et al., 2015). Working memory is linked with the ability to control attention and it is common for youth with ADHD to have weaknesses in working memory (Kasper et al., 2012). A possible explanation for the specific effect on inattention (Dugbartey, 1998) is that fluoride exposure is contributing to thyroid hormone insufficiency. Recent studies demonstrate that even relatively subtle changes in circulating levels of TH in pregnancy (i.e. subclinical hypothyroidism) can have adverse outcomes, including preterm birth, lowered IQ (Hollowell et al., 1999; Murphy et al., 2015; Stagnaro-Green and Rovet, 2016; Thompson et al., 2018) (Yang et al., 2008) and increased risk for attention disorders (Modesto et al., 2015; Pakkila et al., 2014) (Rovet and Hepworth, 2001). Some (Swarup et al., 1998) (Swarup et al., 1998), but not all (Mcpherson et al., 2018), animal studies showed reductions in T3 and T4 levels from fluoride exposure, even at low doses. Several human studies have shown that elevated levels of fluoride in drinking water predict higher TSH and lower T3 levels (Bachinskii et al., 1985; Kheradpisheh et al., 2018; Singh et al., 2014), especially among children (Yasmin et al., 2013), as well as an increased likelihood for a diagnosis of hypothyroidism. However, further research is needed to examine how exposure to fluoride may affect thyroid function in pregnancy. Another potential mechanism through which fluoride may contribute to ADHD relates to the dopamine system. Animal studies have shown that fluoride exposure can alter the levels of dopamine (Pal and Sarkar, 2014). Dopamine is an important modulatory

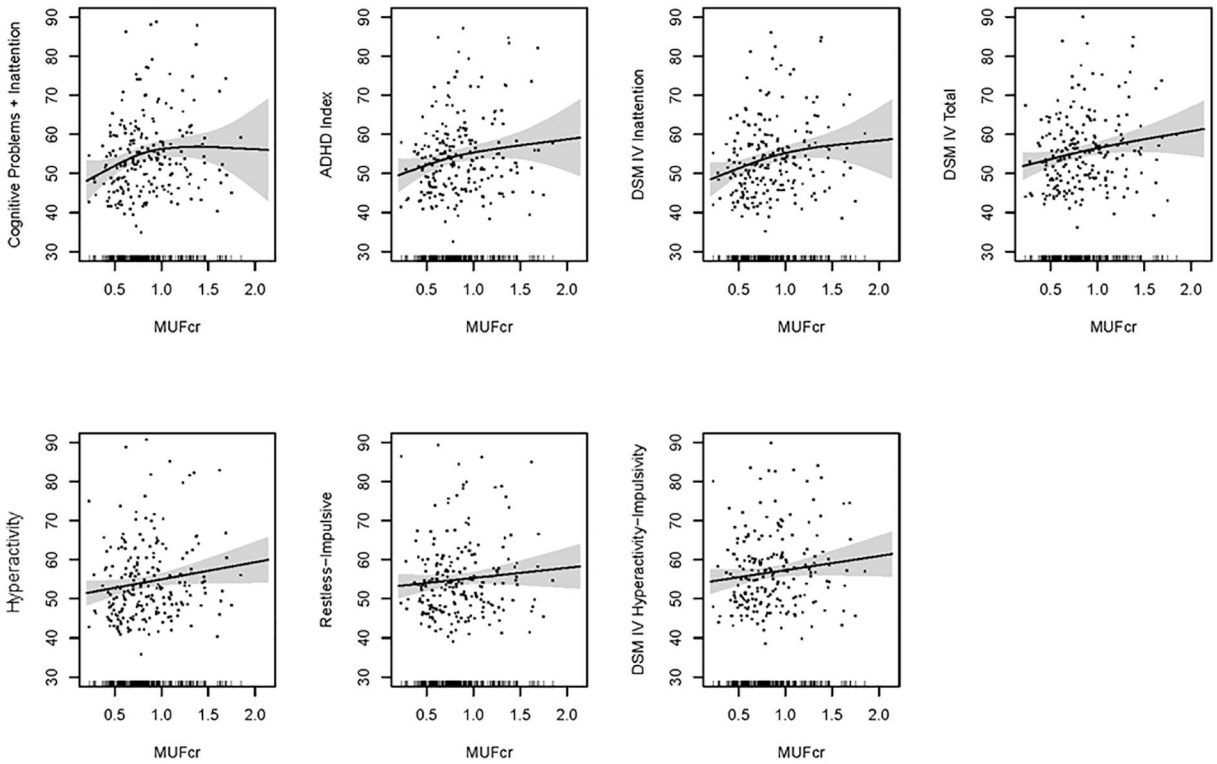

Fig. 2. Association of maternal creatinine-adjusted urinary fluoride (MUF_{cr}) and Conners' Parent Rating Scales-Revised (CRS-R) measures in children at age 6 to 12 years.

Outcome data are adjusted for gestational age, birth weight, sex, parity (being the first child), age at outcome measurement, and maternal characteristics including smoking history (ever smoked vs. non-smoker), marital status (married vs. others), education, socioeconomic status and cohort (Cohort 3 Ca, Cohort 3 placebo and Cohort 2A). Shaded area is the 95% confidence interval. The short vertical bars on the x-axis reflect the density of urinary fluoride measures. Individual data points are individual observations, n = 210.

neurotransmitter in planning and initiation of motor responses, activation, switching, reaction to novelty and processing of reward (Faraone et al., 2015).

The stronger association between prenatal fluoride exposure and parent-reports of attention problems may be explained by the CRS-R measuring distinct and more extensive constructs that rely on attention (e.g. new learning, ability to hold information and complete tasks, organizational skills, etc.) than those assessed by the CPT-II. Additionally, it has been shown that CPT-II performance and parent rating scales are moderately correlated at best (Gualtieri and Johnson, 2005), suggesting that these measures are assessing different constructs. Other studies examining prenatal Polychlorinated Biphenyis (PCBs) exposure and sustained attention (Vreugdenhil et al., 2004) that report using the computerized continuous performance test with children aged 4 and 11 years also fail to show an association with this measure, but do find an association using other psychometric tests of attention (e.g. digit cancellation task). Further investigation of cognitive performance on other domains, such as learning and memory, is warranted in our study cohort.

Key strengths of this study include the relatively large pregnancy cohort that has a biorepository with a capacity to capture high quality individual biomarker exposure across multiple developmental time points, longitudinal follow-up, detailed assessment of ADHD-like behaviors using both rating scales and performance based measures, as well as measurement of additional health outcomes and potential covariates using validated techniques (Thomas et al., 2016). Assessment of attention, hyperactivity, and impulsivity using multiple measures allowed us to examine different types of ADHD-like behaviors using continuous scales that are sensitive to both clinical and subclinical symptoms of ADHD. This approach minimizes the limitations of viewing ADHD as a categorical and conceptually distinct disorder.

Our study also has some limitations. The cohort was not initially designed to study fluoride exposure and so we are missing some aspects of fluoride exposure assessments (e.g., detailed assessments of diet, water, etc.) that are now underway. The urinary samples were not available for all trimesters of pregnancy for majority of the participants; in particular, for those with only one sample, we cannot rule out the possibility that some samples may reflect acute exposure. Differences in the proportion of fluoride that is excreted in the urine have been described for different age groups as well as for pregnant women. Data on the percentage of fluoride excreted for children and adults are available, which makes estimation of intake feasible. On the other hand, data for pregnant women are sparse, making estimates of intake from urinary concentration not feasible. Therefore, while urinary fluoride is a valid biomarker to identify differences in exposure levels in pregnant women, it is not possible, with the currently available data, to estimate how concentration levels relate to intake. With regards to our outcome measure, we did not have information on family history or genetic markers associated with ADHD, nor were children assessed clinically for a diagnosis of ADHD. Only parent reports were used for the CRS-R, and not teacher reports. This is a limitation to our study as previous studies have shown that there can be considerable variation between the two sources in terms of identifying ADHD-associated behaviors (Lavigne et al., 2012). Nevertheless, parents were unaware of their offspring's fluoride exposure status, removing reporting bias as a limitation. Although elevated scores on behavioral checklists like the CRS-R may be associated with a diagnosis of ADHD, the functional consequences of the symptoms must also be characterized for clinically diagnosing the disorder.

5. Conclusion

In summary, we observed a positive association between higher prenatal fluoride exposure and more behavioral symptoms of inattention, but not hyperactivity or impulse control, in a large Mexican cohort of children aged 6 to 12 years. The current findings provide further evidence suggesting neurotoxicity of early-life exposure to fluoride. Replication of these findings is warranted in other population-based studies employing biomarkers of prenatal and postnatal exposure to fluoride.

Acknowledgements

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All authors contributed to the final interpretation of the results and final manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2018.09.017.

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Supplementary Material

Table S1. Multivariate* Gamma regression models of differences across CRS-R and CPT-II scores per 0.5 mg/L higher maternal creatinine-adjusted urinary fluoride (MUFcr). [+] further adjusted for maternal bone Pb, maternal blood Hg and Home score for subset of subjects with available child urinary fluoride measurements.

CRS-R Scores	**Pb [*] (N=178)**	**Hg [**](N=144)**	**Home[***](N=127)**	**CUF_{sg} [****] (N=175)**
DSM-IV Inattention	3.12(0.98 ,5.25)	3.78 (1.26 ,6.31)	2.73 (0.16 ,5.3)	3.19 (1.06 ,5.31)
Cognitive Problems + Inattention	2.78(0.53 ,5.03)	3.49 (0.9 ,6.07)	3.06 (0.51 ,5.61)	2.53 (0.29 ,4.77)
DSM-IV ADHD Total	2.91(0.78 ,5.03)	3.61 (1.06 ,6.16)	2.42 (-0.06 ,4.9)	2.74 (0.66 ,4.82)
ADHD Index	2.66(0.43 ,4.9)	3.75 (1.11 ,6.4)	3.3 (0.75 ,5.84)	2.76 (0.62 ,4.91)
Restless-Impulsive	2.53(0.36 ,4.7)	2.79 (0.11 ,5.46)	1.37 (-1.09 ,3.83)	1.83 (-0.29 ,3.94)
DSM-IV Hyperactivity-Impulsivity	2.51(0.36 ,4.66)	3.36 (0.71 ,6.01)	2.02 (-0.54 ,4.58)	2.02 (-0.11 ,4.15)
Hyperactivity	1.94(-0.14 ,4.02)	1.76 (-0.87 ,4.38)	0.41 (-2.05 ,2.86)	1.37 (-0.71 ,3.44)
CPT-II Scores				
Omission Errors	0.14(-2.58 ,2.85)	0.95 (-2.24 ,4.13)	-0.26 (-3.54 ,3.04)	-0.21 (-2.22 ,1.8)
Commission Errors	-0.55(-2.57 ,1.48)	-0.68 (-3.19 ,1.83)	-0.55 (-2.91 ,1.81)	-0.21 (-2.22 ,1.8)
Hit Reaction Time	1.1(-1.27 ,3.47)	2.49 (-0.42 ,5.39)	2.23 (-0.42 ,4.89)	0.85 (-1.42 ,3.12)

+ Adjusted for gestational age, weight at birth, sex, parity (being the first child), age at outcome measurement, and maternal characteristics including smoking history (ever smoked vs. non-smoker), marital status (married vs. others), education, socioeconomic status and cohort (Cohort 3 --Ca, Cohort 3 --placebo and Cohort 2A).* Regression model further adjusted for maternal bone lead on subset of participants with data on maternal bone lead.

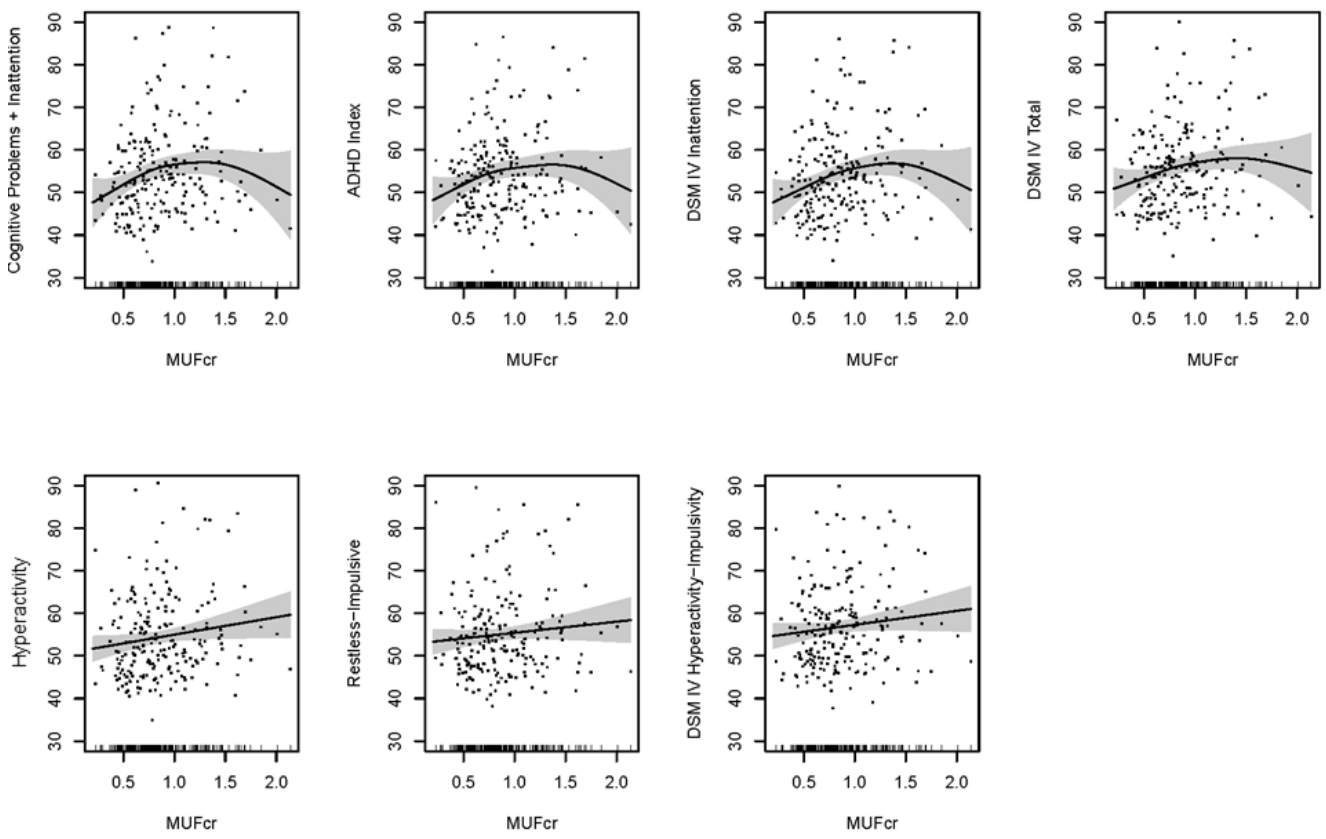
** Regression model further adjusted for maternal blood mercury on participants of cases with data on maternal blood mercury.

*** Regression model further adjusted for Home Observation for the Measurement of the Environment (HOME) scores on subset of participants with data on HOME.

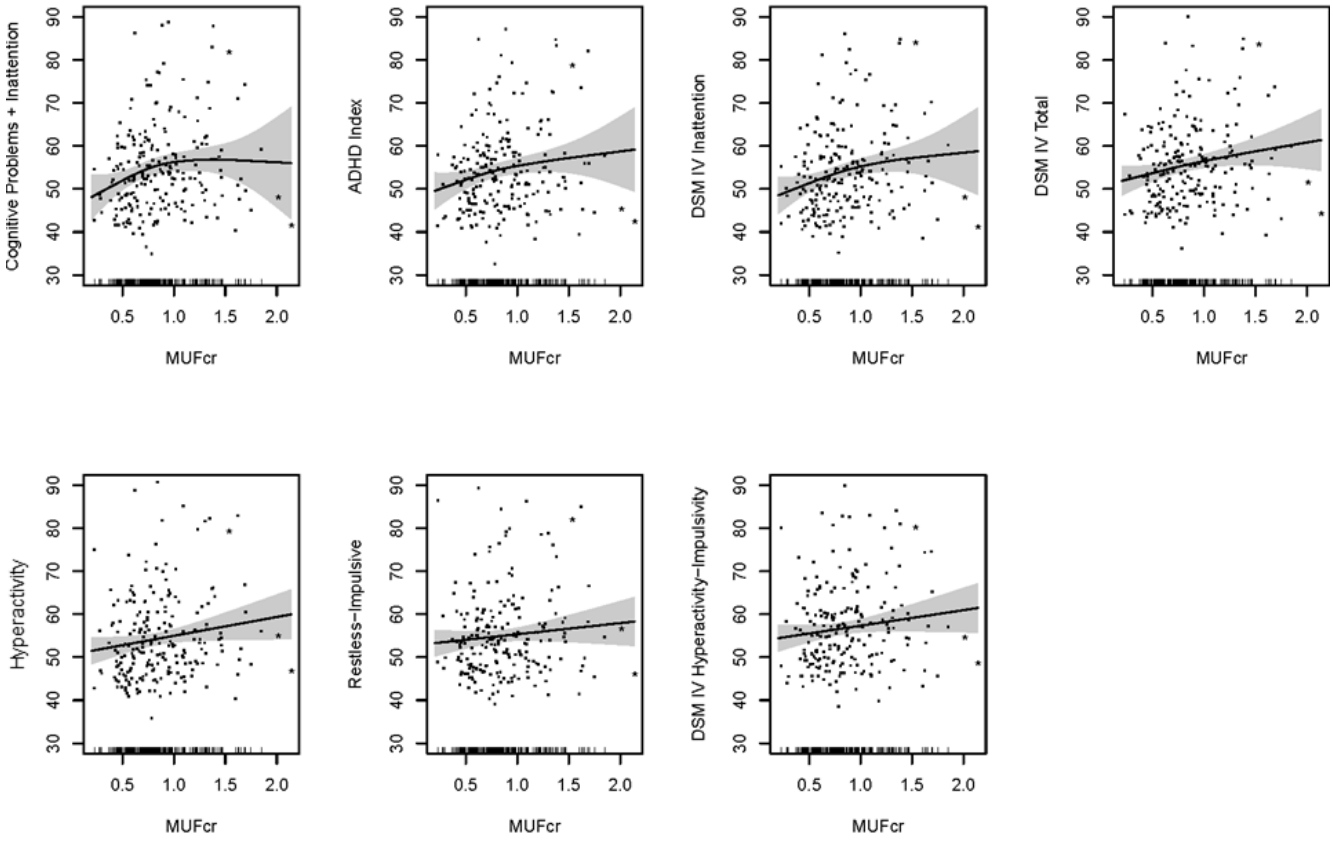
**** Regression model further adjusted for child urinary fluoride (specific gravity adjusted) on subset of participants with data on CUF_{sg}.

Figure S1. Association of maternal creatinine-adjusted urinary fluoride (MUF_{cr}) and Conners' Parent Rating Scales-Revised (CRS-R) including (A) and excluding (B) 3 influential observation in the model

A)


Cognitive Problems + Inattention
ADHD Index
DSM IV Inattention
DSM IV Total
Hyperactivity
Restless−Impulsive
DSM IV Hyperactivity−Impulsivity
MUFcr
30
40
50
60
70
80
90
0.5
1.0
1.5
2.0

B*)

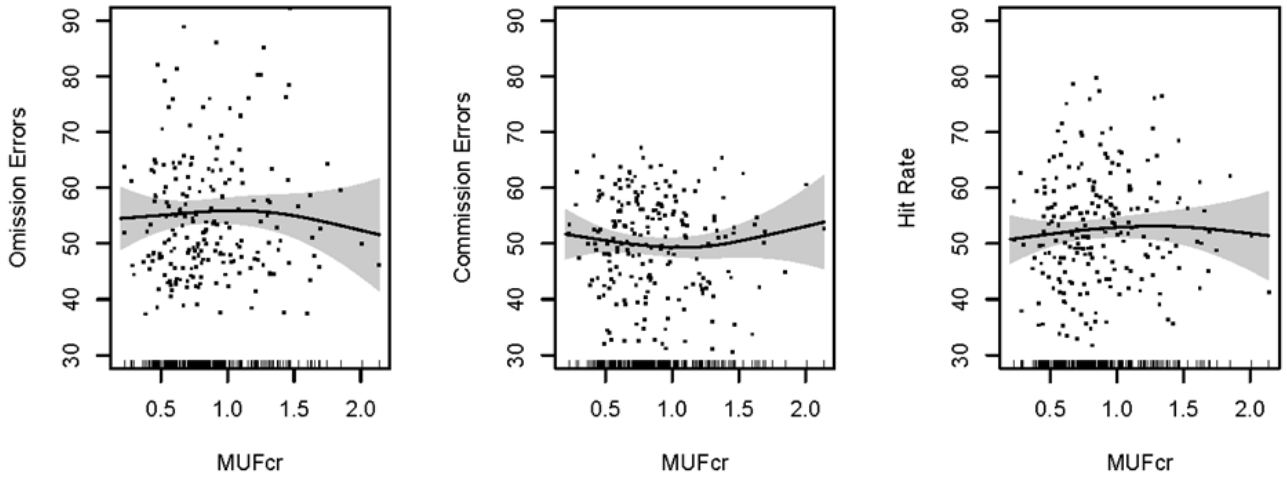


Adjusted for gestational age, weight at birth, sex, parity (being the first child), age at outcome measurement, and maternal characteristics including smoking history (ever smoked vs. non-smoker), marital status (married vs. others), education, socioeconomic status and cohort (Cohort 3 --Ca, Cohort 3 --placebo and Cohort 2A). Shaded area is 95% confidence interval. Short vertical bars on the x-axis reflect the density of the urinary fluoride measures.

*Excluded from the model and only shown as stars

Individual data points are individual observations, (N=213)

Figure S2) Adjusted association of maternal creatinine-adjusted urinary fluoride (MUF_{cr}) and The Conners' Continuous Performance Test (CPT-II) measures.



Adjusted for gestational age, weight at birth, sex, parity (being the first child), age at outcome measurement, and maternal characteristics including smoking history (ever smoked vs. non-smoker), marital status (married vs. others), education, socioeconomic status and cohort (Cohort 3 --Ca, Cohort 3 --placebo and Cohort 2A). Shaded area is 95% confidence interval. Short vertical bars on the x-axis reflect the density of the urinary fluoride measures.

Individual data points are individual observations, n= 210

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Community Water Fluoridation and Urinary Fluoride Concentrations in a National Sample of Pregnant Women in Canada

Christine Till,[1] Rivka Green,[1] John G. Grundy,[1] Richard Hornung,[2] Raichel Neufeld,[1] E. Angeles Martinez-Mier,[3] Pierre Ayotte,[4,5] Gina Muckle,[5,6] and Bruce Lanphear[7,8]

[1]Faculty of Health, York University, Toronto, Ontario, Canada
[2]Pediatrics and Environmental Health, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio, USA
[3]School of Dentistry, Indiana University, Indianapolis, Indiana, USA
[4]Department of Social and Preventive Medicine, Laval University, Quebec, Quebec, Canada
[5]Centre de Recherche du CHU de Québec, Université Laval, Quebec, Quebec, Canada
[6]School of Psychology, Laval University, Quebec, Quebec, Canada
[7]Faculty of Health Sciences, Simon Fraser University, Vancouver, British Columbia, Canada
[8]Child & Family Research Institute, BC Children's Hospital, University of British Columbia, Vancouver, British Columbia, Canada

Background: Fluoride exposures have not been established for pregnant women who live in regions with and without community water fluoridation.

Objective: Our aim was to measure urinary fluoride levels during pregnancy. We also assessed the contribution of drinking-water and tea consumption habits to maternal urinary fluoride (MUF) concentrations and evaluated the impact of various dilution correction standards, including adjustment for urinary creatinine and specific gravity (SG).

Methods: We measured MUF concentrations in spot samples collected in each trimester of pregnancy from 1,566 pregnant women in the Maternal–Infant Research on Environmental Chemicals cohort. We calculated intraclass correlation coefficients (ICCs) to assess variability in MUF concentrations across pregnancy. We used regression analyses to estimate associations between MUF levels, tea consumption, and water fluoride concentrations as measured by water treatment plants.

Results: Creatinine-adjusted MUF values (mean ± SD; milligrams per liter) were almost two times higher for pregnant women living in fluoridated regions (0.87 ± 0.50) compared with nonfluoridated regions (0.46 ± 0.34; $p < 0.001$). MUF values tended to increase over the course of pregnancy using both unadjusted values and adjusted values. Reproducibility of the unadjusted and adjusted MUF values was modest (ICC range = 0.37–0.40). The municipal water fluoride level was positively associated with creatinine-adjusted MUF (B = 0.52, 95% CI: 0.46, 0.57), accounting for 24% of the variance after controlling for covariates. Higher MUF concentrations correlated with numbers of cups of black ($r = 0.31$–0.32 but not green tea ($r = 0.04$–0.06). Urinary creatinine and SG correction methods were highly correlated ($r = 0.91$) and were interchangeable in models examining predictors of MUF.

Conclusion: Community water fluoridation is a major source of fluoride exposure for pregnant women living in Canada. Urinary dilution correction with creatinine and SG were shown to be interchangeable for our sample of pregnant women. https://doi.org/10.1289/EHP3546

Introduction

The public health benefits associated with fluoridated dental products and optimally fluoridated drinking water are cited widely (Brunelle and Carlos 1990; CDC 2014; Featherstone 1999; Newbrun 1989; O'Mullane et al. 2016). Fluoride exposure can also cause potential adverse effects, such as dental fluorosis and skeletal fluorosis, both of which are observed at elevated fluoride exposure levels over a long period of time (Health Canada 2010). Fluoride exposure may also be neurotoxic, especially for the developing fetus (Grandjean and Landrigan 2014). Still, few developmental neurotoxicology studies have measured biomarkers of gestational fluoride exposure (Bashash et al. 2017; Valdez Jiménez et al. 2017). Instead, most studies use water fluoride concentrations (An et al. 1992; Broadbent et al. 2015; Eswar et al. 2011; Karimzade et al. 2014; Khan et al. 2015; Kundu et al. 2015; Liu et al. 2014; Trivedi et al. 2007; Xiang et al. 2003) or children's urinary fluoride level (Das and Mondal 2016; Fan et al. 2007; Trivedi et al. 2007) as measures of contemporaneous exposure.

Fluoride exposure is widespread in North America. Water and water-based beverages are the main sources of systemic ingestion, accounting for approximately 75% of dietary fluoride intake among adults living in communities that fluoridate their water supply in the United States (U.S. EPA 2010). Community water fluoridation (CWF) is the process of adjusting the amount of fluoride found in public drinking water to a level that provides optimal dental benefits. Nearly three-fourths of the U.S. population on community water systems receive fluoridated water (https://www.cdc.gov/fluoridation/statistics/index.htm) compared with approximately one-third of Canadians and only 3% of Europeans. In Canada and the United States, the optimal concentration of fluoride in drinking water is set at 0.7 mg/L to protect against tooth decay (DHHS 2015; Health Canada 2017). Other sources of fluoride include foods, dental products (e.g., toothpastes, mouth rinses), supplements, industrial emissions, and fluoride-containing pharmaceuticals. Certain dietary products, like tea, have been identified to have high concentrations of natural fluoride (Fung et al. 1999; Malinowska et al. 2008; Waugh et al. 2016; USDA 2005).

The National Toxicology Program (NTP) at the National Institutes of Health (NRC 2006) and others (Grandjean and Landrigan 2014) have concluded that prenatal exposure to high levels of fluoride can alter neurodevelopment. Fluoride readily crosses the placenta (Forestier et al. 1990) and, in animal studies, accumulates in critical brain regions involved in learning and memory (Bhatnagar et al. 2002; Dong et al. 1997; Pereira et al. 2011). Results of a meta-analysis (Choi et al. 2012) of 27 epidemiologic studies showed that children who lived in areas

Address correspondence to C. Till, Department of Psychology, York University, 4700 Keele St., M3J 1P3 Toronto, ON, Canada. Telephone: (416) 736-2100, ext. 20776. Email: ctill@yorku.ca

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with high-fluoride exposure had lower intelligence quotient (IQ) scores than those who lived in low-exposure areas. However, these findings are controversial because most of the studies were conducted in China where fluoride exists as a natural contaminant and exposure levels are often higher than the 0.70 mg/L "optimal" level for water fluoridation in North America. Notably, 13 of the 18 waterborne fluoride studies included in the 2012 meta-analysis consisted of fluoride concentrations of less than the maximum contaminant level goal of 4 ppm (mean water fluoride level of 2.3 mg/L). A more recent study (Bashash et al. 2017) conducted in a non-endemic fluorosis area in Mexico City of nearly 300 mother-child pairs demonstrated an inverse association between maternal urinary fluoride (MUF) concentration during pregnancy and child IQ.

Biomarkers of fluoride (e.g., urinary fluoride, serum fluoride) are directly correlated with fluoride exposure levels (e.g., water fluoride concentration, fluoride supplement exposures) in children (dela Cruz et al. 2008; Kumar et al. 2016; LeGeros et al. 1985; Singh et al. 2007; Zipkin et al. 1956), adults (Ahmed et al. 2012; Mansfield 1999; McClure and Likins 1951; Yadav et al. 2007; Zipkin et al. 1956), and pregnant women (Opydo-Szymaczek and Borysewicz-Lewicka 2007; Gardner et al. 1952). To our knowledge, however, no studies have directly compared urinary or serum fluoride concentrations with water fluoride concentration in pregnant women living in North America. Moreover, the impact of using urinary creatinine and urinary SG as correction standards for measuring urinary fluoride concentrations during pregnancy remains unclear.

We measured urinary fluoride concentrations during pregnancy from 1,566 women living in 10 cities across Canada and tested whether MUF concentrations were associated with sociodemographic factors, tea consumption habits, and water fluoride concentrations in public drinking water. We also examined how various methods of adjusting for urinary dilution affected the within-person reliability of MUF concentrations and the relationship of MUF concentrations to water fluoride concentration.

Methods

Study Sample

Between 2008 and 2011, Maternal–Infant Research on Environmental Chemicals (MIREC) Study staff recruited a population-based sample of 2001 pregnant women from 10 cities across different geographical regions of Canada, 7 of which have CWF (Toronto, Hamilton, Ottawa, Sudbury, Halifax, Edmonton, Winnipeg; $n = 1{,}259$) and 3 of which do not (Vancouver, Montreal, Kingston; $n = 742$). To enhance the accuracy of fluoride exposure, we included only women who provided spot samples across all three trimesters. Women were recruited from prenatal clinics during their first trimester to participate in a longitudinal birth cohort study and provided written informed consent after the study was described to them. Participants were included if they could provide consent, communicate in English or French, were older than 18 y of age, and were at <14 wk of gestation. Participants were excluded if there was a known fetal abnormality, if they had any medical complications (i.e., cancer, renal disease, heart disease), or if there was known maternal alcohol or drug abuse during pregnancy. Participant recruitment and further demographic details and birth outcomes on the cohort can be found elsewhere (Arbuckle et al. 2013). Health Canada's Research Ethics Board and all participating recruitment sites approved the MIREC Study. The present study also received ethics approval from the York University Research Ethics Board in Toronto.

Measure of Fluoride

Fluoride concentrations were assessed in archived spot urine samples obtained from Trimester 1 at 11.57 ± 1.57 [mean ± standard deviation(SD)] wk ($n = 1{,}885$), Trimester 2 at 19.11 ± 2.39 wk ($n = 1{,}738$), and Trimester 3 at 33.11 ± 1.50 wk ($n = 1{,}660$) of gestation. Urine was collected in Nalgene® containers that were lot tested for phthalates and bisphenol A. Samples were labeled with a unique identification and barcode and then aliquoted into smaller Cryovials® and stored at appropriate temperatures until they were shipped to the Indiana University School of Dentistry for analysis.

Fluoride concentration was analyzed using a modification of the hexamethyldisiloxane (HMDS; Sigma Chemical Co.) microdiffusion procedure of Taves (1968), as modified by Martínez-Mier et al. (2011). A measured and recorded volume of each sample (0.850 mL) was dispensed into 15-cm plastic Petri dishes (Falcon; Fisher Scientific Co.); a sodium hydroxide (NaOH, anhydrous; Sigma Chemical Co.) trap solution was loaded onto the Petri dish lid and after adding sulfuric acid (H_2SO_4; Sigma Chemical Co.) saturated with HMDS, each dish was tightly sealed. Fluoride was released by acid hydrolysis and trapped in the NaOH trap. The fluoride-containing trap was then removed and buffered to pH 5.2 with perchloric acid ($HClO_4$; Sigma Chemical Co.). The resulting solution was adjusted to a final volume of 100 μL with total ionic strength buffer (TISAB II; Fisher Scientific Co.). Sets of approximately 30 samples were analyzed at any one time. Fluoride levels were determined by comparing the millivolt reading of each sample to standard curves, covering the range of the samples' values, prepared from the data for standard solutions of diffused fluoride determined at the time the samples were analyzed. Reference standard solutions were monitored daily by a quality assurance (QA) officer for stability; technicians reanalyzed, on a rotating basis, one of three standards daily. In addition, urine-based certified reference materials [PC-U-F1305; Institut National de Santé Publique Québec, (INSPQ)/Laboratoire de Toxicologie] were analyzed every 200–300 samples. Finally, the QA officer checked for errors in the sample numbers, recorded results and cell formula errors, and checked results in millivolt readings on source documents versus Microsoft Excel spreadsheets. In neutral solutions, fluoride concentrations can be measured down to 0.02 mg/L fluoride. This method has been shown to yield the highest recoveries of fluoride for undiluted samples. The precision and validity of this analysis technique has been reported elsewhere (Martínez-Mier et al. 2011). Compared with the total sample of spot urines that were available for fluoride analysis, only 0.002% (two samples) of readings was removed at the first trimester due to readings being higher than that of the highest concentration standard. No observations were removed in subsequent trimesters.

Measure of Urinary Creatinine

Urinary creatinine [CRE; in grams (g creatinine)] was measured using colorimetric end-point (Jaffe) tests on an Indiko instrument (Indiko Plus; ThermoFisher Scientific). An alkaline solution of sodium picrate was used to react with creatinine in urine to form a red Janovski complex using the Mircogenics DRI® Creatinine-Detect® Test. The absorbance was read at 510 nm on an Indiko chemistry autoanalyzer (Indiko Plus, ThermoFisher Scientific) with a detection limit of 0.069 mmol/L, reporting limit of 0.23 mmol/L, and reproducibility of 2.2%. Analyses of creatinine levels for Trimester 1 and 2 urines were conducted at an internationally recognized toxicology lab (Institut National de Santé Publique du Québec), which is accredited by the Standards Council of Canada under ISO 17025. Analyses of creatinine levels for Trimester 3 urines were conducted by another lab overseen by

a Health Canada scientist. Both labs completed CRE analyses for Trimester 1, and there was a very high level of agreement between the values from each lab ($r = 0.95$, $p < 0.01$, $n = 1{,}477$); because of the consistency of CRE levels across the two labs, we chose to use the available Trimester 3 CRE results analyzed by this separate lab.

Correction for Variations in Urine Dilution

To account for variations in urine dilution at the time of measurement, MUF concentrations were adjusted for either CRE or specific gravity (SG). We used different methods to correct for hydration status because there is no established standard for estimating fluoride exposure among pregnant women. We used the average MUF concentration taken over all three trimesters for all adjustment methods. The three primary correction methods included adjustment for specific gravity (MUF_{SG}) and two methods of adjustment for creatinine (MUF_{CRE_1} and MUF_{CRE_2}).

MUF_{SG}

The Indiana University laboratory measured SG for all urine samples. Urine samples ($\sim$2.0 mL) were transferred to a 36 × 36 mm weighing dish (catalog no. 08-732-112; Fisher Scientific). After performing zero setting per the instrument instruction manual, the prism head of a pen refractometer (ATAGO Co., Ltd.) was submerged into the sample and a reading was obtained and recorded. Following the SG measurements, a 0.850-mL aliquot of each sample was then removed for fluoride analysis. All readings were conducted in a darkened room and the refractometer prism head was rinsed in deionized water after each reading. Less than 0.02% of SG values fell below the limit of detection; these values were replaced by 1.001 so that we did not lose these data points when we computed the averaged MUF concentration adjusted for SG. Ad hoc analyses were also conducted to show that the use of the imputed SG values of 1.001 did not alter the mean values for MUF adjusted for SG. MUF values were corrected for SG using Equation 1:

$$MUF_{SG(mg/L)} = \frac{MUF_i \times (SG_M - 1)}{(SG_i - 1)} \quad (1)$$

where MUF_{SG} is the SG-adjusted chemical concentration (in milligrams per liter), MUF_i is the observed chemical (fluoride) concentration, SG_i is the specific gravity of the urine sample, and SG_M is the median SG for the cohort (Hauser et al. 2004).

MUF_{CRE_1}

The first method of creatinine adjustment used Equation 2:

$$MUF_{CRE\_1(mg/g)} = MUF_i / CRE_i \quad (2)$$

where MUF_i concentration (in milligrams fluoride per gram creatinine) is the observed fluoride concentration and CRE_i is the observed creatinine concentration for that individual. The second method of CRE adjustment that was adopted by the Early Life Exposures in Mexico to ENvironmental Toxicants (ELEMENT) study (Bashash et al. 2017; Thomas et al. 2016) was based on the following equation:

$$MUF_{CRE\_2(mg/L)} = (MUF_i / CRE_i) \times CRE_{avg} \quad (3)$$

where MUF_{CRE_2} is the creatinine-adjusted fluoride concentration (in milligrams fluoride per liter), MUF_i is the observed fluoride concentration, CRE_i is the observed CRE concentration for that individual, and CRE_{avg} is the average CRE concentration of the samples available at each trimester. This method was included in order to permit comparison with a prior study examining urinary fluoride levels in a large sample of pregnant women (Bashash et al. 2017; Thomas et al. 2016).

In addition to the three methods indicated above, we also adjusted for creatinine (MUF_{CRE_cov}) and SG (MUF_{SG_cov}) as covariates in regression models. This approach was recommended by Barr et al. (2005) as a method to control for confounding between factors—such as age, race, sex, and body mass index (BMI)—that may affect both exposure-related outcomes (e.g., disease risk) and variations in urine dilution.

Measurement of Municipal Drinking-water Fluoride

Municipal drinking-water reports were solicited from each city that was included in the MIREC Study. For each city included in the study, we determined water treatment plant (WTP) boundary regions and then linked the first three letters of the postal code for each participant [as reported in Trimester 3 (note that postal codes were identical between Trimester 1 and 3 for 89% of the participants)]. In some cases, participants were linked with multiple WTPs because water distribution boundaries may overlap. Water fluoride data were obtained for 1,359 of the 1,566 women (86.8%); of these, 813 participants lived in cities with CWF and 546 lived in cities without. The primary source of drinking water (i.e., public water system or private well) was assessed by questionnaires completed by the participants during pregnancy. Of the 1,566 women with MUF and SG analyses, 1,451 who reported drinking tap water from a public source were included in the study, whereas 110 (7%) who reported drinking well water, 4 (0.2%) who reported other, and 1 (<0.06%) with missing data were excluded (see Figure S1). Women who reported a drinking-water source other than a public water supply were more likely to be white and born in Canada relative to the sample of women who reported a public drinking-water source; all other demographic characteristics were similar between the groups (see Table S1). Of the 1,451 women who reported drinking water from the tap, 1,147 (79%) lived within the WTP distribution areas for each city sampled.

Fluoridation was defined according to current national drinking-water guidelines (Health Canada 2010), which are implemented by drinking-water authorities in the affected jurisdiction. A range of 0.6–0.8 mg/L fluoride in water is recommended by the Ministry of Health and Long-Term Care in Ontario (consistent with Health Canada's recommendation of 0.7 mg/L; https://www.hc-sc.gc.ca/hl-vs/iyh-vsv/environ/fluor-eng.php). In practice, fluoridated water levels may correspond to a wider range, with a maximum acceptable concentration of 1.5 mg/L (Health Canada 2010). The present study defined a nonfluoridated site as having water fluoride levels (both adjusted and natural fluoride levels) of <0.3 mg/L.

We calculated each participant's average fluoridated drinking-water value for the duration of their pregnancy by taking the average of three quarterly means. For example, births in Quarter 1 (January, February, March) were calculated by computing the average of Quarters 3 and 4 of the year before birth and Quarter 1 of the birth year. We calculated geometric means (GMs) given the large range of water fluoride values. For participants who received water from more than one WTP, the fluoridated drinking-water value was calculated by computing the average of the three quarterly GMs from each relevant WTP (see Table S2). Some cities (e.g., Montreal) had both fluoridated and nonfluoridated zones (see sample map showing distributions for each WTP in Figure S2). Participants living in each region were coded accordingly. Finally, for cities that reported fluoride concentrations that were equivalent to the limit of detection (LOD), we used an imputed value of the LOD divided by the square root of 2 (Hornung and Reed 1990) to

calculate the water fluoride level. Average water fluoride levels reported by the municipal WTPs during the years that the participants were in the study are provided in Table S3.

Fluoride Intake

We estimated fluoride intake via drinking-water habits and consumption of beverages that are known to be high in fluoride content by asking participants about daily water and tea (black or green) consumption. Black and green tea leaves have both been identified as natural sources of fluoride via absorption through the soil (Fung et al. 1999; Malinowska et al. 2008). Participants were asked at the first and third trimester the following question: "Since the beginning of your pregnancy, how much did you drink the following: water (number of glasses; 1 glass = 8 oz); regular tea (cups); green tea (number of cups; 1 cup = 6 oz)?" Participants could answer "none" or insert a number of glasses/cups and select a frequency (day/week/month).

Statistical Analyses

We performed statistical analyses for women who had all three urine samples corresponding to each trimester using RStudio (version 1.1.383) and SAS (version 9.3; SAS Institute Inc.). We used a two-sided $\alpha = 0.05$ for hypothesis testing. Because the distributions of the MUF levels were right-skewed, values were $\log_{10}$-transformed to obtain a more normal distribution.

We first calculated crude descriptive statistics for each trimester, averaged over the entire pregnancy (ignoring dilution effects) and using each of the three urinary dilution correction methods. Possible differences between the MUF levels for the different trimesters were evaluated with an analysis of variance (ANOVA) test. To assess the reliability of MUF levels over the course of pregnancy, we calculated partial correlation coefficients (adjusted for covariates) between each trimester and for each MUF measurement (MUF_{SG}, MUF_{CRE_1}, MUF_{CRE_2}) to examine whether the method of accounting for urine dilution influenced the results. In addition, we calculated intraclass correlation coefficients (ICCs), and their 95% confidence intervals (CIs). The ICC can be interpreted as a measure of test–retest reliability and uses a pooled mean and standard deviation to center and scale each variable. Values can range from 0 (no reproducibility) to 1 (perfect reproducibility). As a final step, we computed Pearson correlation coefficients to examine the relationship between each of the methods for adjusting for $\log_{10}$-transformed MUF concentration, averaged over the pregnancy.

Covariates of interest were based on literature review (Buzalaf and Whitford 2011; Buzalaf et al. 2015) and consultation with fluoride experts on factors that may influence fluoride metabolism and intake or creatinine (Gerchman et al. 2009). These variables included prepregnancy BMI, maternal age, mother's smoking status (current smoker vs. former or never smoked), alcohol consumption (no alcohol, <1 alcoholic beverage per month, ≥1 alcoholic beverage per month), caffeine consumption (≥1 caffeinated beverage per day vs. did not drink caffeinated beverage), time of urine sample and time since last void (data only available for Trimesters 1 and 3), maternal education (high school or less, some college, college university degree), annual household income (less than vs. more than $70,000 Canadian), and race (white vs. other). Covariates were chosen based on inclusion criteria where p values fell below 0.2 or changed the regression coefficient by more than 10% for the association between the covariate and MUF. Covariates that reached these criteria were prepregnancy BMI (available for 99% of the total sample), maternal age, and mother's smoking status. We used Pearson correlations to examine the associations between average $\log_{10}$-transformed MUF concentration and these three covariates. We also used Pearson correlations to examine the associations between numbers of glasses of water and cups of green and black tea consumed (using averaged data collected at Trimesters 1 and 3) with average $\log_{10}$-transformed MUF concentration. We included these variables in the final models because they are sources of fluoride. We also used Pearson correlations to examine the relationship between MUF $\log_{10}$-transformed values (both averaged and trimester-specific) and time-dependent spot sampling variables (i.e., time since last void and time at void). Next, we used one-way analysis of covariance (ANCOVA) to test differences in average $\log_{10}$-transformed MUF by residential CWF status, adjusted for covariates. To ensure that multivariate interactions between covariates were not contributing to our findings, we then examined a propensity score matching algorithm in a supplemental analysis (Rosenbaum and Rubin 1983) to match the two groups on the covariates and any multivariate interactions that may exist. This approach used logistic regression to first predict the probability of all people belonging to one group. Then, a second step matched individuals from one group to those in the other based on the probability scores. Thus, individuals that contribute to an unequal match between groups were removed and the n between groups was equated. Given our large sample size, the reduction in n between groups was not a concern. It was more important to show that this procedure and the analyses including everyone provided converging evidence for our conclusions.

Finally, we used linear regression analyses to examine the association between the average $\log_{10}$-transformed MUF concentrations and sources of fluoride-related variables (e.g., WTP fluoride levels, number of cups of tea drunk), with and without covariates. Hierarchical regression was first used to assess the relative contribution of WTP fluoride concentrations on MUF concentration after controlling for all covariates and the other sources of fluoride. Next, we conducted forward regression to examine whether any variables other than water fluoride concentrations were contributing significantly to the model. Separate regression models were run for each method of accounting for urinary dilution of MUF concentration. We also conducted secondary analyses adjusted for urinary dilution by modeling urinary creatinine and SG at each trimester as a time-dependent covariate. The best dilution standard was deemed to be the one that had the highest partial R^2 value and beta coefficient for WTP fluoride levels regressed on the MUF level.

Results

Of the women who had at least one valid measure of MUF level, 1,566 (81.6%) women had a urinary spot sample for all three trimesters, whereas 418 women were excluded because they had <3 samples (including 215, 137, and 66 women with 2, 1, and 0 urine samples, respectively) (Table 1). Of the women who had samples for all three trimesters, the mean age was 32.3 y (SD = 4.94, range 28–48 y). Eighty-six percent of the sample identified as white, and 81% of the women were born in Canada. Almost 96% were married or common-law, and almost 85% had a college diploma or university degree. At the time of pregnancy, 86% of the women were employed either full or part time. Specific gravity was measured in all 1,566 urine samples, whereas urine creatinine was available for 1,236 of the 1,566 (78.9%) urine samples (Table 1). Women who were excluded from the analyses of MUF_{SG} because they had <3 samples ($n = 418$) tended to have a lower level of education and household income, a slightly higher BMI, and were more likely to be younger, unmarried, and to smoke as compared with the women who were included in the analysis (Table 1). Women who were

Table 1. Characteristics of women with data from three trimesters that were included in the analyses of MUF_{SG} ($n = 1{,}566$) and MUF_{CRE_1} ($n = 1{,}236$) and women who were excluded because they had data from two or fewer trimesters. Values are mean ± SD or *n* (%) unless otherwise indicated.

Variables	MUF_{SG}			MUF_{CRE_1}		
	Included[a]	Excluded	*p*-Value[b]	Included	Excluded	*p*-Value[b]
n	1,566[c]	418[c]	—	1,236[c]	748[c]	—
Age of mother at enrollment (y)	32.3 ± 4.9	31.7 ± 5.5	0.03	32.3 ± 4.9	32.0 ± 5.3	0.20
Race						
White	1,347 (86.0)	304 (84.0)	0.32	1,074 (86.9)	577 (83.4)	0.03
Other	219 (14.0)	58 (16.0)		162 (13.1)	115 (16.6)	
Marital Status						
Married or common law	1,501 (95.9)	335 (92.5)	0.008	1,182 (95.6)	654 (94.5)	0.27
Not married	65 (4.1)	27 (7.5)		54 (4.4)	38 (5.5)	
Country of birth						
Born in Canada	1,269 (81.0)	300 (82.9)	0.42	1,011 (81.8)	558 (80.6)	0.53
Born outside of Canada	297 (19.0)	62 (17.1)		225 (18.2)	134 (19.4)	
Maternal Education						
High school or less	124 (7.9)	44 (12.2)	0.005	95 (7.7)	73 (10.6)	0.007
Some college	78 (5.0)	22 (6.1)		59 (4.8)	41 (5.9)	
College diploma	356 (22.7)	97 (26.8)		274 (22.2)	179 (25.9)	
University degree	1,007 (64.3)	199 (55.0)		807 (65.3)	398 (57.6)	
Prepregnancy BMI	24.8 ± 5.4	25.5 ± 5.7	0.02	24.7 (5.34)	25.4 (5.7)	0.006
Employment status at time of pregnancy						
Employed	1,349 (86.0)	298 (82.3)	0.06	1,064 (86.1)	583 (84.2)	0.27
Unemployed	217 (14.0)	64 (17.7)		172 (13.9)	109 (15.8)	
Net household income						
Net household income >$70,000 CDN	1,067 (70.8)	217 (64.2)	0.02	839 (70.4)	445 (68.1)	0.06
Net <$70,000 CDN	440 (29.2)	121 (35.8)		353 (29.6)	208 (31.9)	
Smoking during pregnancy						
Trimester 1	77 (5.0)	35 (8.4)	0.007	49 (4.0)	63 (8.9)	<0.001
Trimester 3	69 (4.4)	11 (2.6)	0.10	46 (3.7)	34 (4.8)	0.37

Note: BMI, body mass index; CRE, creatinine; MUF, maternal urinary fluoride; SD, standard deviation; SG, specific gravity.
[a]The total sample of women who had a valid value for unadjusted MUF at each trimester included two additional participants ($n = 1{,}568$). These two additional participants would have a negligible impact on the means shown for MUF_{SG}, and hence data are not shown for the unadjusted MUF.
[b]Comparisons of percentages/count data were done using the chi-square test; comparisons of means were done using Student's *t*-test. The total sample of women who had a valid value for unadjusted MUF at each trimester included two additional participants ($n = 1{,}568$), which had a negligible impact on the means (data not shown).
[c]Sample size may be lower for some of the characteristics listed in each group because of missing data.

excluded from the analyses of MUF_{CRE} because they had <3 samples ($n = 748$) tended to be of nonwhite race, smoking at Trimester 1 (but not Trimester 3), and to have a slightly lower level of education and a higher prepregnancy BMI.

Of the sample of women who provided three urine samples, 114 (7.3%) participants reported a primary drinking-water source other than the public water supply (i.e., reverse osmosis system, well water, or bottled water) in their first trimester visit; these women were excluded from the analysis, as was one participant who did not report what type of water source she used (see Table S1).

Consistency of MUF Levels over Pregnancy and across Dilution Correction Methods

MUF values increased from Trimester 1 to Trimester 3 across all methods used to correct urinary dilution (Figure 1; see also Table S4). Linear contrast tests were all highly significant ($p < 0.0001$) for all of the MUF values, suggesting a linear increase over time. MUF concentrations across each trimester of pregnancy were weakly to moderately correlated, with the correlation coefficients ranging from 0.31 to 0.52 (all $p < 0.0001$) (Figure 2). We observed stronger correlations between measurements closer in time (e.g., T1 and T2 or T2 and T3). Correlations with T1 were lower for all of the urinary dilution adjusted methods. Overall, serial MUF measurements indicated modest reproducibility across all methods of adjustment (ICC range: 0.37–0.40). The highest ICC was observed using the MUF_{CRE_2} measurement (ICC = 0.40; 95% CI: 0.36, 0.43). A slightly lower ICC value was observed for the unadjusted MUF value (ICC = 0.37; 95% CI: 0.34, 0.40).

Averaging over the course of pregnancy, $\log_{10}$-transformed unadjusted MUF values were moderately correlated with SG- ($r = 0.68$, $p < 0.001$) and CRE-adjusted MUF values ($r = 0.56$, $p < 0.001$), whereas SG- and CRE-adjusted values were strongly correlated ($r = 0.91$, $p < 0.001$). The CRE-adjustment methods were perfectly correlated ($r = 1.00$) given that MUF_{CRE_2} was derived by multiplying MUF_{CRE_1} by a constant.

MUF levels as a function of fluoridated versus nonfluoridated status. Mean MUF levels were almost two times higher among women living in fluoridated than nonfluoridated communities (Figure 3; see also Table S4), even after controlling for covariates (Table 2) or using propensity score matching on the covariates (see Table S5). The pattern was consistent across all three methods used to adjust for dilution status, but the mean values were highest using the creatinine correction adjustment methods, particularly MUF_{CRE_1}. As expected, water fluoride levels were also significantly higher among fluoridated sites than nonfluoridated sites (Table 2). Specific gravity measurements and creatinine values were also higher among pregnant women living in fluoridated as compared with nonfluoridated areas (Table 2).

Correlations between MUF and Covariates

Correlations between mean $\log_{10}$-transformed MUF concentrations for both SG- and creatinine-adjusted values and covariates are shown in Table 3. MUF_{SG} and MUF_{CRE} levels were not correlated or were very weakly associated (r values <0.10) with BMI, smoking status during pregnancy, parity, level of education, and income level. Weak positive correlations were found between both MUF_{SG} and MUF_{CRE} concentrations and maternal age ($r = 0.12$ to 0.17). Moderate correlations were found between MUF_{SG} and MUF_{CRE} concentrations and water fluoride level as reported by the WTP ($r = 0.50$ to 0.52); these moderate correlations remained after we multiplied the number of glasses of water

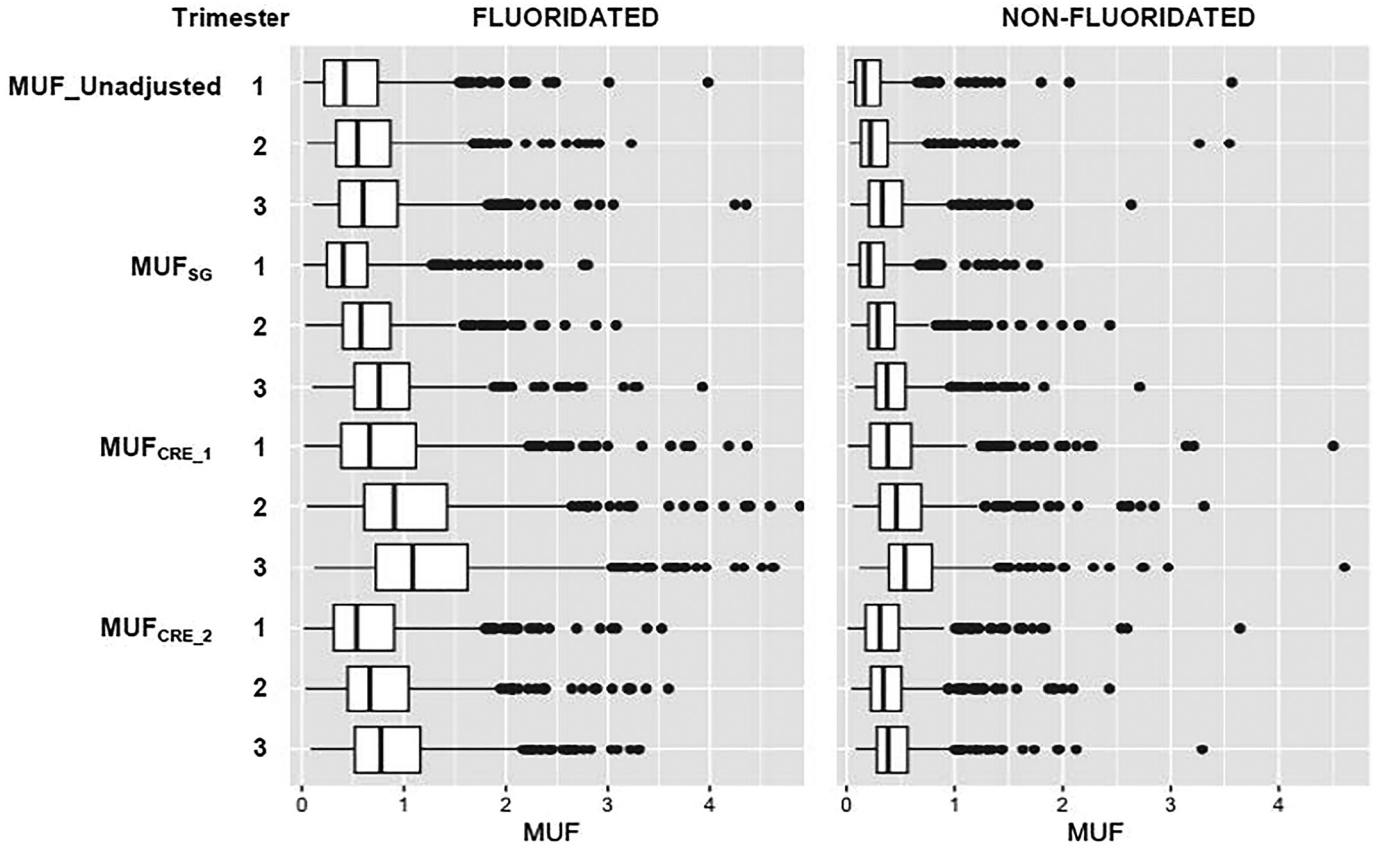

Figure 1. Fluoride concentrations by trimester in the urine of pregnant women from the MIREC cohort living in fluoridated versus nonfluoridated communities. MUF (maternal urinary fluoride) levels are shown unadjusted and adjusted for specific gravity (MUF_{SG}; Equation 1) and creatinine using two different methods (MUF_{CRE_1} and MUF_{CRE_2}; Equations 2 and 3, respectively). Box plots display the upper and lower quartiles of the data; the median is marked by the vertical line inside the box. The whiskers show the 5th and 95th percentile, whereas the individual data points represent values that exceed the 95th percentile. Box plots were produced after removing outliers defined as a MUF concentration ≥5.

consumed by the estimated amount of fluoride that would be found in a 200-mL cup of tap water ($r = 0.47$–0.48). Finally, higher MUF_{SG} and MUF_{CRE} concentrations correlated with number of cups of black ($r = 0.31$ to 0.32) but not green tea ($r = 0.04$–0.06); the estimated amount of fluoride intake from tea consumption (factoring in fluoride from an average cup of black tea as well as from a 200-mL cup of tap water) was also correlated with both MUF_{SG} and MUF_{CRE} concentrations ($r = 0.16$–0.18).

Linear Regression Using Water Fluoridation Levels

To determine whether the relationship between WTP fluoride level and MUF concentration differed as a function of the different urinary dilution correction methods that were used, we fit separate regression models using unadjusted MUF, MUF_{SG}, and $MUF_{CRE1/2}$ concentration. WTP fluoride level significantly predicted $\log_{10}$-transformed MUF_{SG} concentrations and accounted for approximately 24% of the variance, Model 1: $R^2 = 0.24$, $F(1,1134) = 361.9$, $p < 0.0001$ (Table 4). After controlling for covariates, WTP fluoride levels remained a significant predictor of $\log_{10}$-transformed MUF_{SG} (B = 0.48, 95% CI: 0.43, 0.53), accounting for approximately 22% of the variance. Model 2 was slightly stronger (B = 0.52, 95% CI: 0.46, 0.57) than Model 1, accounting for 24% of the variance in predicting $\log_{10}$-transformed MUF_{CRE} concentration after adjusting for covariates (Table 4). These findings show that a 0.5-mg/L increase in water fluoride, which is roughly the difference in water fluoride level among cities that are fluoridated versus nonfluoridated in our study, would result in an increase of 73.8% and 82.0% in MUF_{SG} and MUF_{CRE} concentrations, respectively.

We examined models predicting log-transformed MUF (unadjusted) levels at each trimester by WTP fluoride levels before and after controlling for covariates, including the addition of urinary CRE and urinary SG (i.e., MUF_{CRE_cov}, MUF_{SG_cov}). Partial R^2 values for WTP fluoride levels ranged from 0.10 to 0.16 when creatinine was used a covariate versus 0.09 to 0.14 when SG was used a covariate (Table 5). These values were lower than the partial R^2 in Models 1 and 2 (0.22 and 0.24, respectively), although this difference can be explained by the high correlation between CRE and SG with MUF concentration. The associations were somewhat stronger between WTP fluoride and MUF concentration for CRE than for SG as a covariate.

Discussion

We measured fluoride levels in urine samples collected during each trimester from 1,566 pregnant women living in fluoridated and nonfluoridated communities in a Canadian pregnancy cohort. We found that mean urinary fluoride values were almost two times higher for pregnant women living in fluoridated regions than for those in nonfluoridated regions (Table 2). The differences in MUF concentration remained significant after adjustment for relevant covariates. Urinary fluoride levels were significantly lower among women living in nonfluoridated regions, despite the so-called diffusion or halo effect (Griffin et al. 2001; Ripa 1993), which refers to the extension of fluoridation to residents of nonfluoridated communities as a result of foods and beverages that

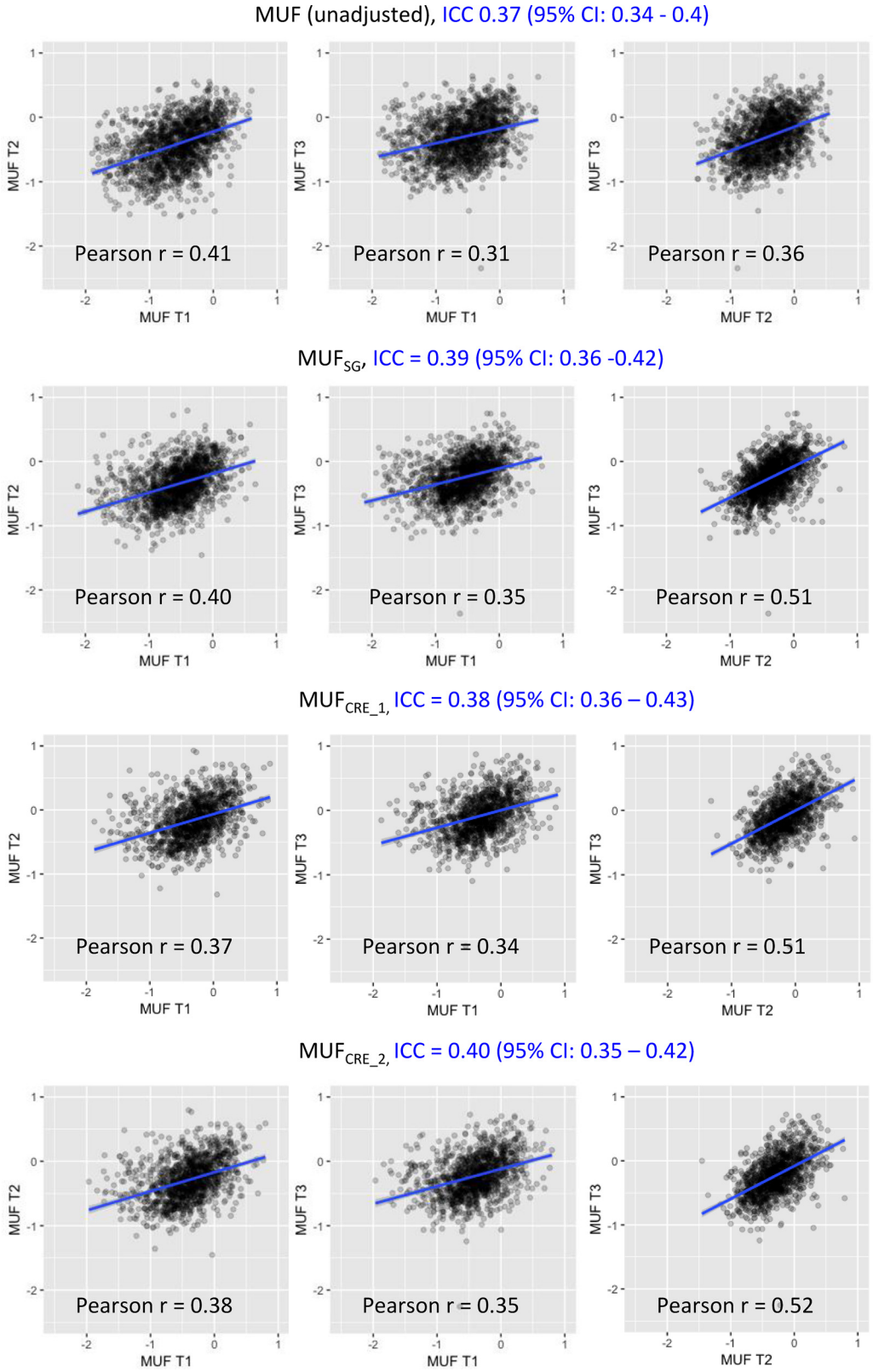

Figure 2. Pearson *r* correlations between pairs of trimesters (T1, T2, T3) and intraclass correlation coefficients (ICCs) across trimesters [with 95% confidence interval (CI)] for $\log_{10}$-transformed maternal urinary fluoride (MUF) levels without adjustment, with adjustment for specific gravity (MUF_{SG}), and with adjustment for creatinine using two methods of adjustment (MUF_{CRE_1} and MUF_{CRE_2}). Individual data points represent individual observations, solid lines represent regression lines.

are commercially processed in fluoridated areas and consumed in nonfluoridated communities. Measuring fluoride exposure as a function of CWF status is therefore essential, especially given that the prevalence and severity of dental fluorosis (evidence for excessive ingestion of fluoride) is higher among youth living in fluoridated regions (Beltrán-Aguilar et al. 2010; Warren

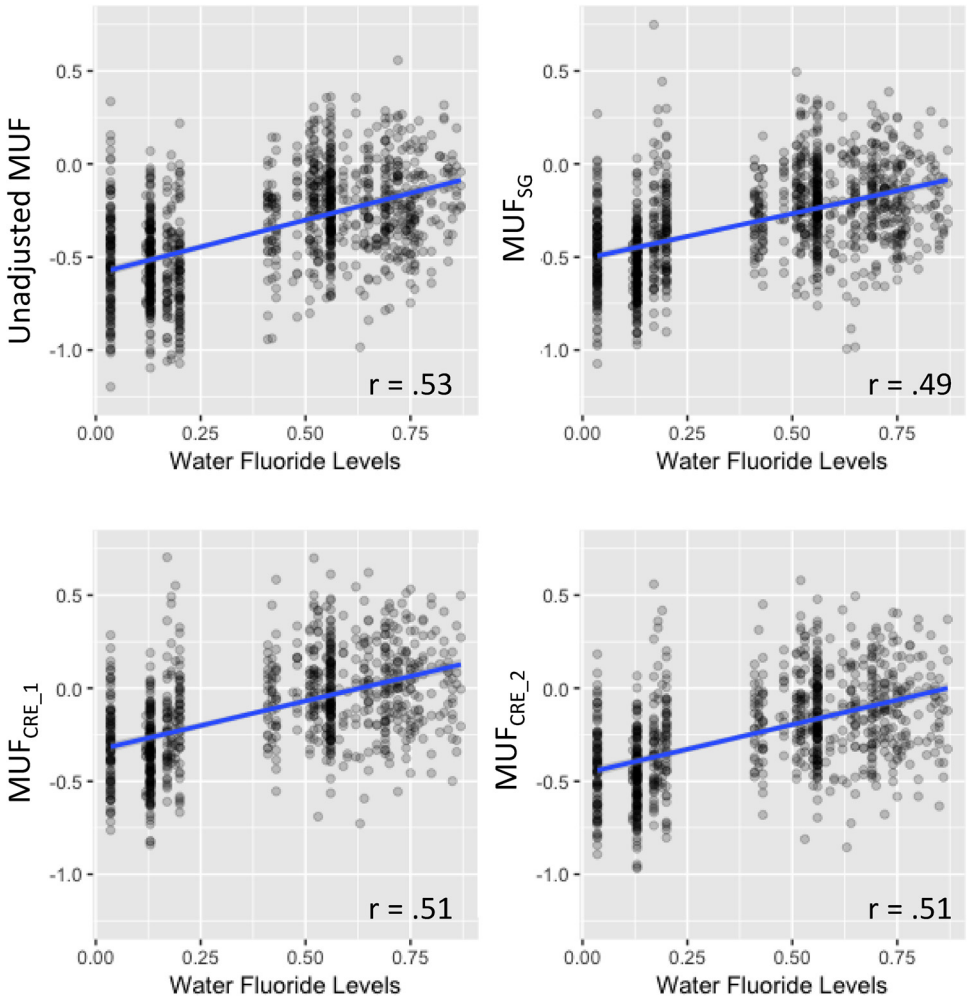

Figure 3. Log_{10}-transformed maternal urinary fluoride (MUF) exposure levels as a function of water treatment plant fluoride levels. MUF levels are shown unadjusted and adjusted for specific gravity (MUF_{SG}) and creatinine using two different methods (MUF_{CRE_1} and MUF_{CRE_2}). Individual data points represent individual observations. Solid lines represent regression lines.

et al. 1999), reflecting the widespread availability of fluoride. Differences in urinary fluoride level as a function of CWF status are consistent with those reported in another Canadian sample of respondents, 3 to 79 y of age, who participated in Cycle 2 (2009–2011) of the Chemical Health Measures Survey (CHMS) (McLaren 2016).

MUF levels increased from Trimesters 1 to 3 for all of the methods used to correct for urinary dilution, consistent with prior studies conducted in pregnant women in urine (Opydo-Szymaczek and Borysewicz-Lewicka 2005; Valdez Jiménez et al. 2017) and blood plasma (Opydo-Szymaczek and Borysewicz-Lewicka 2006). This linear increase may reflect a number of potential mechanisms that change over the course of fetal development and pregnancy, such as the higher uptake of fluoride into fetal bone in the first trimester compared with the third trimester when fetal bone tissues are mineralized. In contrast with our study, some other studies have reported decreasing levels of MUF over the course of pregnancy (Gedalia et al. 1959; Thomas et al. 2016). In the ELEMENT cohort, MUF concentrations were measured (Thomas et al. 2016) in over 500 women living in Mexico City. However, the time points included broad and overlapping intervals that were defined as early (0–26 wk), mid (15–

Table 2. Comparison of maternal urinary fluoride adjusted for covariates (BMI, maternal age, smoking status, glasses of water, as well as black and green tea consumption) as a function of residential fluoridation status. Values reported represent data from individuals who had valid urinary fluoride measurements at all three time points.

	Fluoridated					Non-fluoridated						
Fluoride measures	*n*	Mean	Median	SD	Range	*n*	Mean	Median	SD	Range	*F*-Value	*p*-Value
MUF_Unadjusted (mg/L)	672	0.70	0.61	0.40	0.10–3.61	464	0.34	0.28	0.24	0.06–2.17	465.8	<0.0001
MUF_{SG} (mg/L)	672	0.71	0.62	0.38	0.10–3.12	463	0.41	0.34	0.28	0.08–2.78	347.1	<0.0001
MUF_{CRE_1} (mg/g)	528	1.15	0.99	0.65	0.19–4.18	369	0.60	0.50	0.40	0.14–3.56	309.7	<0.0001
MUF_{CRE_2} (mg/L)	530	0.87	0.74	0.50	0.14–3.80	370	0.46	0.38	0.34	0.11–3.62	305.4	<0.0001
WTP fluoride level (mg/L)	813	0.61	0.56	0.11	0.41–0.87	546	0.12	0.13	0.06	0.04–0.20	8562.6	<0.0001
SG	729	1.014	1.013	0.005	1.002–1.030	476	1.012	1.012	0.005	1.002–1.028	31.29	<0.0001
Creatinine (grams)	536	6.66	6.17	3.61	0.93–23.84	374	6.03	5.51	3.50	1.07–32.67	6.75	0.01

Note: BMI, body mass index; CRE, creatinine; MUF, maternal urinary fluoride; SD, standard deviation; SG, specific gravity; WTP, water treatment plant.

Table 3. Pearson correlations between different derivations of $\log_{10}$-transformed MUF averaged across three trimesters.

Covariates	Log MUF_{SG}	*p*-Value	Log MUF_{CRE_1}[a]	*p*-Value
Prepregnancy BMI	0.03	0.33	−0.10	0.001
Maternal age at delivery	0.12	<0.0001	0.17	<0.0001
Smoking during pregnancy (yes/no)	0.05	0.03	0.02	0.61
Level of maternal education[d]	0.05	0.03	0.09	0.001
Income level[e]	−0.02	0.36	−0.03	0.31
Water fluoride level (reported by WTP)	0.50	<0.0001	0.52	<0.0001
No. glasses of water	0.14	<0.0001	0.15	<0.0001
Estimated amount of fluoride in a 200-mL cup of tap water multiplied by no. of glasses of water reported to be consumed per day	0.47	<0.0001	0.48	<0.0001
No. cups of black tea	0.32	<0.0001	0.31	<0.0001
Estimated amount of fluoride in a 200-mL cup of black tea multiplied by no. of cups of tea reported to be consumed per day[b]	0.18	<0.0001	0.16	<0.0001
No. cups of green tea	0.06	0.01	0.04	0.13
Time since last void[c]				
Trimester 1	−0.08	0.004	−0.12	<0.0001
Trimester 3	−0.12	<0.0001	−0.16	<0.0001
Time at void[c]				
Trimester 1	0.07	0.002	0.09	0.0002
Trimester 3	0.07	0.005	0.09	0.01

Note: BMI, body mass index; CRE, creatinine; MUF, maternal urinary fluoride; SD, standard deviation; SG, specific gravity; WTP, water treatment plant.
[a]Data not shown for MUF_{CRE_2} because the results were identical to MUF_{CRE_1}.
[b]Mean fluoride concentration of tea is based on the average fluoride content in tea when made with deionized water (i.e., 2.6 mg/L; Waugh et al. 2016); thus the fluoride intake from one cup of tea (assuming 200 mL) would be 0.52 mg. We then added the amount of fluoride that would be found if tap water was used to make the tea (i.e., if water is fluoridated at 0.7 mg F/L, then an additional 0.14 mg F would be added to the tea for a total of 0.66 mg F per 200-mL cup of tea).
[c]Urine collection time variables were not collected at Trimester 2.
[d]Education categories were based on the following seven classifications: 0, ≤Grade 8; 1, some high school; 2, high school diploma; 3, some college; 4, college diploma; 5, trade school diploma; 6, undergraduate degree, 7, graduate degree.
[e]Income categories were based on the following nine classifications: 0, ≤$10,000; 1, $10,001–20,000; 2, $20,001–30,000; 3, $30,001–40,000; 4, $40,001–50,000; 5, $50,001–60,000; 6, $60,001–70,000; 7, $70,001–80,000; 8, $80,001–100,000; 9, ≥$100,000.

37 wk), and late (22–43 wk), which may have diluted trimester-specific effects. Moreover, only 71 of the women provided samples at all three time points and some of the women were sampled at zero weeks of pregnancy (i.e., not yet pregnant), which would inflate measurements in the early stage of pregnancy given that nonpregnant women have higher levels of urinary fluoride compared with pregnant women (Opydo-Szymaczek and Borysewicz-Lewicka 2005).

We found that pregnant women who lived in fluoridated communities in Canada had mean $MUF_{\_2}$ creatinine-adjusted concentrations (0.87 mg/L; range: 0.14–3.80) that fall within a similar range as the creatinine-adjusted levels reported among pregnant women living in nonendemic fluorosis areas in Mexico City (0.91 mg/L; range: 0.02–3.67) (Thomas et al. 2016). The similarity in MUF concentrations between the Canadian and Mexican pregnancy cohorts is of scientific and public health relevance given recent findings showing an inverse association between prenatal fluoride exposure and child IQ at 4 y of age and between 6 and 12 y of age in nearly 300 mother–child pairs (Bashash et al. 2017). At the time of the publication of the paper by Bashash et al., there were no available data on urinary fluoride exposure of pregnant women exposed to fluoridated water to assess the applicability of their findings. Our results therefore provide an important comparison point. However, the Mexican population was mainly exposed to fluoride through ingestion of salt (fluoridated to 230 ppm), not artificially fluoridated water. This difference in fluoride source does not permit direct comparison of fluoride exposure among the two populations because we were unable to determine whether people in communities with fluoridated water had the same level of fluoride ingestion as those who consumed fluoridated salt in Mexico.

We also examined factors that could contribute to fluoride exposure or metabolism, including women's age, prepregnancy BMI, education, income level, water and tea consumption, and fluoride level of the woman's drinking-water supply. Older age was associated with higher urinary fluoride concentration, consistent with prior findings showing higher fluoride content in bone with increasing age in women (Mostafaei et al. 2015). Higher education was weakly and positively associated with urinary fluoride concentration ($r < 0.10$), whereas income level and

Table 4. Comparison of beta coefficients for dilution-adjusted MUF linear regression models. Models 1 and 2 predict MUF_{SG} and MUF_{CRE_2} concentrations by water treatment plant fluoridation levels before and after controlling for covariates.

	Model 1 Log MUF_{SG} ($n = 1{,}136$)			Model 2[a] Log MUF_{CRE_2} ($n = 900$)		
Covariates	R^2	B coefficient	95% CI	R^2	B coefficient	95% CI
Unadjusted Model						
WTP fluoride level	0.24	0.49	0.44, 0.54	0.26	0.53	0.47, 0.59
Adjusted Model	0.33			0.35		
WTP fluoride level	0.22	0.48	0.43, 0.53	0.24	0.52	0.46, 0.57
Prepregnancy BMI	0.00	0.00	−0.00, 0.00	0.01	−0.01	−0.01, 0.00
Maternal age at delivery	0.02	0.01	0.00, 0.01	0.02	0.01	0.00, 0.01
Smoking during pregnancy	0.00	0.01	−0.01, 0.03	0.00	−0.00	−0.03, 0.02
No. glasses of water	0.02	0.02	0.01, 0.03	0.03	0.02	0.01, 0.02
No. cups of black tea	0.07	0.12	0.10, 0.14	0.05	0.10	0.08, 0.13
No. cups of green tea	0.00	0.08	0.01, 0.15	0.00	0.06	−0.02, 0.13

Note: BMI, body mass index; CI, confidence interval; CRE, creatinine; MUF, maternal urinary fluoride; SG, specific gravity; WTP, water treatment plant.
[a]Data not shown for MUF_{CRE_1} because the results were identical to MUF_{CRE_2}.

Table 5. Beta coefficients and R^2 for linear regression models predicting log-transformed MUF (unadjusted) levels by water fluoride levels before and after controlling for covariates. Model 3 includes creatinine level at each trimester (i.e., MUF_{CRE_cov}) and Model 4 includes specific gravity level (MUF_{SG_cov}) at each trimester.

	Log $MUF_{\_trimester1}$ ($n = 1{,}317$)			Log $MUF_{\_trimester2}$ ($n = 1{,}251$)			Log $MUF_{\_trimester3}$ ($n = 1{,}203$)		
Covariates	R^2	B coefficient	95% CI	R^2	B coefficient	95% CI	R^2	B coefficient	95% CI
Unadjusted WTP fluoride level	0.17	0.75	0.66, 0.84	0.23	0.70	0.63, 0.77	0.13	0.47	0.40, 0.54
Model 3: After covariate adjustment	0.33			0.45			0.48		
Creatinine	0.21	0.04	0.04, 0.04	0.25	0.04	0.04, 0.04	0.29	0.04	0.04, 0.05
WTP fluoride level	0.10	0.58	0.50, 0.66	0.16	0.59	0.53, 0.65	0.16	0.50	0.44, 0.56
Prepregnancy BMI	0.00	−0.00	−0.01, 0.00	0.00	−0.00	−0.01, 0.00	0.00	−0.00	−0.00, 0.00
Maternal age at delivery	0.01	0.01	0.00, 0.01	0.01	0.01	0.00, 0.01	0.01	0.01	0.00, 0.08
Smoking during pregnancy	0.00	0.02	−0.00, 0.05	0.00	0.01	−0.01, 0.03	0.00	0.02	−0.00, 0.04
No. glasses of water	0.00	0.01	0.00, 0.02	0.00	0.01	0.00, 0.01	0.00	0.01	0.00, 0.01
No. cups of black tea	0.01	0.10	0.06, 0.14	0.03	0.11	0.08, 0.14	0.02	0.08	0.06, 0.11
No. cups of green tea	0.00	0.07	−0.03, 0.16	0.00	0.02	0.04, 0.04	0.00	−0.02	−0.09, 0.05
Model 4: After covariate adjustment	0.31			0.48			0.50		
SG	0.21	29.35	26.58, 32.12	0.30	30.15	28.03, 32.30	0.32	29.00	26.83, 31.16
WTP fluoride level	0.09	0.57	0.49, 0.65	0.14	0.57	0.51, 0.62	0.13	0.47	0.42, 0.53
Prepregnancy BMI	0.00	0.00	−0.01, 0.00	0.00	0.00	−0.00, 0.00	0.00	0.00	−0.00, 0.00
Maternal age at delivery	0.00	0.00	0.00, 0.01	0.01	0.01	0.00, 0.01	0.01	0.01	0.00, 0.01
Smoking during pregnancy	0.00	0.02	−0.00, 0.05	0.00	0.01	−0.01, 0.03	0.00	0.02	0.00, 0.04
No. glasses of water	0.00	0.01	0.00, 0.02	0.00	0.01	0.00, 0.02	0.01	0.01	0.01, 0.02
No. black cups of tea	0.01	0.10	0.07, 0.14	0.03	0.11	0.09, 0.14	0.03	0.11	0.08, 0.13
No. cups of green tea	0.00	0.07	−0.03, 0.16	0.00	0.04	−0.04, 0.12	0.00	0.01	−0.05, 0.08

Note: BMI, body mass index; CI, confidence interval; COV, covariance; CRE, creatinine; MUF, maternal urinary fluoride; SG, specific gravity; WTP, water treatment plant.
*$p < 0.01$.

prepregnancy BMI were not associated. The strongest correlate of MUF_{SG} and MUF_{CRE} concentration was water fluoride level, indicating that artificially fluoridated drinking water is a major source of fluoride intake. Specifically, for every 0.5-mg/L increase in water fluoride level, we would expect to see a 74–82% increase in urinary fluoride concentration. These findings are consistent with prior studies showing that fluoride levels in drinking water are closely related to those in urine in adults (Paez and Dapas 1983), children and adults (Zipkin et al. 1956), and pregnant women (Opydo-Szymaczek and Borysewicz-Lewicka 2005).

Black tea consumption was also a significant predictor of MUF levels, accounting for approximately 5% of the variance. Black teas have high concentrations of natural fluoride due to the accumulation of fluoride in tea leaves from the soil (Fung et al. 1999), and the bioavailability of fluoride in tea is close to that of sodium fluoride (U.S. EPA 2010; Waugh et al. 2016). In the Republic of Ireland, where consumption of black tea is among the highest in the world per capita, the total dietary intake of fluoride from tea can exceed the upper tolerable intake limit for both adults and children (Waugh et al. 2016). In our sample, however, the contribution of tea consumption to MUF was minor compared with fluoride intake from public drinking water.

Comparison across the different methods of controlling for urinary dilution revealed several important observations. First, the unadjusted MUF concentration (fluoridated: 0.70 mg/L) was similar with the SG-adjusted MUF concentration (fluoridated: 0.71 mg/L) whereas both creatinine-adjustment methods produced the highest MUF concentrations (i.e., $MUF_{CRE1} = 1.16$ mg/g and $MUF_{CRE2} = 0.87$ mg/L). Second, ICC values for consistency across trimesters were slightly higher when correction methods were used (either SG- or CRE-adjustment ratios) relative to no adjustment for variations in urine dilution. The ICC values between SG and creatinine were about the same (0.39 vs. 0.40), suggesting that the two urinary dilution correction factors are interchangeable. Notably, the ICC in the present study was considerably higher than that reported in a Mexican study of pregnant women (i.e., ICC = 0.25) (Thomas et al. 2016), which is likely related to the tight control of sampling at each time point in the present study and our larger sample size. Moreover, the correlation between MUF_{SG} and $MUF_{CRE1/2}$ concentration was high ($r = 0.91$), suggesting minimal variability between these two correction factors. Third, WTP fluoride level regressed on MUF concentration revealed only a slight advantage for the model, adjusting for urinary creatinine, as compared with SG (R^2: 0.35 vs. 0.33). These findings suggest that both correction standards are appropriate methods, with MUF concentration adjusted for creatinine being slightly stronger in terms of predicting water fluoride level. The same pattern was revealed when creatinine was added as a covariate to the model as compared with SG as a covariate.

Our ability to compare the urinary fluoride data with an external source of fluoride (public drinking water) is an important strength of the study. It is notable that minimum and maximum concentrations of fluoride in public drinking-water supplies differed substantially across cities and from year to year (see Table S3). Water fluoride concentrations were lower in 2011–2012 than in 2008–2010. To reduce time-varying changes in water fluoride data, our methods carefully matched water fluoride data with the 9-month period that overlapped with each woman's pregnancy. Our ability to take the average of repeated (in most cases daily) fluoride measurements from each WTP outweighs individual measurement of fluoride from the water tap in the home. WTPs that did not add fluoridation chemicals to public drinking-water supplies did not measure fluoride levels as frequently as the sites that added fluoridation chemicals. However, it is unlikely that the reduced frequency of testing fluoride levels affected our results given that the range of exposure levels was much lower in non-fluoridated areas.

There are several limitations of this study. First, overnight fasting or 24-h urine samples are considered to be the optimal dosimeter for measuring chronic fluoride exposure (WHO 2014). In contrast, the present study measured the concentration of fluoride in a spot urine sample that did not control for recent fluoride ingestion. Urinary fluoride concentration does not measure total exposure (intake) nor did we estimate the 24-h daily urinary fluoride excretion level, which would require multiplying our fluoride:creatinine ratio by a standard creatinine value (not established to our

knowledge for each trimester of pregnancy). A spot urine sample is limited due to diurnal variations and the influence of diet (e.g., high vegetable intake associated with higher fluoride excretion) or intake of high-fluoride foods or beverages immediately before sample collection. In general, the measurement of urinary fluoride concentration may be influenced by the rapid elimination of fluoride from the body (biologic half life of $\sim$6 h) (Whitford 1994), urinary pH levels (Buzalaf et al. 2015), as well as variation in creatinine excretion by muscle mass, age, sex, and other factors (Barr et al. 2005; Aylward et al. 2015). Assessment of fluoride during pregnancy introduces additional challenges because many physiological changes (e.g., maternal bone metabolism) are occurring that can affect the interpretation of urinary fluoride analyses (Andra et al. 2015). To enhance our measurement, we therefore measured urinary fluoride at three time points, providing a more sensitive measurement of MUF concentration than if only one measurement were used. We only included participants who had valid fluoride measurements at each trimester in the analysis in order to control for trimester-related differences in urinary fluoride level. The modest ICC for MUF concentration in the present study suggests that exposure to fluoride (through typical water/beverage consumption habits and dental product use) occurred throughout the day in our sample, which in turn, minimized the degree of within- and between-subject variation. This notion is further supported by the relatively weak correlations between both time since last void and time at void and MUF concentration. Indeed, strong correlations ($r = 0.87$–0.94) have been reported between the fluoride:creatinine ratio on a morning spot urine sample and fluoride excretion in a 24-h urine sample for preschool children (Villa et al. 2010; Zohouri et al. 2006), indicating that adjustment for urinary dilution approximates a 24-h biomarker. A second limitation is that the MIREC Study is not a nationally representative sample of the Canadian population of pregnant women. Nonetheless, the MIREC Study, which involves women from 10 major cities across Canada, is the largest study to date assessing fluoride exposures in pregnant women. Third, water fluoride concentrations were assigned to each woman based on the aggregation of quarterly GMs and matched to the woman's postal codes at the third trimester. This method may have introduced some variability if the pregnancy period did not align exactly with each quarter or if the woman moved her residence to a new WTP zone during the pregnancy. We noted that 11% of women had different postal codes between trimesters, although we presume some of these women moved within the same WTP zone. It should also be noted that variability in our water fluoride measurement may have been introduced by combining water fluoride data across multiple municipal drinking-water systems when there was overlap in the distribution systems, as found in Toronto. However, the mean water fluoride values were similar across these overlapping WTPs (i.e., mean water fluoride values ranging within ± 0.10 mg/L of each other). Finally, information about oral hygiene product use, topical fluoride procedures, or consumption of certain foods (e.g., shellfish) and other beverages (e.g., coffee, juices) may represent other important sources of fluoride but were not measured in the present study.

In summary, the modest ICC across serial time points and the strong relationship between MUF concentration and WTP fluoride levels supports the biomarker potential of urinary fluoride concentration in pregnant women, using either SG or CRE to account for urine dilution. Given the widespread exposure to fluoride and recent findings (Bashash et al. 2017) showing reductions in child IQ with gestational exposure to fluoride, the present study is an important step in quantifying fluoride exposure, patterns of exposure, and major sources of fluoride exposure in pregnant women. Research is urgently needed to determine whether prenatal exposure to fluoride contributes to neurodevelopmental outcomes in the offspring of these women.

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Supplemental Material

Community Water Fluoridation and Urinary Fluoride Concentrations in a National Sample of Pregnant Women in Canada

Christine Till, Rivka Green, John G. Grundy, Richard Hornung, Raichel Neufeld, E. Angeles Martinez-Mier, Pierre Ayotte, Gina Muckle, and Bruce Lanphear

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Figure S2. Sample map showing regions serviced by each WTP in Montreal http://ville.montreal.qc.ca/pls/portal/docs/PAGE/EAU_FR/MEDIA/DOCUMENTS/USINE-MOD-18-SEPT.PDF

References

Table S1. Characteristics of participants who drank tap water from a public water source and were included in the study and participants who were excluded because they reported a non-public drinking water source (well water or other). Data shown only for participants who provided three urine samples. Values are means ± SD or n (%) unless otherwise indicated.

Variable	Women reporting a public water drinking source	Women reporting a drinking water source other than public water source
N*	1451	114
Age (yrs) of mother at enrollment	32.26 ± 4.98	32.82 ± 4.4
Race		
Caucasian	1234 (85.0)	112 (98.2)
Other	217 (15.0)	2 (1.8)
Marital Status		
Married or Common law	1389 (95.7)	111 (97.4)
Widowed	0 (0.0)	0 (0.0)
Divorced	4 (0.3)	1 (0.9)
Separated	3 (0.2)	0 (0.0)
Single	54 (3.7)	2 (1.8)
Other	1 (0.07)	0 (0.0)
Birth Country		
Born in Canada	1160 (79.9)	108 (94.7)
Born outside of Canada	291 (20.1)	6 (5.3)
Maternal Education		
High school or less	116 (8.0)	8 (7.1)
Some college	74 (5.1)	4 (3.5)
College diploma	322 (22.2)	34 (30.1)
University degree	938 (64.7)	67 (59.3)
Missing	1	1
Pre-pregnancy BMI, mean ± SD	24.76 ± 5.41	25.24 ± 5.58

Employed at time of pregnancy	1248 (86.0)	100 (87.7)
Net income household		
>$70,000	983 (67.7)	83 (72.8)
<$70,000	410 (28.3)	30 (26.3)
Refuse to answer or don't know	58 (4.0)	1 (0.9)
Smoking during trimester 1		
Current	70 (4.8)	7 (6.1)
Former or never smoked	1381 (95.2)	107 (93.9)
Smoking during trimester 3		
Current	64 (4.4)	5 (4.4)
Former or never smoked	1387 (95.6)	109 (95.6)
Gestational diabetes	16 (1.1)	1 (0.9)
Caffeine consumption (per day)	0.69 (0.87)	0.71 (0.75)
1 or more caffeinated beverage	1202 (83.0)	99 (
Did not drink caffeinated beverage	246 (17.0)	15 (13.1)
Alcohol consumption (beer, wine, liquor)		
No alcohol	1188 (82.0)	96 (84.2)
<1 alcoholic drink per month	169 (11.7)	12 (10.5)
≥1 alcoholic beverage per month	91 (6.3)	6 (5.3)

Abbreviations: BMI = body mass index; SD = standard deviation; yrs = years

***** One participant did not report type of water they drank; this person was omitted from this table because it was not known which category they belonged to. Data not available for some covariates.

Table S2. Water treatment plant (WTP) reports of fluoride treatment by city. Lab analysis data reported for all sites.

City	Notes about data collection
Vancouver	Fluoride levels for each WTP was documented at <0.05 mg/L for every time point measured between 2008 to 2011. The limit of detection (LoD) for fluoride at the WTPs was 0.05 mg/L. We used a correction factor, LoD/√2 (L.D. 1990), to calculate the water fluoride level for the Vancouver sites.
Edmonton	The geomean (GM) was calculated for each WTP in quarters. Because the distribution zones are only general estimates that fluctuate over time and because distribution zones often overlap, mean fluoride measurements were calculated using an average of the GMs from the 2 WTPs. Raw data used to calculate the GM were daily fluoride measurements.
Winnipeg	The GM was calculated for each pumping station in quarters. Fluoridated drinking water values for participants that live in zones that receive water from more than one pumping station were calculated by averaging the GMs from the relevant stations. Raw data used to calculate the GM were daily fluoride measurements.
Toronto	The GM was calculated for each WTP in quarters. Because the distribution zones are only general estimates that fluctuate over time and because distribution zones often overlap, fluoride measurements were calculated using an average of the GMs from the four WTPs. Raw data used to calculate the GM were daily fluoride measurements.
Sudbury	The GM was calculated for each WTP in quarters. Water distributed is a combination of water from two WTPs; therefore, fluoride measurements were calculated using an average of the GMs from both WTPs. Raw data used to calculate the GM were daily fluoride measurements
Kingston	The GM was calculated for each WTP in quarters. Fluoridated drinking water values for participants that live in zones that receive water from more than one WTP were calculated by averaging the GMs from the relevant plants. Raw data used to calculate the GM from King St WTP were fluoride measurements taken approximately five times per month in 2009 and less frequently, four times per year in the following years. Raw data used to calculate the GM from Point Pleasant WTP were fluoride measurements taken four times per year.
Montreal[a]	The GM was calculated for each WTP in quarters. Fluoridated drinking water values for participants that live in zones that receive water from more than one WTP were calculated by averaging the GMs from the relevant plants. Data used to calculate the GM from Atwater and Charles J Des Baillets WTPs were yearly average fluoride measurements provided by the city. Raw data used to calculate the GM for Dorval and Pointe-Claire WTPs were taken randomly, approximately every five days.
Halifax	The GM was calculated for each WTP in quarters. Fluoridated drinking water values

	for participants that live in zones that receive water from more than one WTP were calculated by averaging the GMs from the relevant plants. Raw data used to calculate the GM were daily fluoride measurements.
Hamilton	The GM was calculated for each WTP in quarters. Raw data used to calculate the GM were fluoride measurements taken twice daily.
Ottawa	The GM was calculated for each WTP in quarters. Water distributed is a combination of water from two WTPs, therefore fluoride measurements were calculated using an average of the GMs from the two WTPs. Raw data used to calculate the GM from Lemieux WTP were daily fluoride measurements. Raw data used to calculate the GM from the East End WTP were fluoride measurements taken approximately twice per month in 2008 and less frequently, approximately once a month in the following years.

[a] Two out of four of the WTPs in Montreal reported large gaps of time in which no fluoride measurements were taken. However, these two plants only supply water to three participants in our sample.

Abbreviations: GM = geometric mean; LoD = limit of detection; WTP = water treatment plant

Table S3. Geometric means (GM) (geometric standard deviation; GSD) by city and by year. Bolded N value refers to total number of participants in each city matched with WTP fluoride data. Non-bolded N refers to the number of participants receiving water from the specific WTP site.

Water Treatment Plant	Year	GM (GSD)	Range	N
Fluoridated				
Edmonton				— [a]
E.L. Smith	2010	0.71 (1.03)	0.65-0.75	
E.L. Smith	2011	0.55 (1.82)	0.09-0.78	
Rossdale	2010	0.71 (1.03)	0.62-0.77	
Rossdale	2011	0.70 (1.03)	0.63-0.76	
Hamilton				**184**
Highlift	2008	0.68 (1.10)	0.37-0.96	
Highlift	2009	0.56 (1.07)	0.46-0.69	
Highlift	2010	0.56 (1.05)	0.46-0.64	
Highlift	2011	0.56 (1.05)	0.38-0.66	
Highlift	2012	0.57 (1.10)	0.36-0.81	
Halifax				**138**
J.D Kline	2008	0.82 (1.17)	0.26-1.32	93 [b]
J.D Kline	2009	0.77 (1.16)	0.33-1.10	
J.D Kline	2010	0.71 (1.19)	0.27-1.04	
J.D Kline	2011	0.75 (1.18)	0.28-1.00	
Lake Major	2008	0.76 (1.12)	0.33-0.93	46 [b]
Lake Major	2009	0.69 (1.30)	0.05-1.03	
Lake Major	2010	0.62 (1.17)	0.25-0.93	
Lake Major	2011	0.65(1.20)	0.11-1.18	

Montreal				— [a]
Pointe-Claire	2009	0.67 (1.10)	0.47-0.75	
Pointe-Claire	2010	0.69 (1.08)	0.53-0.79	
Dorval	2009	0.62 (1.25)	0.21-0.82	
Dorval	2010	0.61 (1.10)	0.44-0.77	
Ottawa				**71**
Lemieux	2008	0.73 (1.04)	0.60-0.85	
Lemieux	2009	0.73 (1.05)	0.61-0.83	
Lemieux	2010	0.68 (1.04)	0.58-0.80	
Lemieux	2011	0.67 (1.04)	0.54-0.76	
East End	2008	0.69 (1.46)	0.07-0.81	
East End	2009	0.74 (1.07)	0.69-0.84	
East End	2010	0.71 (1.03)	0.67-0.74	
East End	2011	0.71 (1.04)	0.65-0.74	
Sudbury				**44**
Wanapitei	2008	0.61 (1.56)	0.08-1.15	
Wanapitei	2009	0.67 (1.26)	0.26-0.97	
Wanapitei	2010	0.59 (1.20)	0.37-0.91	
Wanapitei	2011	0.56 (1.40)	0.21-0.91	
David Street	2008	0.61 (1.56)	0.08-1.15	
David Street	2009	0.67 (1.26)	0.26-0.97	
David Street	2010	0.59 (1.20)	0.37-0.91	
David Street	2011	0.57 (1.40)	0.21-0.91	
Toronto				**283**
R.L. Clark	2008	0.41 (1.57)	0.10-0.59	
R.L. Clark	2009	0.40 (1.74)	0.12-0.62	

R.L. Clark	2010	0.51 (1.52)	0.10-0.73	
R.L. Clark	2011	0.50 (1.51)	0.12-0.70	
R.L. Clark	2012	0.56 (1.21)	0.17-0.69	
Island	2008	0.53 (1.31)	0.13-2.0	
Island	2009	0.46 (1.78)	0.11-0.68	
Island	2010	0.57 (1.40)	0.12-0.78	
Island	2011	0.61 (1.10)	0.21-0.70	
Island	2012	0.58 (1.27)	0.15-0.70	
F.J. Horgan	2008	0.48 (1.25)	0.13-1.51	
F.J. Horgan	2009	0.41 (1.64)	0.12-1.93	
F.J. Horgan	2010	0.46 (1.51)	0.13-0.68	
F.J. Horgan	2011	0.36 (1.77)	0.13-0.68	
F.J. Horgan	2012	0.40 (1.64)	0.14-0.63	
R.C. Harris	2008	0.48 (1.29)	0.10-0.60	
R.C. Harris	2009	0.38 (1.94)	0.11-0.63	
R.C. Harris	2010	0.60 (1.13)	0.18-0.73	
R.C. Harris	2011	0.56 (1.32)	0.14-0.68	
R.C. Harris	2012	0.47 (1.75)	0.12-0.70	
Winnipeg				**72**
Maclean	2009	0.83 (1.05)	0.59-0.92	38[b]
Maclean	2010	0.84 (1.03)	0.79-0.88	
Maclean	2011	0.72 (1.08)	0.63-0.86	
Maclean	2012	0.70 (1.03)	0.66-0.75	
McPhillips	2009	0.84 (1.05)	0.73-0.94	25[b]
McPhillips	2010	0.83 (1.03)	0.78-0.88	
McPhillips	2011	0.72 (1.07)	0.59-0.86	
McPhillips	2012	0.70 (1.03)	0.66-0.74	

Hurst	2009	0.83 (1.05)	0.74-0.93	46[b]
Hurst	2010	0.86 (1.04)	0.78-1.01	
Hurst	2011	0.71 (1.10)	0.57-0.90	
Hurst	2012	0.7 (1.03)	0.64-0.76	
Non-fluoridated				
Vancouver				**154**
Seymour	2008-11	0.035 [c]	n/a	
Capilano	2008-11	0.035 [c]	n/a	
Coquitlam	2008-11	0.035 [c]	n/a	
Kingston				**184**
King Street	2009	0.16 (2.04)	0.02-0.43	143[b]
King Street	2010	0.20 (1.00)	0.20-0.20	
King Street	2011	0.17 (1.41)	0.10-0.20	
King Street	2012	0.20 (1.00)	0.20-0.20	
Point Pleasant	2009	0.20 (1.00)	0.20-0.20	113[b]
Point Pleasant	2010	0.20 (1.00)	0.20-0.20	
Point Pleasant	2011	0.19 (1.58)	0.10-0.30	
Point Pleasant	2012	0.20 (1.22)	0.20-0.30	
Montreal				**208**
Atwater & Charles-J Des Baillets[d]	2008	0.13	0.11-0.15	
Atwater & Charles-J Des Baillets[d]	2009	0.13	0.13-0.13	
Atwater & Charles-J Des Baillets[d]	2010	0.13	0.13-0.13	
Atwater & Charles-J Des Baillet[d]	2011	0.11	0.11-0.11	

Total WTP fluoride values				**1359**

[a] MIREC minimum sample size requirements precluded reporting of small sample sizes for these sites.

[b] Refers to number of participants receiving water from the WTP. Participants can receive water from more than one WTP because some water distribution zones overlap.

[c] Limit of detection (LoD) is 0.05 mg/L for Vancouver sites. Values reported in the table for measurements below the LoD used an imputed value of (LoD/√2) (Hornung and Reed 1990) to calculate the water fluoride level. No variation reported because only LoD was provided for this site.

[d] Annual average reported by this WTP site

Table S4. Fluoride concentrations in the urine of pregnant women from the MIREC cohort living in fluoridated versus non-fluoridated communities.

	Trimester	N	Arith Mean	Arith SD	Geo Mean	Geo SD	Min	5%	25%	50%	75%	95%	Max
NON-FLUORIDATED													
MUF_Unadjusted	1	541	0.24	0.29	0.15	2.65	0.01	0.03	0.08	0.15	0.30	0.69	3.56
	2	509	0.32	0.33	0.23	2.22	0.03	0.06	0.13	0.22	0.38	0.90	3.54
	3	476	0.47	0.39	0.36	2.05	0.04	0.11	0.22	0.36	0.60	1.23	3.77
MUF_{SG}	1	541	0.31	0.39	0.20	2.56	0.01	0.04	0.12	0.20	0.35	0.84	4.67
	2	507	0.39	0.32	0.31	1.89	0.04	0.12	0.21	0.29	0.46	0.96	2.44
	3	475	0.48	0.32	0.40	1.78	0.08	0.17	0.28	0.38	0.56	1.09	2.71
MUF_{CRE_1}	1	533	0.50	0.50	0.35	2.40	0.01	0.08	0.22	0.37	0.60	1.41	4.5
	2	502	0.58	0.44	0.48	1.85	0.06	0.19	0.31	0.46	0.69	1.47	3.31
	3[a]	386	0.67	0.47	0.56	1.75	0.12	0.24	0.40	0.54	0.79	1.45	4.61
MUF_{CRE_2}	1	534	0.41	0.45	0.29	2.42	0.01	0.06	0.18	0.30	0.49	1.15	4.81
	2	502	0.43	0.32	0.35	1.85	0.04	0.14	0.23	0.34	0.51	1.08	2.43
	3[a]	386	0.48	0.33	0.40	1.75	0.08	0.17	0.29	0.39	0.56	1.04	3.29
FLUORIDATED													
MUF_Unadjusted	1	762	0.57	0.49	0.40	2.57	0.02	0.06	0.23	0.43	0.79	1.48	3.98
	2	728	0.71	0.53	0.56	2.03	0.04	0.17	0.35	0.56	0.89	1.68	3.77
	3	712	0.82	0.60	0.63	2.04	0.11	0.19	0.39	0.64	1.06	1.99	4.36
MUF_{SG}	1	762	0.52	0.46	0.37	2.44	0.01	0.07	0.25	0.4	0.64	1.30	3.84
	2	728	0.71	0.47	0.59	1.84	0.03	0.23	0.40	0.58	0.87	1.63	3.78
	3	711	0.88	0.55	0.74	1.81	0.08	0.27	0.51	0.77	1.08	1.89	3.97
MUF_{CRE_1}	1	757	0.83	0.68	0.60	2.44	0.01	0.12	0.39	0.65	1.09	2.19	4.89
	2	723	1.13	0.77	0.93	1.91	0.05	0.32	0.61	0.91	1.42	2.63	4.89
	3[a]	546	1.30	0.82	1.10	1.86	0.12	0.41	0.72	1.08	1.63	3.10	4.63
MUF_{CRE_2}	1	759	0.68	0.58	0.49	2.46	0.01	0.09	0.31	0.53	0.88	1.80	4.61
	2	727	0.85	0.60	0.69	1.92	0.04	0.24	0.45	0.67	1.05	2.00	4.66
	3[a]	553	0.97	0.68	0.80	1.90	0.09	0.29	0.52	0.78	1.18	2.41	4.78

[a] Trimester 3 creatinine was analyzed at a separate lab, which reflects the lower sample size relative to trimesters 1 and 2

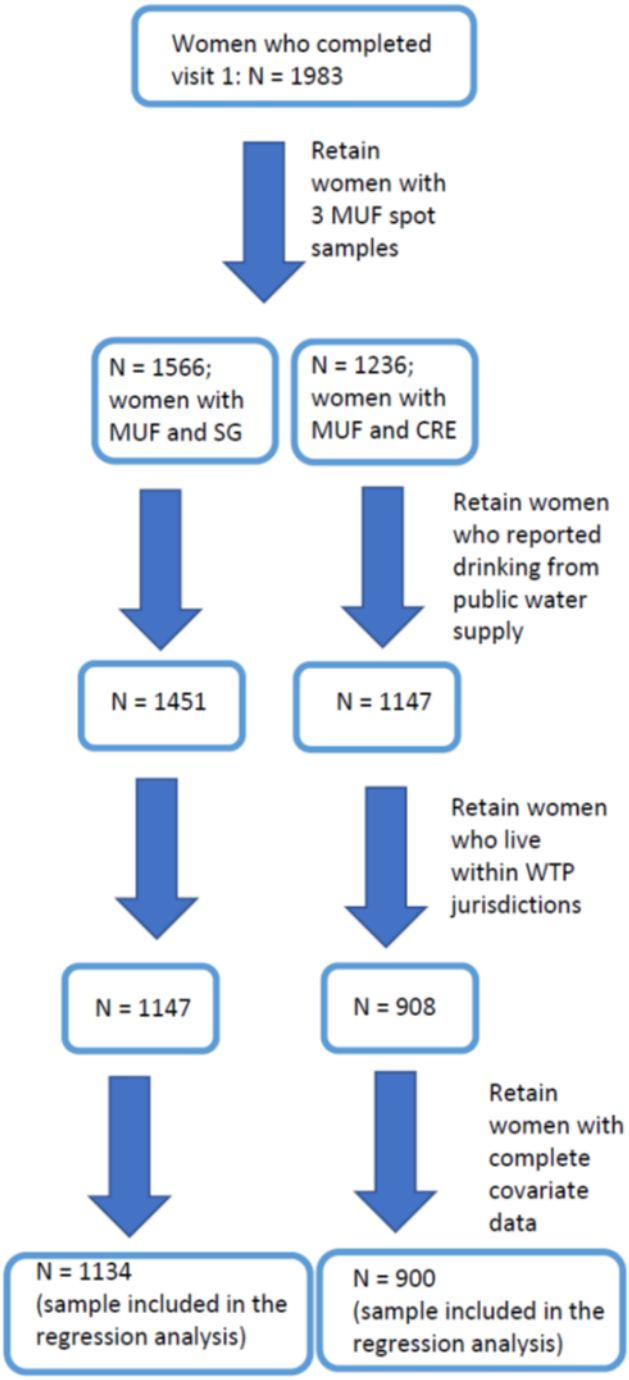
Abbreviations: MUF_{SG}: maternal urinary fluoride adjusted for specific gravity; MUF_{CRE_1}: maternal urinary fluoride adjusted for creatinine using the Hauser et al. (2004) method; MUF_{CRE_2}: maternal urinary fluoride adjusted for creatinine using the WHO (2014) method

Note: Means were calculated after removing outliers defined as a MUF concentration ≥5. The calculation of MUF_{CRE_1} was more prone to outliers relative to MUF_{CRE_2} which explains the slight differences in sample size between the two methods.

Table S5. Comparison of maternal urinary fluoride using propensity-score matching (Rosenbaum and Rubin 1983) as a function of residential fluoridation status matching on the covariates (BMI, maternal age, smoking status, of glasses of water, as well as amount of green and regular tea consumption).

Fluoride measure	Fluoridated				Non-fluoridated					
	N	Mean	Median	SD	N	Mean	Median	SD	*F*	*p*
MUF_Unadjusted	426	0.68	0.58	0.41	426	0.35	0.28	0.24	340.7	< 0.0001
MUF_{SG}	339	0.73	0.63	0.41	339	0.42	0.34	0.38	279.6	< 0.0001
MUF_{CRE_1}	339	1.22	1.03	0.69	339	0.63	0.51	0.48	277.0	< 0.0001
MUF_{CRE_2}	339	0.91	0.77	0.52	339	0.47	0.39	0.35	273.6	< 0.0001

*Units: MUF_Unadjusted =mg/L; MUF_{SG} = mg/L; MUF_{CRE_1} = mg/g; MUF_{CRE_2} = mg/L


Women who completed visit 1: N = 1983
Retain women with 3 MUF spot samples
N = 1566; women with MUF and SG
N = 1236; women with MUF and CRE
Retain women who reported drinking from public water supply
N = 1451
N = 1147
Retain women who live within WTP jurisdictions
N = 1147
N = 908
Retain women with complete covariate data
N = 1134 (sample included in the regression analysis)
N = 900 (sample included in the regression analysis)

1

Figure S1. Sample flow chart accounting for participants that were excluded from the regression analyses predicting maternal urinary fluoride adjusted for specific gravity (SG) or creatinine (CRE).

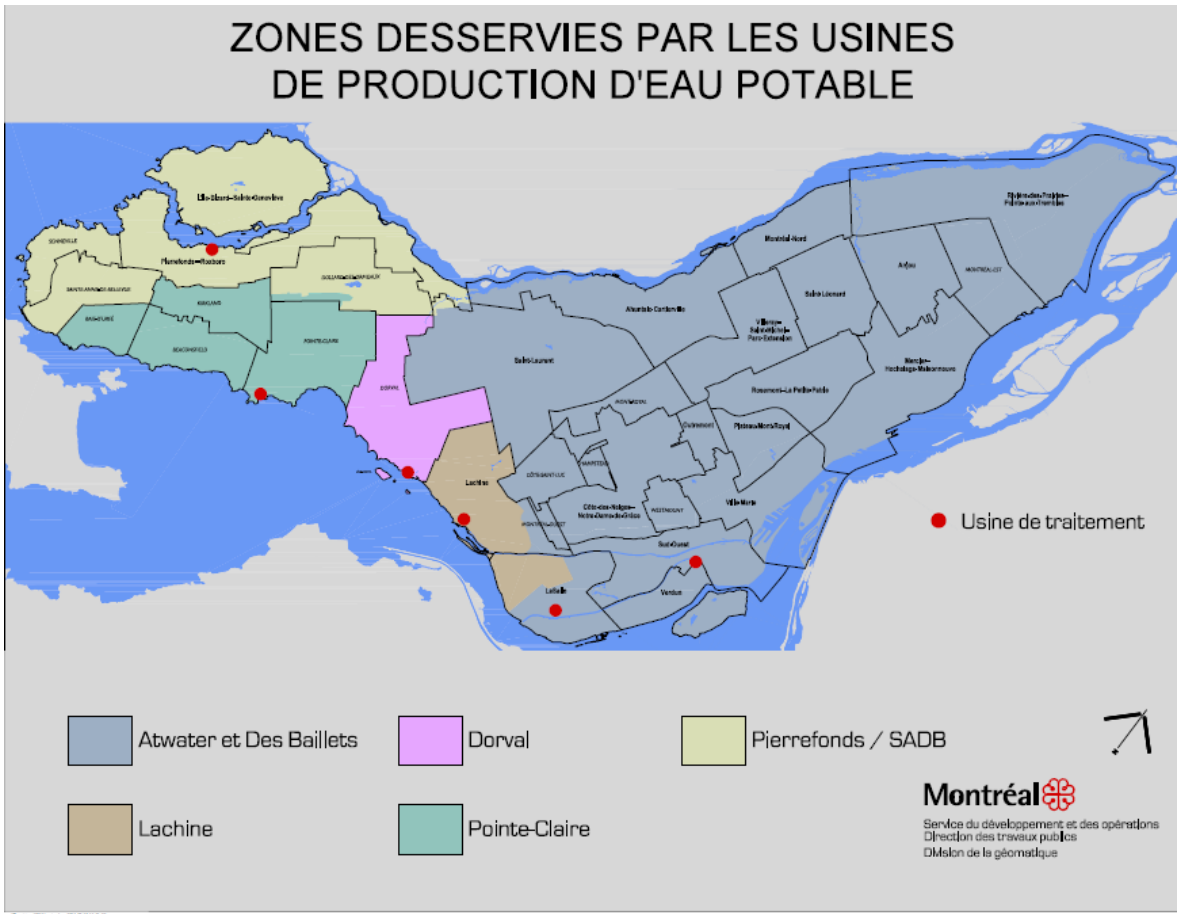


Figure S2. Sample map showing regions serviced by each WTP in Montreal

http://ville.montreal.qc.ca/pls/portal/docs/PAGE/EAU_FR/MEDIA/DOCUMENTS/USINE-MOD-18-SEPT.PDF

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JAMA Pediatrics | Original Investigation

Association Between Maternal Fluoride Exposure During Pregnancy and IQ Scores in Offspring in Canada

Rivka Green, MA; Bruce Lanphear, MD; Richard Hornung, PhD; David Flora, PhD; E. Angeles Martinez-Mier, DDS; Raichel Neufeld, BA; Pierre Ayotte, PhD; Gina Muckle, PhD; Christine Till, PhD

IMPORTANCE The potential neurotoxicity associated with exposure to fluoride, which has generated controversy about community water fluoridation, remains unclear.

OBJECTIVE To examine the association between fluoride exposure during pregnancy and IQ scores in a prospective birth cohort.

DESIGN, SETTING, AND PARTICIPANTS This prospective, multicenter birth cohort study used information from the Maternal-Infant Research on Environmental Chemicals cohort. Children were born between 2008 and 2012; 41% lived in communities supplied with fluoridated municipal water. The study sample included 601 mother-child pairs recruited from 6 major cities in Canada; children were between ages 3 and 4 years at testing. Data were analyzed between March 2017 and January 2019.

EXPOSURES Maternal urinary fluoride (MUF_{SG}), adjusted for specific gravity and averaged across 3 trimesters available for 512 pregnant women, as well as self-reported maternal daily fluoride intake from water and beverage consumption available for 400 pregnant women.

MAIN OUTCOMES AND MEASURES Children's IQ was assessed at ages 3 to 4 years using the Wechsler Primary and Preschool Scale of Intelligence-III. Multiple linear regression analyses were used to examine covariate-adjusted associations between each fluoride exposure measure and IQ score.

RESULTS Of 512 mother-child pairs, the mean (SD) age for enrollment for mothers was 32.3 (5.1) years, 463 (90%) were white, and 264 children (52%) were female. Data on MUF_{SG} concentrations, IQ scores, and complete covariates were available for 512 mother-child pairs; data on maternal fluoride intake and children's IQ were available for 400 of 601 mother-child pairs. Women living in areas with fluoridated tap water (n = 141) compared with nonfluoridated water (n = 228) had significantly higher mean (SD) MUF_{SG} concentrations (0.69 [0.42] mg/L vs 0.40 [0.27] mg/L; $P = .001$; to convert to millimoles per liter, multiply by 0.05263) and fluoride intake levels (0.93 [0.43] vs 0.30 [0.26] mg of fluoride per day; $P = .001$). Children had mean (SD) Full Scale IQ scores of 107.16 (13.26), range 52-143, with girls showing significantly higher mean (SD) scores than boys: 109.56 (11.96) vs 104.61 (14.09); $P = .001$. There was a significant interaction ($P = .02$) between child sex and MUF_{SG} (6.89; 95% CI, 0.96-12.82) indicating a differential association between boys and girls. A 1-mg/L increase in MUF_{SG} was associated with a 4.49-point lower IQ score (95% CI, −8.38 to −0.60) in boys, but there was no statistically significant association with IQ scores in girls ($B = 2.40$; 95% CI, −2.53 to 7.33). A 1-mg higher daily intake of fluoride among pregnant women was associated with a 3.66 lower IQ score (95% CI, −7.16 to −0.14) in boys and girls.

CONCLUSIONS AND RELEVANCE In this study, maternal exposure to higher levels of fluoride during pregnancy was associated with lower IQ scores in children aged 3 to 4 years. These findings indicate the possible need to reduce fluoride intake during pregnancy.

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Audio and Supplemental content

Author Affiliations: Author affiliations are listed at the end of this article.

Corresponding Author: Christine Till, PhD, Department of Psychology, York University, 4700 Keele St, Toronto, ON M3J 1P3, Canada (ctill@yorku.ca).

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For decades, community water fluoridation has been used to prevent tooth decay. Water fluoridation is supplied to about 66% of US residents, 38% of Canadian residents, and 3% of European residents.[1] In fluoridated communities, fluoride from water and beverages made with tap water makes up 60% to 80% of daily fluoride intake in adolescents and adults.[2]

Fluoride crosses the placenta,[3] and laboratory studies show that it accumulates in brain regions involved in learning and memory[4] and alters proteins and neurotransmitters in the central nervous system.[5] Higher fluoride exposure from drinking water has been associated with lower children's intelligence in a meta-analysis[6] of 27 epidemiologic studies and in studies[7,8] including biomarkers of fluoride exposure. However, most prior studies were cross-sectional and conducted in regions with higher water fluoride concentrations (0.88-31.6 mg/L; to convert to millimoles per liter, multiply by 0.05263) than levels considered optimal (ie, 0.7 mg/L) in North America.[9] Further, most studies did not measure exposure during fetal brain development. In a longitudinal birth cohort study involving 299 mother-child pairs in Mexico City, Mexico, a 1-mg/L increase in maternal urinary fluoride (MUF) concentration was associated with a 6-point (95% CI, −10.84 to −1.74) lower IQ score among school-aged children.[10] In this same cohort, MUF was also associated with more attention-deficit/hyperactivity disorder–like symptoms.[11] Urinary fluoride concentrations among pregnant women living in fluoridated communities in Canada are similar to concentrations among pregnant women living in Mexico City.[12] However, it is unclear whether fluoride exposure during pregnancy is associated with cognitive deficits in a population receiving optimally fluoridated water.

This study examined whether exposure to fluoride during pregnancy was associated with IQ scores in children in a Canadian birth cohort in which 40% of the sample was supplied with fluoridated municipal water.

Key Points

Question Is maternal fluoride exposure during pregnancy associated with childhood IQ in a Canadian cohort receiving optimally fluoridated water?

Findings In this prospective birth cohort study, fluoride exposure during pregnancy was associated with lower IQ scores in children aged 3 to 4 years.

Meaning Fluoride exposure during pregnancy may be associated with adverse effects on child intellectual development, indicating the possible need to reduce fluoride intake during pregnancy.

Methods

Study Cohort

Between 2008 and 2011, the Maternal-Infant Research on Environmental Chemicals (MIREC) program recruited 2001 pregnant women from 10 cities across Canada. Women who could communicate in English or French, were older than 18 years, and were within the first 14 weeks of pregnancy were recruited from prenatal clinics. Participants were not recruited if there was a known fetal abnormality, if they had any medical complications, or if there was illicit drug use during pregnancy. Additional details are in the cohort profile description.[13]

A subset of 610 children in the MIREC Study was evaluated for the developmental phase of the study at ages 3 to 4 years; these children were recruited from 6 of 10 cities included in the original cohort: Vancouver, Montreal, Kingston, Toronto, Hamilton, and Halifax. Owing to budgetary restraints, recruitment was restricted to the 6 cities with the most participants who fell into the age range required for the testing during the data collection period. Of the 610 children, 601 (98.5%) completed neurodevelopmental testing; 254 (42.3%) of these children lived in nonfluoridated regions and 180 (30%) lived in fluoridated regions; for 167 (27.7%) fluoridation status was unknown owing to missing water data or reported not drinking tap water (**Figure 1**).

This study was approved by the research ethics boards at Health Canada, York University, and Indiana University. All women signed informed consent forms for both mothers and children.

Maternal Urinary Fluoride Concentration

We used the mean concentrations of MUF measured in urine spot samples collected across each trimester of pregnancy at a mean (SD) of 11.57 (1.57), 19.11 (2.39), and 33.11 (1.50) weeks of gestation. Owing to the variability of urinary fluoride measurement and fluoride absorption during pregnancy,[14] we only included women who had all 3 urine samples. In our previous work, these samples were moderately correlated; intraclass correlation coefficient (ICC) ranged from 0.37 to 0.40.[12]

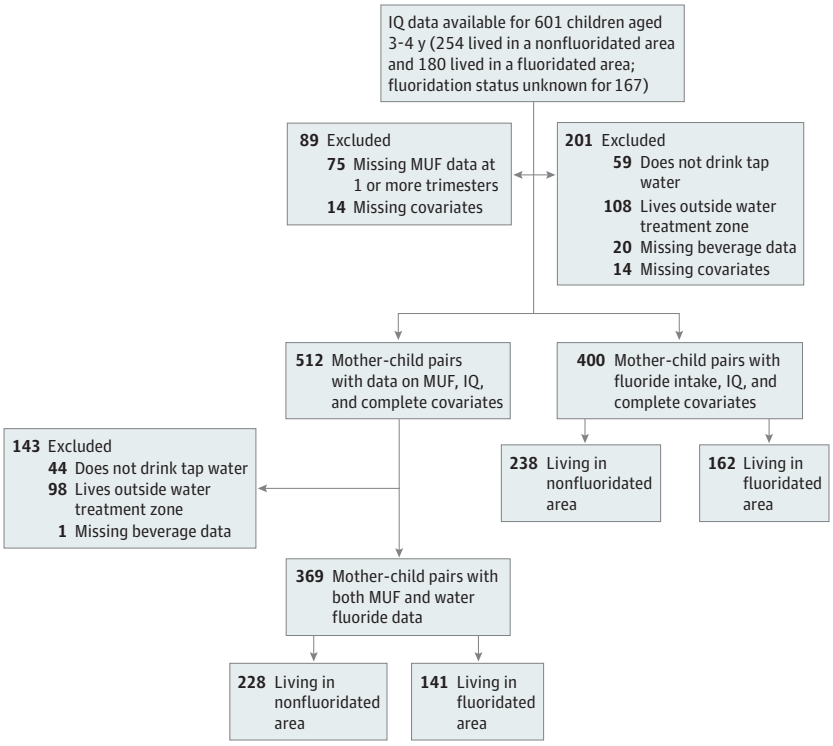
Urinary fluoride concentration was analyzed at the Indiana University School of Dentistry using a modification of the hexamethyldisiloxane (Sigma Chemical Co) microdiffusion procedure[15] and described in our previous work.[12] Fluoride concentration could be measured to 0.02 mg/L. We excluded 2 samples (0.002%) because the readings exceeded the highest concentration standard (5 mg/L) and there was less certainty of these being representative exposure values.

To account for variations in urine dilution at the time of measurement, we adjusted MUF concentrations for specific gravity (SG) using the following equation: $MUF_{SG} = MUF_i \times (SG_M\text{-}1)/(SG_i\text{-}1)$, where MUF_{SG} is the SG-adjusted fluoride concentration (in milligrams of fluoride per liter), MUF_i is the observed fluoride concentration, SG_i is the SG of the individual urine sample, and SG_M is the median SG for the cohort.[16] For comparison, we also adjusted MUF using the same creatinine adjustment method that was used in the 2017 Mexican cohort.[10]

Water Fluoride Concentration

Water treatment plants measured fluoride levels daily if fluoride was added to municipal drinking water and weekly or monthly if fluoride was not added to water.[12] We matched participants' postal codes with water treatment plant zones, allowing an estimation of water fluoride concentration for each woman by averaging water fluoride concentrations (in milligrams per liter) dur-

Figure 1. Flowchart of Inclusion Criteria



MUF indicates maternal urinary fluoride.

ing the duration of pregnancy. We only included women who reported drinking tap water during pregnancy.

Daily Fluoride Intake in Mothers

We obtained information on consumption of tap water and other water-based beverages (tea and coffee) from a self-report questionnaire completed by mothers during the first and third trimesters. This questionnaire was used in the original MREC cohort and has not been validated. Also, for this study, we developed methods to estimate and calculate fluoride intake that have not yet been validated. To estimate fluoride intake from tap water consumed per day (milligrams per day), we multiplied each woman's consumption of water and beverages by her water fluoride concentration (averaged across pregnancy) and multiplied by 0.2 (fluoride content for a 200-mL cup). Because black tea contains a high fluoride content (2.6 mg/L),[17,18] we also estimated the amount of fluoride consumed from black tea by multiplying each cup of black tea by 0.52 mg (mean fluoride content in a 200-mL cup of black tea made with deionized water) and added this to the fluoride intake variable. Green tea also contains varying levels of fluoride; therefore, we used the mean for the green teas listed by the US Department of Agriculture (1.935 mg/L).[18] We multiplied each cup of green tea by 0.387 mg (fluoride content in a 200-mL cup of green tea made with deionized water) and added this to the fluoride intake variable.

Primary Outcomes

We assessed children's intellectual abilities with the Wechsler Preschool and Primary Scale of Intelligence, Third Edition. Full Scale IQ (FSIQ), a measure of global intellectual functioning, was the primary outcome. We also assessed verbal IQ (VIQ), representing verbal reasoning and comprehension, and performance IQ (PIQ), representing nonverbal reasoning, spatial processing, and visual-motor skills.

Covariates

We selected covariates from a set of established factors associated with fluoride metabolism (eg, time of void and time since last void) and children's intellectual abilities (eg, child sex, maternal age, gestational age, and parity) (**Table 1**). Mother's race/ethnicity was coded as white or other, and maternal education was coded as either bachelor's degree or higher or trade school diploma or lower. The quality of a child's home environment was measured by the Home Observation for Measurement of the Environment (HOME)-Revised Edition[19] on a continuous scale. We also controlled for city and, in some models, included self-reported exposure to secondhand smoke (yes/no) as a covariate.

Statistical Analyses

In our primary analysis, we used linear regression analyses to estimate the associations between our 2 measures of fluoride exposure (MUF_{SG} and fluoride intake) and children's FSIQ scores. In addition to providing the coefficient corresponding to a 1-mg difference in fluoride exposure, we also estimated coefficients corresponding to a fluoride exposure difference spanning the 25th to 75th percentile range (which corresponds to a 0.33 mg/L and 0.62 mg F/d difference in MUF_{SG} and fluoride intake, respectively) as well as the 10th

Table 1. Demographic Characteristics and Exposure Outcomes for Mother-Child Pairs With MUF_{SG} (n = 512) and Fluoride Intake Data (n = 400) by Fluoridated and Nonfluoridated Status[a]

Variable[b]	No. (%)		
		Maternal-Child Pairs With Fluoride Intake, IQ, and Complete Covariate Data (n = 400)	
	MUF_{SG} Sample (n = 512)[c]	Nonfluoridated (n = 238)	Fluoridated (n = 162)
Mothers			
Age of mother at enrollment, mean (SD), y	32.33 (5.07)	32.61 (4.90)	32.52 (4.03)
Prepregnancy BMI, mean (SD)	25.19 (6.02)	25.19 (6.35)	24.33 (5.10)
Married or common law	497 (97)	225 (95)	159 (98)
Born in Canada	426 (83)	187 (79)	131 (81)
White	463 (90)	209 (88)	146 (90)
Maternal education			
Trade school diploma/high school	162 (32)	80 (34)	38 (24)
Bachelor's degree or higher	350 (68)	158 (66)	124 (76)
Employed at time of pregnancy	452 (88)	205 (86)	149 (92)
Net income household >$70 000 CAD	364 (71)	162 (68)	115 (71)
HOME total score, mean (SD)	47.32 (4.32)	47.28 (4.48)	48.14 (3.90)
Smoked in trimester 1	12 (2)	7 (3)	2 (1)
Secondhand smoke in the home	18 (4)	9 (4)	2 (1)
Alcohol consumption, alcoholic drink/mo			
None	425 (83)	192 (81)	136 (84)
<1	41 (8)	23 (10)	11 (7)
≥1	46 (9)	23 (10)	15 (9)
Parity (first birth)	233 (46)	119 (50)	71 (44)
Children			
Female	264 (52)	118 (50)	83 (51)
Age at testing, mean (SD), y	3.42 (0.32)	3.36 (0.31)	3.49 (0.29)
Gestation, mean (SD), wk	39.12 (1.57)	39.19 (1.47)	39.17 (1.81)
Birth weight, mean (SD), kg	3.47 (0.49)	3.48 (0.48)	3.47 (0.53)
FSIQ	107.16 (13.26)	108.07 (13.31)	108.21 (13.72)
Boys[d]	104.61 (14.09)	106.31 (13.60)	104.78 (14.71)
Girls[d]	109.56 (11.96)	109.86 (12.83)	111.47 (11.89)
Exposure variables			
MUF_{SG} concentration, mg/L[e]			
No.	512	228	141
Mean (SD)	0.51 (0.36)	0.40 (0.27)	0.69 (0.42)
Fluoride intake level per day, mg			
No.	369[a]	238	162
Mean (SD)	0.54 (0.44)	0.30 (0.26)	0.93 (0.43)
Water fluoride concentration, mg/L			
No.	369[a]	238	162
Mean (SD)	0.31 (0.23)	0.13 (0.06)	0.59 (0.08)

Abbreviations: BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); CAD, Canadian dollars; FSIQ, Full Scale IQ; HOME, Home Observation for Measurement of the Environment; MUF_{SG}, maternal urinary fluoride adjusted for specific gravity.

SI conversion factor: To convert fluoride to millimoles per liter, multiply by 0.05263.

[a] Owing to missing water treatment plant data and/or MUF data, the samples are distinct with some overlapping participants in both groups (n = 369).

[b] All of the listed variables were tested as potential covariates, as well as the following: paternal variables (age, education, employment status, smoking status, and race/ethnicity); maternal chronic condition during pregnancy and birth country; breastfeeding duration; and time of void and time since last void.

[c] Maternal urinary fluoride (averaged across all 3 trimesters) and corrected for specific gravity.

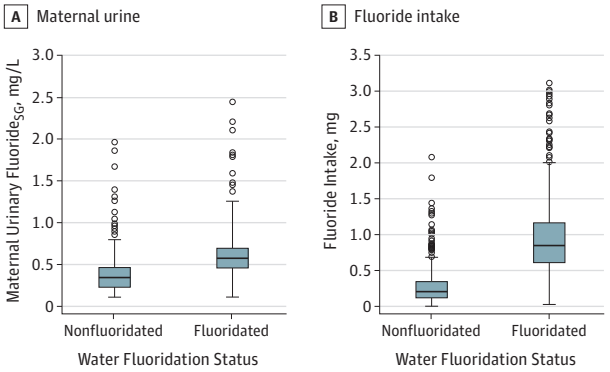
[d] The FSIQ score has a mean (SD) of 100 (15); US population norms used.

[e] Owing to missing water treatment plant data, the samples in the fluoridated and nonfluoridated regions do not add up to the MUF sample size.

to 90th percentile range (which corresponds to a 0.70 mg/L and 1.04 mg F/d difference in MUF_{SG} and fluoride intake, respectively).

We retained a covariate in the model if its *P* value was less than .20 or its inclusion changed the regression coefficient of the variable associated factor by more than 10% in any of the IQ models. Regression diagnostics confirmed that there were no collinearity issues in any of the IQ models with MUF_{SG} or fluoride intake (variance inflation factor <2 for all covariates). Residuals from each model had approximately normal distributions, and their Q-Q plots revealed no extreme outliers. Plots of residuals against fitted values did not suggest any assumption violations and there were no substantial influential observations as measured by Cook distance. Including quadratic or natural-log effects of MUF_{SG} or fluoride intake did not significantly improve the regression models. Thus, we present the more easily interpreted estimates from linear regression models. Additionally, we examined separate models with 2 linear splines to test whether the MUF_{SG} association significantly differed between lower and higher levels of MUF_{SG} based on 3 knots, which were set at 0.5 mg/L (mean MUF_{SG}), 0.8 mg/L (threshold seen in the Mexican birth cohort),[10] and 1 mg/L (op-

Figure 2. Distribution of Fluoride Levels in Maternal Urine and for Estimated Fluoride Intake by Fluoridation Status



To convert fluoride to millimoles per liter, multiply by 0.05263.

timal concentration in the United States until 2015).[20] For fluoride intake, knots were set at 0.4 mg (mean fluoride intake), 0.8 mg, and 1 mg (in accordance with MUF_{SG}). We also examined sex-specific associations in all models by testing the interactions between child sex and each fluoride measure.

In sensitivity analyses, we tested whether the associations between MUF_{SG} and IQ were confounded by maternal blood concentrations of lead,[21] mercury,[21] manganese,[21,22] perfluoro-octanoic acid,[23] or urinary arsenic.[24] We also conducted sensitivity analyses by removing IQ scores that were greater than or less than 2.5 standard deviations from the sample mean. Additionally, we examined whether using MUF adjusted for creatinine instead of SG affected the results.

In additional analyses, we examined the association between our 2 measures of fluoride exposure (MUF_{SG} and fluoride intake) with VIQ and PIQ. Additionally, we examined whether water fluoride concentration was associated with FSIQ, VIQ, and PIQ scores.

For all analyses, statistical significance tests with a type I error rate of 5% were used to test sex interactions, while 95% confidence intervals were used to estimate uncertainty. Analyses were conducted using R software (the R Foundation).[25] The *P* value level of significance was .05, and all tests were 2-sided.

Results

For the first measure of fluoride exposure, MUF_{SG}, 512 of 601 mother-child pairs (85.2%) who completed the neurodevelopmental visit had urinary fluoride levels measured at each trimester of the mother's pregnancy and complete covariate data (Figure 1); 89 (14.8%) were excluded for missing MUF_{SG} at 1 or more trimesters (n = 75) or missing 1 or more covariates included in the regression (n = 14) (Figure 1). Of the 512 mother-child pairs with MUF_{SG} data (and all covariates), 264 children were female (52%).

For the second measure of fluoride exposure, fluoride intake from maternal questionnaire, data were available for 400 of the original 601 mother-child pairs (66.6%): 201 women (33.4%) were excluded for reporting not drinking tap water (n = 59), living outside of the predefined water treatment plant zone (n = 108), missing beverage consumption data (n = 20), or missing covariate data (n = 14) (Figure 1).

Children had mean FSIQ scores in the average range (population normed) (mean [SD], 107.16 [13.26], range = 52-143), with girls (109.56 [11.96]) showing significantly higher scores than boys (104.61 [14.09]; *P* < .001) (Table 1). The demographic characteristics of the 512 mother-child pairs included in the primary analysis were not substantially different from the original MIREC cohort or subset of mother-child pairs without 3 urine samples (eTable 1 in the Supplement). Of the 400 mother-child pairs with fluoride intake data (and all covariates), 118 of 238 (50%) in the group living in a nonfluoridated region were female and 83 of 162 (51%) in the group living in a fluoridated region were female.

Fluoride Measurements

The median MUF_{SG} concentration was 0.41 mg/L (range, 0.06-2.44 mg/L). Mean MUF_{SG} concentration was significantly higher among women (n = 141) who lived in communities with fluoridated drinking water (0.69 [0.42] mg/L) compared with women (n=228) who lived in communities without fluoridated drinking water (0.40 [0.27] mg/L; *P* < .001) (Table 1; **Figure 2**).

The median estimated fluoride intake was 0.39 mg per day (range, 0.01-2.65 mg). As expected, the mean (SD) fluoride intake was significantly higher for women (162 [40.5%]) who lived in communities with fluoridated drinking water (mean [SD], 0.93 [0.43] mg) than women (238 [59.5%]) who lived in communities without fluoridated drinking water (0.30 [0.26] mg; *P* < .001) (Table 1; Figure 2). The MUF_{SG} was moderately correlated with fluoride intake (*r* = 0.49; *P* < .001) and water fluoride concentration (*r* = 0.37; *P* < .001).

Maternal Urinary Fluoride Concentrations and IQ

Before covariate adjustment, a significant interaction (*P* for interaction = .03) between MUF_{SG} and child sex (*B* = 7.24; 95% CI, 0.81-13.67) indicated that MUF_{SG} was associated with FSIQ in boys; an increase of 1 mg/L MUF_{SG} was associated with a 5.01 (95% CI, −9.06 to −0.97; *P* = .02) lower FSIQ score in boys. In contrast, MUF_{SG} was not significantly associated with FSIQ score in girls (*B* = 2.23; 95% CI, −2.77 to 7.23; *P* = .38) (**Table 2**).

Adjusting for covariates, a significant interaction (*P* for interaction = .02) between child sex and MUF_{SG} (*B* = 6.89; 95% CI, 0.96-12.82) indicated that an increase of 1 mg/L of MUF_{SG} was associated with a 4.49 (95% CI, −8.38 to −0.60; *P* = .02) lower FSIQ score for boys. An increase from the 10th to 90th percentile of MUF_{SG} was associated with a 3.14 IQ decrement among boys (Table 2; **Figure 3**). In contrast, MUF_{SG} was not significantly associated with FSIQ score in girls (*B* = 2.43; 95% CI, −2.51 to 7.36; *P* = .33).

Estimated Fluoride Intake and IQ

A 1-mg increase in fluoride intake was associated with a 3.66 (95% CI, −7.16 to −0.15; *P* = .04) lower FSIQ score among boys and girls (Table 2; Figure 3). The interaction between child sex and fluoride intake was not statistically significant (*B* = 1.17; 95% CI, −4.08 to 6.41; *P* for interaction = .66).

Table 2. Unadjusted and Adjusted Associations Estimated From Linear Regression Models of Fluoride Exposure Variables and FSIQ Scores

	Difference (95% CI)			
		Adjusted Estimates, Regression Coefficients Indicate Change in Outcome per[a]		
Variable	Unadjusted	1 mg	25th to 75th Percentiles	10th to 90th Percentiles
MUF_{SG}[b,c]	−2.60 (−5.80 to 0.60)	−1.95 (−5.19 to 1.28)	−0.64 (−1.69 to 0.42)	−1.36 (−3.58 to 0.90)
Boys	−5.01 (−9.06 to −0.97)	−4.49 (−8.38 to −0.60)	−1.48 (−2.76 to −0.19)	−3.14 (−5.86 to −0.42)
Girls	2.23 (−2.77 to 7.23)	2.40 (−2.53 to 7.33)	0.79 (−0.83 to 2.42)	1.68 (−1.77 to 5.13)
Fluoride intake[d,e]	−3.19 (−5.94 to −0.44)	−3.66 (−7.16 to −0.15)	−2.26 (−4.45 to −0.09)	−3.80 (−7.46 to −0.16)

Abbreviations: FSIQ, Full Scale IQ; HOME, Home Observation for Measurement of the Environment; MUF_{SG}, maternal urinary fluoride adjusted for specific gravity.

[a] Adjusted estimates pertain to predicted FSIQ difference for a value spanning the interquartile range (25th to 75th percentiles) and 80th central range (10th to 90th percentiles): (1) MUF_{SG}: 0.33 mg/L, 0.70 mg/L, respectively; (2) fluoride intake: 0.62 mg, 1.04 mg, respectively.

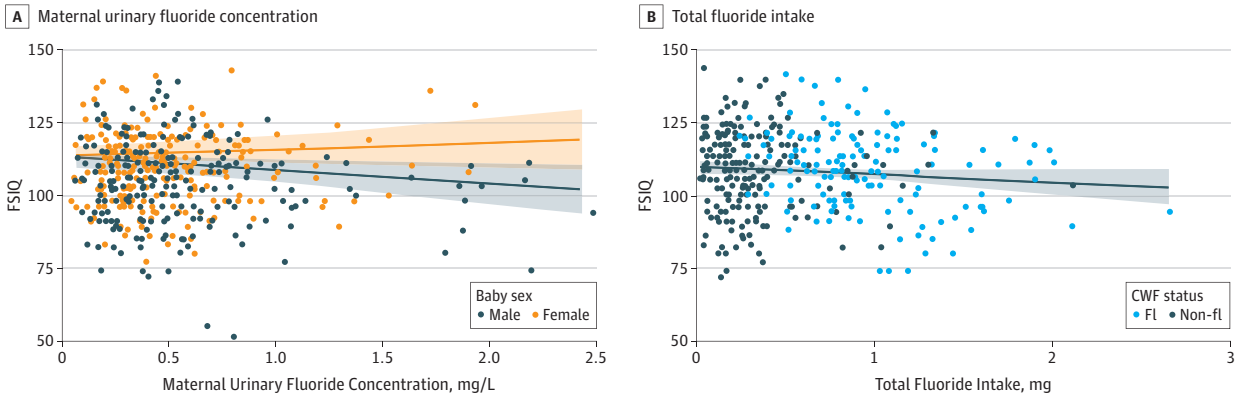
[b] n = 512.

[c] Adjusted for city, HOME score, maternal education, race/ethnicity, and including child sex interaction.

[d] n = 400.

[e] Adjusted for city, HOME score, maternal education, race/ethnicity, child sex, and prenatal secondhand smoke exposure.

Figure 3. Covariate Results of Multiple Linear Regression Models of Full Scale IQ (FSIQ) from Maternal Urinary Fluoride Concentration by Child Sex (n = 512) and Total Fluoride Intake Estimated from Daily Maternal Beverage Consumption (n = 400)



B, Community fluoridation status (CWF) is shown for each woman; black dots represent women living in nonfluoridated (non-Fl) communities and blue dots represent women living in fluoridated (Fl) communities.

Sensitivity Analyses

Adjusting for lead, mercury, manganese, perfluorooctanoic acid, or arsenic concentrations did not substantially change the overall estimates of MUF_{SG} for boys or girls (eTable 2 in the Supplement). Use of MUF adjusted for creatinine did not substantially alter the associations with FSIQ (eTable 2 in the Supplement). Including time of void and time since last void did not substantially change the regression coefficient of MUF_{SG} among boys or girls.

Estimates for determining the association between MUF_{SG} and PIQ showed a similar pattern with a statistically significant interaction between MUF_{SG} and child sex (P for interaction = .007). An increase of 1 mg/L MUF_{SG} was associated with a 4.63 (95% CI, −9.01 to −0.25; P = .04) lower PIQ score in boys, but the association was not statistically significant in girls (B = 4.51; 95% CI, −1.02 to 10.05; P = .11). An increase of 1 mg/L MUF_{SG} was not significantly associated with VIQ in boys (B = −2.85; 95% CI, −6.65 to 0.95; P = .14) or girls (B = 0.55; 95% CI, −4.28 to 5.37; P = .82); the interaction between MUF_{SG} and child sex was not statistically significant (P for interaction = .25) (eTable 3 in the Supplement).

Consistent with the findings on estimated maternal fluoride intake, increased water fluoride concentration (per 1 mg/L) was associated with a 5.29 (95% CI, −10.39 to −0.19) lower FSIQ score among boys and girls and a 13.79 (95% CI, −18.82 to −7.28) lower PIQ score (eTable 4 in the Supplement).

Discussion

Using a prospective Canadian birth cohort, we found that estimated maternal exposure to higher fluoride levels during pregnancy was associated with lower IQ scores in children. This association was supported by converging findings from 2 measures of fluoride exposure during pregnancy. A difference in MUF_{SG} spanning the interquartile range for the entire sample (ie, 0.33 mg/L), which is roughly the difference in MUF_{SG} concentration for pregnant women living in a fluoridated vs a nonfluoridated community, was associated with a 1.5-point IQ decrement among boys. An increment of 0.70 mg/L in MUF_{SG} concentration was associated with a 3-point IQ decrement in boys; about half of the women living in a fluoridated commu-

nity have a MUF_{SG} equal to or greater than 0.70 mg/L. These results did not change appreciably after controlling for other key exposures such as lead, arsenic, and mercury.

To our knowledge, this study is the first to estimate fluoride exposure in a large birth cohort receiving optimally fluoridated water. These findings are consistent with that of a Mexican birth cohort study that reported a 6.3 decrement in IQ in preschool-aged children compared with a 4.5 decrement for boys in our study for every 1 mg/L of MUF.[10] The findings of the current study are also concordant with ecologic studies that have shown an association between higher levels of fluoride exposure and lower intellectual abilities in children.[7,8,26] Collectively, these findings support that fluoride exposure during pregnancy may be associated with neurocognitive deficits.

In contrast with the Mexican study,[10] the association between higher MUF_{SG} concentrations and lower IQ scores was observed only in boys but not in girls. Studies of fetal and early childhood fluoride exposure and IQ have rarely examined differences by sex; of those that did, some reported no differences by sex.[10,27-29] Most rat studies have focused on fluoride exposure in male rats,[30] although 1 study[31] showed that male rats were more sensitive to neurocognitive effects of fetal exposure to fluoride. Testing whether boys are potentially more vulnerable to neurocognitive effects associated with fluoride exposure requires further investigation, especially considering that boys have a higher prevalence of neurodevelopmental disorders such as ADHD, learning disabilities, and intellectual disabilities.[32] Adverse effects of early exposure to fluoride may manifest differently for girls and boys, as shown with other neurotoxicants.[33-36]

The estimate of maternal fluoride intake during pregnancy in this study showed that an increase of 1 mg of fluoride was associated with a decrease of 3.7 IQ points across boys and girls. The finding observed for fluoride intake in both boys and girls may reflect postnatal exposure to fluoride, whereas MUF primarily captures prenatal exposure. Importantly, we excluded women who reported that they did not drink tap water and matched water fluoride measurements to time of pregnancy when estimating maternal fluoride intake. None of the fluoride concentrations measured in municipal drinking water were greater than the maximum acceptable concentration of 1.5 mg/L set by Health Canada; most (94.3%) were lower than the 0.7 mg/L level considered optimal.[37]

Water fluoridation was introduced in the 1950s to prevent dental caries before the widespread use of fluoridated dental products. Originally, the US Public Health Service set the optimal fluoride concentrations in water from 0.7 to 1.2 mg/L to achieve the maximum reduction in tooth decay and minimize the risk of enamel fluorosis.[38] Fluorosis, or mottling, is a symptom of excess fluoride intake from any source occurring during the period of tooth development. In 2012, 68% of adolescents had very mild to severe enamel fluorosis.[39] The higher prevalence of enamel fluorosis, especially in fluoridated areas,[40] triggered renewed concern about excessive ingestion of fluoride. In 2015, in response to fluoride overexposure and rising rates of enamel fluorosis,[39,41,42] the US Public Health Service recommended an optimal fluoride concentration of 0.7 mg/L, in line with the recommended level of fluoride added to drinking water in Canada to prevent caries. However, the beneficial effects of fluoride predominantly occur at the tooth surface after the teeth have erupted.[43] Therefore, there is no benefit of systemic exposure to fluoride during pregnancy for the prevention of caries in offspring.[44] The evidence showing an association between fluoride exposure and lower IQ scores raises a possible new concern about cumulative exposures to fluoride during pregnancy, even among pregnant women exposed to optimally fluoridated water.

Strengths and Limitations

Our study has several strengths and limitations. First, urinary fluoride has a short half-life (approximately 5 hours) and depends on behaviors that were not controlled in our study, such as consumption of fluoride-free bottled water or swallowing toothpaste prior to urine sampling. We minimized this limitation by using 3 serial urine samples and tested for time of urine sample collection and time since last void, but these variables did not alter our results. Second, although higher maternal ingestion of fluoride corresponds to higher fetal plasma fluoride levels,[45] even serial maternal urinary spot samples may not precisely represent fetal exposure throughout pregnancy. Third, while our analyses controlled for a comprehensive set of covariates, we did not have maternal IQ data. However, there is no evidence suggesting that fluoride exposure differs as a function of maternal IQ; our prior study did not observe a significant association between MUF levels and maternal education level.[12] Moreover, a greater proportion of women living in fluoridated communities (124 [76%]) had a university-level degree compared with women living in nonfluoridated communities (158 [66%]). Nonetheless, despite our comprehensive array of covariates included, this observational study design could not address the possibility of other unmeasured residual confounding. Fourth, fluoride intake did not measure actual fluoride concentration in tap water in the participant's home; Toronto, for example, has overlapping water treatment plants servicing the same household. Similarly, our fluoride intake estimate only considered fluoride from beverages; it did not include fluoride from other sources such as dental products or food. Furthermore, fluoride intake data were limited by self-report of mothers' recall of beverage consumption per day, which was sampled at 2 points of pregnancy, and we lacked information regarding specific tea brand.[17,18] In addition, our methods of estimating maternal fluoride intake have not been validated; however, we show construct validity with MUF. Fifth, this study did not include assessment of postnatal fluoride exposure or consumption. However, our future analyses will assess exposure to fluoride in the MIREC cohort in infancy and early childhood.

Conclusions

In this prospective birth cohort study from 6 cities in Canada, higher levels of fluoride exposure during pregnancy were associated with lower IQ scores in children measured at age 3 to 4 years. These findings were observed at fluoride levels typically found in white North American women. This indicates the possible need to reduce fluoride intake during pregnancy.

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Author Affiliations: Faculty of Health, York University, Toronto, Ontario, Canada (Green, Flora, Neufeld, Till); Faculty of Health Sciences, Simon Fraser University, Burnaby, British Columbia, Canada (Lanphear); Child and Family Research Institute, British Columbia Children's Hospital, University of British Columbia, Vancouver, British Columbia, Canada (Lanphear); Pediatrics and Environmental Health, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio (Hornung); School of Dentistry, Indiana University, Indianapolis (Martinez-Mier); Department of Social and Preventive Medicine, Laval University, Québec City, Québec, Canada (Ayotte); Centre de Recherche du CHU de Québec, Université Laval, Québec City, Québec, Canada (Ayotte, Muckle); School of Psychology, Laval University, Québec City, Québec, Canada (Muckle).

Author Contributions: Ms Green and Dr Till had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.
Concept and design: Green, Lanphear, Martinez-Mier, Ayotte, Muckle, Till.
Acquisition, analysis, or interpretation of data: All authors.
Drafting of the manuscript: Green, Flora, Martinez-Mier, Muckle, Till.
Critical revision of the manuscript for important intellectual content: All authors.
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Supervision: Flora, Till.

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Editor's Note

Decision to Publish Study on Maternal Fluoride Exposure During Pregnancy

Dimitri A. Christakis, MD, MPH

The decision to publish this article was not easy.[1] Given the nature of the findings and their potential implications, we subjected it to additional scrutiny for its methods and the presentation of its findings. The mission of the journal is to ensure that child health is optimized by bringing the best available evidence to the fore. Publishing it serves as testament to the fact that *JAMA Pediatrics* is committed to disseminating the best science based entirely on the rigor of the methods and the soundness of the hypotheses tested, regardless of how contentious the results may be. That said, scientific inquiry is an iterative process. It is rare that a single study provides definitive evidence. This study is neither the first, nor will it be the last, to test the association between prenatal fluoride exposure and cognitive development. We hope that purveyors and consumers of these findings are mindful of that as the implications of this study are debated in the public arena.

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Audio

Author Affiliations: University of Washington, Seattle; Seattle Children's Research Institute, Seattle, Washington; Editor, *JAMA Pediatrics*.

Corresponding Author: Dimitri A. Christakis, MD, MPH, 2001 Eighth Ave, Suite 400, Seattle, WA 98121 (dimitri.christakis@seattlechildrens.org).

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Supplementary Online Content

Green R, Lanphear B, Hornung R, et al. Association between maternal fluoride exposure during pregnancy and IQ scores in offspring in Canada. Published online August 19, 2019. *JAMA Pediatr.* doi:10.1001/jamapediatrics.2019.1729

This supplementary material has been provided by the authors to give readers additional information about their work.

eTable 1: Comparison of current sample to other MIREC samples

Variable	Participants in the MIREC cohort with:		
	Live births[a]	Women with 3 urine samples and child IQ scores	Women with <3 urine samples and child IQ scores
n	1983	512	70
Mean (SD) age of mother at enrollment (years)	32.2 (5.1)	32.51 (4.46)	32.43 (5.29)
Caucasian, No. (%)	1651 (85)	463 (90)	56 (80)
Married or Common-law, No. (%)	1890 (95.3)	497 (97)	63 (90)
Born in Canada, No. (%)	1569 (79)	426 (83)	53 (76)
Maternal Education, No. (%)			
High school or less	158 (9)	24 (5)	4 (6)
Some college	100 (5)	17 (3)	5 (7)
College diploma	412 (24)	121 (24)	20 (29)
University degree	1246 (62)	348 (68)	40 (57)
Employed at time of pregnancy, No. (%)	1647 (83)	452 (88)	87.0
Net household income >$70,000, No. (%)	1269 (64)	364 (71)	65.2

Abbreviations: SD = standard deviation

[a]from a total of 2001 women who were recruited

Note: Differences between the analytic sample (n=512), live births sample (n=1983), and excluded participants (n=70) were all considered small (i.e. Cohen's effect size *h* of ≤0.30).

eTable 2: Sensitivity analyses predicting Full Scale IQ (FSIQ).

MLR Models	*N*	*B (SE)* of predictor	*p*	95% CI
Model $_{A}$	512	-4.49 (1.98)	.02	-8.38, -0.60
Model $_{A+lead}$	504	-4.61 (1.98)	.02	-8.50, -0.71
Model $_{A+mercury}$	456	-5.13 (2.05)	.01	-9.16, -1.10
Model $_{A+PFOA}$	503	-4.57 (1.97)	.02	-8.21, -0.50
Model $_{A+arsenic}$	512	-4.44 (1.99)	.03	-8.35, -0.54
Model $_{A+manganese}$	502	-4.55 (1.97)	.02	-8.42, -0.69
Model $_{A+second\ hand\ smoke\ exposure}$	512	-4.18 (1.98)	.03	-8.06, -0.30
Model $_{B}$	510	-4.11 (1.92)	.03	-7.89, -0.33
Model $_{C}$	407	-4.96 (1.83)	.007	-8.56, -1.36
Model $_{D}$	369	-6.25 (2.70)	.02	-11.56, -0.94

Abbreviations: HOME = Home Observation for Measurement of the Environment; PFOA = perfluorooctanoic acid; MLR = multiple linear regression; MUF = maternal urinary fluoride

Model $_{A}$ – MUF_{SG} coefficient for boys controlling for city, HOME total score, race and maternal level of education with baby sex as an interaction term

Model $_{B}$ – Model $_{A}$ without two boys with FSIQ lower than 60

Model $_{C}$ – MUF coefficient for boys adjusted for creatinine with same covariates as Model $_{A}$

Model $_{D}$ – using water fluoride concentration as a predictor for those women who have MUF values only

eTable 3. Unadjusted and adjusted effect estimates from linear regression models of fluoride exposure variables predicting Verbal IQ and Performance IQ scores.

	Performance IQ		Verbal IQ	
	Unadjusted	Adjusted	Unadjusted	Adjusted
Predictor	***B* (95% CI)**	***B* (95% CI)**	***B* (95% CI)**	***B* (95% CI)**
MUF_{SG}[a]	-5.81* (-9.31, -2.30)	-1.24 (-4.88, 2.40)	1.28 (-1.87, 4.43)	-1.60 (-4.74, 1.55)
Boys	-8.81* (-13.29, -4.32)	-4.63* (-9.01, -0.25)[d]	-0.21 (-4.19, 3.77)	-2.82 (-6.62, 0.98)[c]
Girls[b]	-0.56 (-6.09, 4.97)	4.51 (-1.02, 10.05)[d]	4.78 (-0.14, 9.70)	0.50 (-4.32, 5.33)[c]
Fluoride intake[c]	-5.75* (-8.74, -2.76)	-2.74 (-6.82, 1.34)[e]	-0.03 (-2.71, 2.64)	-3.08 (-6.40, 0.25)[d]

Abbreviations: MUF_{SG} = maternal urinary fluoride adjusted for specific gravity; HOME = Home Observation for Measurement of the Environment

[a] N=507 for PIQ; N=509 for VIQ

[b] Girls had significantly higher scores on VIQ ($p < .001$) and PIQ ($p = .03$) compared with boys

[c] N=395 for PIQ; N=399 for VIQ; Missing data due to incomplete questionnaire responses to beverage consumption

[d] adjusted for city, HOME score, maternal education, race and including child sex interaction

[e] adjusted for HOME score, maternal education, race, child sex, prenatal second-hand smoke exposure, and city

*$p < .05$

eTable 4: Unadjusted and adjusted effect estimates from linear regression models of water fluoride concentration (mg/L) predicting FSIQ scores.

			Adjusted estimates[b]	
	Unadjusted (FSIQ)	**Full Scale IQ**	**Performance IQ**	**Verbal IQ**
Predictor	***B* (95% CI)**	***B* (95% CI)**	***B* (95% CI)**	***B* (95% CI)**
Water fluoride concentration[a]	3.49 (-9.04, 2.06)	-5.29* (-10.39, -0.19)[b]	-13.79* (-18.82, -7.28)	3.37 (-1.50, 8.24)

[a] N=420

[b] adjusted for HOME score, maternal education, race, child sex, and prenatal second-hand smoke exposure; because city was strongly multi-collinear with water fluoride concentration (VIF >20), it was excluded from the model

*$p < .05$

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Fluoride exposure from infant formula and child IQ in a Canadian birth cohort

Christine Till[a,*], Rivka Green[a], David Flora[a], Richard Hornung[b], E. Angeles Martinez-Mier[c], Maddy Blazer[a], Linda Farmus[a], Pierre Ayotte[d,e], Gina Muckle[d,f], Bruce Lanphear[g,h]

[a] Faculty of Health, York University, Ontario, Canada
[b] Pediatrics and Environmental Health, Cincinnati Children's Hospital Medical Center, OH, USA
[c] School of Dentistry, Indiana University, IN, USA
[d] Centre de Recherche du CHU de Québec, Université Laval, Québec, Canada
[e] Department of Social and Preventive Medicine, Laval University, Quebec, Canada
[f] School of Psychology, Laval University, Quebec, Canada
[g] Faculty of Health Sciences, Simon Fraser University, British Columbia, Canada
[h] Child & Family Research Institute, BC Children's Hospital, University of British Columbia, Canada

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ABSTRACT

Background: Infant consumption of formula reconstituted with fluoridated water can lead to excessive fluoride intake. We examined the association between fluoride exposure in infancy and intellectual ability in children who lived in fluoridated or non-fluoridated cities in Canada.

Methods: We examined 398 mother-child dyads in the Maternal-Infant Research on Environmental Chemicals cohort who reported drinking tap water. We estimated water fluoride concentration using municipal water reports. We used linear regression to analyze the association between fluoride exposure and IQ scores, measured by the Wechsler Primary and Preschool Scale of Intelligence-III at 3–4 years. We examined whether feeding status (breast-fed versus formula-fed) modified the impact of water fluoride and if fluoride exposure during fetal development attenuated this effect. A second model estimated the association between fluoride intake from formula and child IQ.

Results: Thirty-eight percent of mother-child dyads lived in fluoridated communities. An increase of 0.5 mg/L in water fluoride concentration (approximately equaling the difference between fluoridated and non-fluoridated regions) corresponded to a 9.3- and 6.2-point decrement in Performance IQ among formula-fed (95% CI: −13.77, −4.76) and breast-fed children (95% CI: −10.45, −1.94). The association between water fluoride concentration and Performance IQ remained significant after controlling for fetal fluoride exposure among formula-fed ($B = -7.93$, 95% CI: −12.84, −3.01) and breastfed children ($B = -6.30$, 95% CI: −10.92, −1.68). A 0.5 mg increase in fluoride intake from infant formula corresponded to an 8.8-point decrement in Performance IQ (95% CI: −14.18, −3.34) and this association remained significant after controlling for fetal fluoride exposure ($B = -7.62$, 95% CI: −13.64, −1.60).

Conclusions: Exposure to increasing levels of fluoride in tap water was associated with diminished non-verbal intellectual abilities; the effect was more pronounced among formula-fed children.

1. Introduction

Fluoride can occur naturally in water and, in some communities, is added to water supplies to reach the recommended concentration of 0.7 mg/L for the prevention of tooth decay (Health Canada, 2010). About 74% of Americans and 38% of Canadians on municipal water are supplied with fluoridated drinking water. Water fluoridation has been reported to reduce the prevalence of tooth decay by 26% to 44% (Iheozor-Ejiofor et al., 2015; National Health and Medical Research Council (NHMRC), 2017) in youth and by 26% (Iheozor-Ejiofor et al.,

Abbreviations: BF, breastfed; FF, formula fed; CI, confidence intervals; HOME, home observation for measurement of the environment; IQ, intelligence quotient; FSIQ, full scale IQ; PIQ, performance IQ; VIQ, verbal IQ; MIREC, maternal-infant research on environmental chemicals; MUF, maternal urinary fluoride; SD, standard deviation

* Corresponding author at: Department of Psychology, York University, 4700 Keele Street, M3J 1P3 Toronto, ON, Canada.
E-mail address: ctill@yorku.ca (C. Till).

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2015) to 27% (NHMRC, 2017) in adults. Infants who are fed formula reconstituted with fluoridated water have approximately three to four times greater exposure to fluoride than adults (National Research Council (NRC), 2006) on a per body-weight basis. Formula-fed infants residing in fluoridated areas have an approximate 70-fold higher fluoride intake than exclusively breastfed infants (Ekstrand, 1981; Zohoori et al., 2018; United States Environmental Protection Agency, 2010)

The prevalence of enamel fluorosis, a discoloration of enamel resulting from chronic, excessive ingestion of fluoride during tooth development (Brothwell and Limeback, 2003; Buzalaf et al., 2001), is higher among formula-fed infants than breastfed infants (Buzalaf et al., 2001; Do et al., 2012; Fv et al., 2012; Hong et al., 2006; Walton and Messer, 1981). While enamel fluorosis develops from excess fluoride exposure during the first four years of life, (Levy et al., 2010) the first 12 months are the most vulnerable period (Hong et al., 2006). The risk of fluorosis increases with higher levels of fluoride in the water supply for formula-fed infants (Hujoel et al., 2009).

Breastmilk contains extremely low concentrations of fluoride (0.005–0.01 mg/L) due to the limited transfer of fluoride in plasma into breastmilk (Dabeka et al., 1986; Ekstrand, 1981; Ekstrand and Hardell, 1984; Esala et al., 1982; Faraji et al., 2014; Zohoori et al., 2018). Exclusive breastfeeding for six months, which is recommended by current practice guidelines (Critch, 2013; Eidelman, 2012), is reported by 25% of mothers in the United States (Breastfeeding Report Card. United States, 2018) and Canada (Health Canada, 2001). Ninety percent of bottle-fed infants are fed powdered formula (Infant Feeding Practices Survey II) and 75% of mothers report using tap water to reconstitute formula (Van Winkle et al., 1995). Thus, reconstituted formula is the major source of nutrition for many infants in the United States and Canada.

Despite growing concerns about excessive exposure to fluoride during infancy and the vulnerability of the developing brain (Rice and Barone, 2000; Grandjean and Landrigan, 2006), no studies have tested the potential neurotoxicity of using optimally fluoridated drinking water to reconstitute formula during infancy (Harriehausen et al., 2019). Increased fluoride exposure during fetal brain development was associated with diminished IQ scores in two birth cohort studies (Bashash et al., 2017; Green et al., 2019; Valdez Jiménez et al., 2017), among a number of recent studies conducted in endemic fluorosis areas (Karimzade et al., 2014; Dong et al., 2018; Zhang et al., 2015), as well as a 2012 meta-analysis of 27 ecologic studies (Choi et al., 2012). Increased fluoride exposure has also been linked with ADHD-related behaviors in children (Malin and Till, 2015; Bashash et al., 2018; Riddell et al., 2019).

We investigated the association between water fluoride concentration and intellectual abilities of Canadian children who were formula-fed or breastfed. In addition, we tested whether postnatal effects of fluoride exposure on child IQ remained after controlling for fetal exposure.

2. Materials and methods

2.1. Study population

Between 2008 and 2011, the Maternal-Infant Research on Environmental Chemicals (MIREC) program recruited 2001 pregnant women from ten Canadian cities to participate in a longitudinal pregnancy cohort study. Women who could communicate in English or French, were > 17 years, and were < 14 weeks gestation were recruited from prenatal clinics. Participants were excluded if there was a known fetal abnormality, if they had any medical complications, or if there was known illicit drug use during pregnancy. Additional details are in the cohort profile description (Arbuckle et al., 2013).

Of the 610 children who were recruited to participate in the developmental follow-up phase of the study (MIREC-Child Development Plus), 601 completed all testing. Children were recruited from six of the cities in the original cohort (Vancouver, Toronto, Hamilton, Halifax, Kingston, Montreal); approximately half of the children lived in non-fluoridated cities and half lived in fluoridated cities.

This study received ethics approval from Health Canada and York University.

2.2. Infant feeding assessment

When children were between 30 and 48 months of age, mothers completed an infant feeding questionnaire asking, “How old was your baby when you ceased breastfeeding exclusively? At what age did you introduce other type of milk or food to your baby?”. Women who breastfed exclusively for six months or longer were included in the breastfeeding (BF) group; those who reported introducing formula within the first six months (never breastfed or partial breastfeeding) were included in the formula-feeding (FF) group.

To explore the possibility of recall or response bias of mothers completing the questionnaire, we compared information reported by mothers when their children were between 30 and 48 months of age (i.e. time when the questionnaire was completed for classifying the BF and FF groups) with information reported by a subset of women at an earlier visit when their children were between 6 and 8 months of age. Information about infant feeding was only available for 11% of the sample at the infant visit (note that responses could only be matched for women who had stopped breastfeeding at the time the questionnaire was completed at the infant visit). Among women who provided information at both occasions, the median difference for when breastfeeding was reported to be ceased was 0 months; responses were within 1.5 months of each other for two-thirds of this subsample.

We dichotomized feeding status at six months because the Canadian Pediatric Society and American Academy of Pediatrics both recommend exclusive breastfeeding for six months (Critch, 2013; Eidelman, 2012). Moreover, formula-fed infants who are younger than six-months derive most of their nutrition from formula, placing this group at highest risk of exceeding the recommended upper limit (0.7 mg/d) for fluoride (Harriehausen et al., 2019; Institutes of Medicine, 1997; National Research Council (NRC), 2006). Finally, fluoride intake differences become less evident when other dietary sources of fluoride are introduced at around six months (Zohoori et al., 2018).

2.3. Infant fluoride exposure

We estimated fluoride concentrations in drinking water by accessing daily or monthly reports provided by water treatment plants. Water reports were first linked with mothers’ postal codes and the daily or weekly amounts were averaged over the first six-months of the child’s life. We only included participants whose postal codes could be linked to a water treatment plant that provided water fluoride measurements. We also excluded participants who reported that their primary drinking source was from a well or ‘other’ (e.g. bottled water) (Table S1). Further details can be found in our previous report (Till et al., 2018).

To obtain a continuous fluoride exposure estimate collapsed across the BF and FF groups, we estimated fluoride intake from formula (in mg F/day) by multiplying water fluoride concentration by the amount of time that the infant was not exclusively breastfed in the first year using the following equation:

$$\textit{Fluoride intake from formula} = (\textit{water\_F mg/L}) * (1 - \#\textit{mo\_excl\_BF}/11.99) * 0.80\ \textit{L/day}$$

where *water_F mg/L* refers to the average water fluoride concentration and *1-#mo_excl_BF/11.99* represents the proportion over the 12-month period the infant was not exclusively breastfed. A value near one indicates that an infant was primarily formula-fed over the 12 months whereas a value near zero indicates an infant primarily breastfed. We

estimated fluoride intake based on an average of 0.80 L of water used to reconstitute powdered formula as suggested by an infant food diary completed for infants in a prior study (Carignan et al., 2015); the average milk intake at 3 months of age is 0.812 L per day, ranging from 0.523 to 1.124 L (Dewey et al., 1991). Because we did not know the type of formula used (i.e. soy- or milk-based), we did not add fluoride derived from formula to our fluoride intake estimate. Previous studies have indicated that fluoride from water used in formula is a greater source of fluoride than fluoride found in formula (Buzalaf et al., 2004).

2.4. Fetal fluoride exposure

We used maternal urinary fluoride (MUF) adjusted for specific gravity as a proxy of fetal fluoride exposure. MUF, which was derived by averaging three spot samples collected across all three trimesters of pregnancy, was considered our most reliable measure of exposure (Till et al., 2018). Urinary fluoride concentrations were analyzed at the Indiana University School of Dentistry using a modification (Martínez-Mier et al., 2011) of the hexamethyldisiloxane (Sigma Chemical Co., USA) micro-diffusion procedure previously described (Green et al., 2019).

2.5. Intelligence assessment

We assessed children's intellectual abilities between ages 3.0 and 4.0 years with the Wechsler Preschool and Primary Scale of Intelligence-III (Wechsler, 2002) using United States population-based normative data (*mean* = 100, *SD* = 15). Outcomes included Full Scale IQ (FSIQ), a measure of global intellectual functioning, Verbal IQ (VIQ), a measure of verbal reasoning, and Performance IQ (PIQ), a measure of non-verbal reasoning and visual-motor coordination skills.

2.6. Covariates

We adjusted for potential confounding by selecting covariates *a priori* that have been associated with fluoride, breastfeeding, and children's intellectual abilities. Final covariates included child's sex and age at testing, maternal education (dichotomized as either a bachelor's degree or higher versus trade school diploma or lower), maternal race (white or not), second-hand smoke in the home (yes, no), and quality of the child's home environment (measured at time of testing using the Home Observation for Measurement of the Environment (HOME) - Revised Edition (Caldwell and Bradley, 1984). For each analysis, a covariate was retained in the final model if its *p*-value was < 0.20 or its inclusion changed the regression coefficient of water fluoride concentration or fluoride intake from formula by more than 10% (Kleinbaum et al., 1982). City was not included as a covariate in Model 1 because it was strongly multi-collinear with water fluoride concentration (VIF > 20). City was also excluded from Model 2 because fluoride intake from formula is a function of water fluoride concentration and was therefore deemed redundant.

2.7. Statistical analyses

We used linear regression to model differences in child IQ by water fluoride concentration while controlling for covariates. In our first model, we examined whether feeding status (BF or FF) modified the impact of water fluoride. In our second model, we estimated the association between fluoride intake from formula and child IQ. We controlled potential confounders by including them simultaneously with predictors.

In secondary analyses, we controlled for MUF during pregnancy in both models to account for fetal exposure. We also tested for sex-specific effects because we previously found that MUF concentration was only associated with diminished FSIQ in males (Green et al., 2019).

Regression diagnostics indicated no assumption violations pertaining to linearity, normality, or homogeneity of variance. Specifically, QQ-plots of residuals were consistent with a normal distribution and plots of residuals against fitted values did not suggest any assumption violations. Two observations were investigated based on a plot of Cook's D that suggested they may be influential; these cases had extremely low IQ scores that were more than 2.5 standard deviations from the sample mean. In a sensitivity analyses, we re-estimated the models after removing these two observations. Finally, variance inflation factors indicated no concerns with excessive multicollinearity.

To aid interpretation, we divided all regression coefficients by 2 so that they represent the predicted IQ difference per 0.5 mg/L of fluoride in tap water or 0.5 mg fluoride from formula; 0.5 mg/L corresponds to the approximate difference between mean water fluoride level in fluoridated versus non-fluoridated regions in our sample.

3. Results

Of the 601 children who completed neurodevelopmental testing, 591 (99%) mother–child pairs completed the infant feeding questionnaire and IQ testing (BF: $n = 296$; FF: $n = 295$). Of these, 398 (67.3%) pairs reported drinking tap water, had water fluoride data and complete covariate data (BF: $n = 200$; FF: $n = 198$). The demographic characteristics of women included in the current analyses ($n = 398$) were not substantially different from the original MIREC cohort ($N = 1945$) or the subset without complete water fluoride and covariate data ($n = 203$) (Table S2, Mcknight-hanes et al., 1988).

Among the BF group, more women who lived in a fluoridated region had a bachelor's degree or higher compared with those in a non-fluoridated region (86 vs. 74%, $p = .001$) (Table 1). Compared with the FF group, women in the BF group were more educated, more likely to be married or common law, and had higher HOME scores (all $ps < 0.05$). The BF group had significantly higher FSIQ and VIQ scores relative to the FF group (Table 1; Fig. S1). Children living in a fluoridated region had a significantly lower PIQ score, but higher VIQ score, relative to children living in a non-fluoridated region (Table 1; Fig. S1).

Water fluoride concentration was correlated with MUF (r = 0.37, $p < .001$) and estimated fluoride intake from formula (r = 0.79, $p < .001$); MUF was correlated with fluoride intake from formula (r = 0.55, $p < .001$).

3.1. Feeding status

The mean duration of exclusive breastfeeding was 4.98 months (*SD* = 3.48); 54 (13.6%) women reported never breastfeeding, 32 (8%) reported discontinuing breastfeeding after the first three months, and 200 (50.2%) reported continuing to breastfeed at six months or longer. Water fluoride concentration did not significantly differ between the BF ($M = 0.32$ mg/L) and FF groups ($M = 0.29$ mg/L; $p = .18$).

3.2. Model 1: IQ scores and water fluoride concentration by feeding status

A 0.5 mg/L increase in water fluoride concentration was associated with a decrease of 4.4 FSIQ points (95% CI: −8.34, −0.46, $p = .03$) in the FF group, but it was not significantly associated with FSIQ in the BF group ($B = -1.34$, 95% CI: −5.04, 2.38, $p = .48$) (Table 2; Fig. 1A); the interaction between water fluoride and feeding status was not statistically significant ($p = .26$). Controlling for fetal exposure by adding MUF to the model resulted in non-significant associations between water fluoride concentration and FSIQ in both the FF ($B = -3.58$, 95% CI: −7.83, 0.66, $p = .098$) and BF groups ($B = -1.69$, 95% CI: −5.66, 2.27, $p = .40$). Removing two cases with extreme IQ scores from the models resulted in non-significant associations between water fluoride concentration and FSIQ in both groups (Table S3).

Water fluoride concentration was significantly associated with lower PIQ in the FF ($B = -9.26$, 95% CI: −13.77, −4.76, $p < .001$) and the BF groups ($B = -6.19$, 95% CI: −10.45, −1.94, $p = .004$)

Table 1
Demographic characteristics and exposure outcomes for mother-child pairs by infant feeding status.

Characteristic	Breastfed ≥ 6 mo. (n = 200)		Formula-fed (n = 198)		
	Fluoridated (n = 83) Mean (SD)/%	Non-fluoridated (n = 117) Mean (SD)/%	Fluoridated (n = 68) Mean (SD)/%	Non-fluoridated (n = 130) Mean (SD)/%	*p* value comparing BF and FF groups
Maternal characteristics					
Years of age at delivery	32.54 (3.64)	32.86 (4.79)	32.91 (4.42)	32.39 (5.11)	.73
Net household income > $70 K	70.3	72.9	79.7	68	.88
Caucasian	88	93	88	84	.11
Maternal education					
Trade school diploma/high school	14	26*	28	42*	< .001
Bachelor's degree or higher	86	74*	72	58*	< .001
Employed at time of pregnancy	92	90	94	84*	.40
Married/common-law (at time of testing)	100	99	96	92	.001
Smoked in trimester 1	0	1.7	2.9	3.8	.17
Parity (first birth)	45	51	43	47	.61
Number of months exclusively breastfeeding	7.54 (2.95)	7.45 (2.46)	2.63 (2.08)	2.37 (2.13)	< .001
Child characteristics					
Years of age at IQ testing	3.48 (0.29)	3.34 (0.31)*	3.53 (0.28)	3.37 (0.3)*	.32
Female sex	51	53	54	47	.32
HOME total score	48.71 (3.42)	48.09 (3.86)	47.59 (4.33)	46.55 (4.76)	< .001
Second hand smoke in home	2.5	3.4	4.4	5.4	.43
Gestational age in weeks	39.22 (1.55)	39.17 (1.52)	38.68 (2.48)	39.15 (1.53)	.24
Birth weight (kg)	3.42 (0.50)	3.49 (0.46)	3.43 (0.62)	3.46 (0.52)	.75
Full Scale IQ	109.9 (12.4)	108.9 (13.6)	106.1 (15.8)	106.8 (13.5)	.03[a]
Verbal IQ[b]	115.1 (11.3)	110.4 (12.4)*	110.9 (14.9)	107.1 (13.3)	.00[a]
Performance IQ[b]	102.0 (15.2)	105.6 (15.8)	99.7 (15.1)	105.6 (13.4)*	.69
Exposure variables					
Water fluoride concentration (mg/L)	0.58 (0.08)	0.13 (0.06)*	0.59 (0.07)	0.13 (0.05)*	.18
% living in fluoridated region	41.5		34.3		14
Infant fluoride intake (mg F/day)	0.12 (0.07)	0.02 (0.02)*	0.34 (0.12)	0.08 (0.04)*	< .001
MUF concentration (mg/L)	0.70 (0.39)	0.42 (0.28)*	0.64 (0.37)	0.38 (0.27)*	.07

Abbreviations: HOME = Home Observation for Measurement of the Environment; MUF = Maternal urinary fluoride, adjusted for specific gravity; SD = standard deviation.

* $p < .05$ for comparing participants in the breastfed or formula-fed group living in a fluoridated versus non-fluoridated region.

[a] *p*-value reported for main effect of feeding status from 2 × 2 ANCOVA, adjusting for maternal education (binary), maternal race (binary), child's age at IQ testing (continuous), child's sex, HOME total score (continuous), second-hand smoke status in the child's house (yes, no), and water fluoridation status (fluoridated versus non-fluoridated).

[b] Main effect of fluoridation status, adjusting for maternal education (binary), maternal race (binary), child's age at IQ testing (continuous), child's sex, HOME total score (continuous), second-hand smoke status in the child's house (yes, no), and feeding status (BF vs. FF); VIQ: $p = .02$; PIQ: $p < .001$.

Table 2
Adjusted difference in IQ scores at 3–4 years of age per 0.5 mg/L water fluoride concentration and 0.5 mg infant fluoride intake from formula per day, with and without adjusting for maternal urinary fluoride (MUF).

Exposure variable	N	FSIQ B (95% CI)	N	PIQ B (95% CI)	N	VIQ B (95% CI)
Model 1						
Water Fl (mg/L)	398		393		397	
Formula-fed		−4.40 (−8.34, −0.46)*		−9.26 (−13.77, −4.76)*		0.89 (−2.87, 4.65)
Breastfed		−1.34 (−5.04, 2.38)		−6.19 (−10.45, −1.94)*		3.06 (−0.49, 6.61)
Water Fl (mg/L) adjusted for MUF[a]	350		345		349	
Formula-fed		−3.58 (−7.83, 0.66)		−7.93 (−12.84, −3.01)*		2.60 (−1.98, 7.16)
Breastfed		−1.69 (−5.66, 2.27)		−6.30 (−10.92, −1.68)*		4.20 (−0.06, 8.45)
Model 2						
Fluoride intake from formula	398	−2.69 (−7.38, 2.01)	393	−8.76 (−14.18, −3.34)*	397	3.08 (−1.40, 7.55)
Fluoride intake from formula adjusted for MUF[b]	350	−1.94 (−7.09, 3.21)	345	−7.62 (−13.64, −1.60)*	349	3.05 (−1.89, 7.98)

Abbreviations: Fl = fluoride; MUF = maternal urinary fluoride; Regression model adjusted for maternal education (binary), maternal race (binary), child's age at IQ testing (continuous), child's sex, HOME total score (continuous), and second-hand smoke status in the child's house (yes, no).

* $p < .05$.

[a] MUF was not significantly associated with FSIQ score ($B = -1.08$, 95% CI: −1.54, 0.47, $p = .29$), PIQ score ($B = -1.31$, 95% CI: −3.63, 1.03, $p = .27$), or VIQ score ($B = -0.34$, 95%CI: −2.21, 1.59, $p = .73$). Note: regression coefficients represent the predicted IQ difference per 0.5 mg/L MUF; effect for both sexes is reported. Variance inflation factor (VIF) for water Fl is 2.41 for FSIQ, 2.41 for PIQ, and 2.40 for VIQ when MUF is entered in the model.

[b] MUF is significantly associated with PIQ score ($B = -2.38$, 95% CI: −4.62, −0.27, $p = .04$), but not FSIQ score ($B = -1.50$, 95% CI: −3.41, 0.43, $p = .13$) or VIQ score ($B = -0.11$, 95% CI: −1.94, 1.74, $p = .91$); Note: regression coefficients represent the predicted IQ difference per 0.5 mg/L MUF; effect for both sexes is reported. Variance inflation factor (VIF) for infant fluoride intake is 1.10 for FSIQ, 1.12 for PIQ, and 1.10 for VIQ when MUF is entered in the model

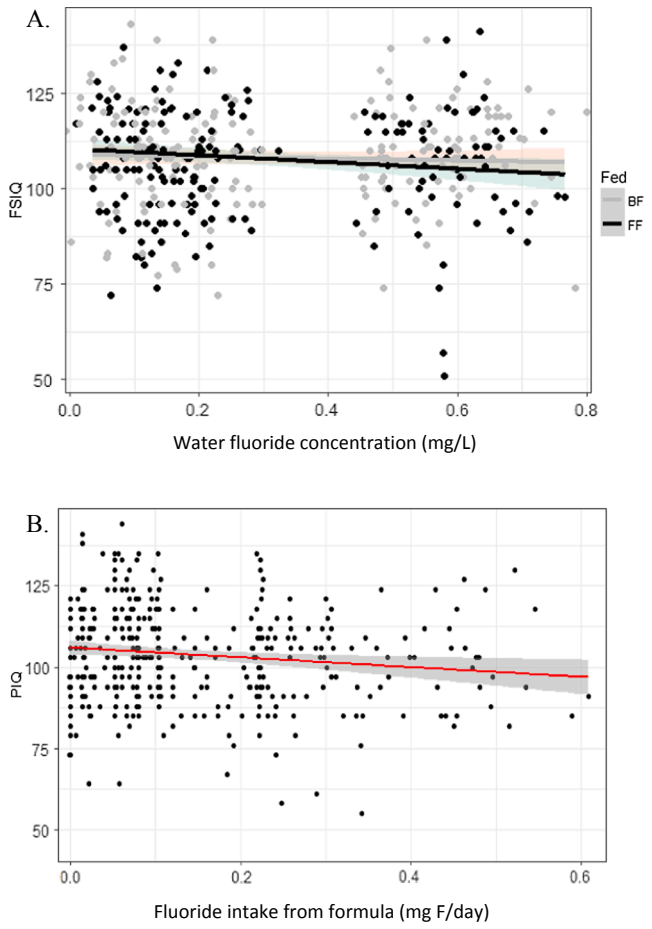

Fig. 1. A. Water fluoride concentration as a predictor of Full Scale IQ with an interaction by formula-fed (FF) vs. breastfed (BF) group. Black data points represent the FF group and grey data points represent the BF group. B. Fluoride intake from formula (mg F/day) as a predictor of Performance IQ score.

(Table 2); the interaction was not significant (p = .26). Controlling for MUF, water fluoride concentration remained significantly associated with PIQ in the FF (B = −7.93 95% CI: −12.84, −3.01, p = .002) and BF groups (B = −6.30, 95% CI: −10.92,-1.68, p = .008). Likewise, the associations between water fluoride concentration and PIQ remained significant for both groups after removing two cases with extreme IQ scores (Table S3).

In contrast, water fluoride concentration was not associated with VIQ in the FF (B = 0.89, 95% CI: −2.87, 4.65, p = .64) or BF group (B = 3.06, 95% CI: −0.49, 6.61, p = .09); these associations remained non-significant after controlling for MUF (Table 2) and removing two cases with extreme IQ scores (Table S3).

3.3. Model 2: IQ scores and fluoride intake from formula

Fluoride intake from formula was not significantly associated with FSIQ (B = −2.69, 95% CI: −7.38, 2.01, p = .26) or VIQ (B = 3.08, 95% CI: −1.40, 7.55, p = .18) (Table 2). In contrast, a 0.5 mg increase in fluoride intake predicted an 8.76-point decrement in PIQ score (95% CI: −14.18, −3.34, p = .002; Fig. 1B). Adding MUF to the PIQ model slightly attenuated the association between fluoride intake and PIQ (B = −7.62, 95% CI: −13.64, −1.60, p = .01) (Table 2). Removing two cases with extreme IQ scores did not appreciably alter the association between fluoride intake and PIQ score, with and without adjustment for MUF (Table S3).

4. Discussion

For each 0.5 mg/L increase in water fluoride concentration, we found a decrease of 4.4 FSIQ points among preschool children who were formula-fed in the first six months of life; 0.5 mg/L is the approximate difference in mean water fluoride level between fluoridated (0.59 mg/L) and non-fluoridated (0.13 mg/L) regions. In contrast, we did not find a significant association between water fluoride concentration and FSIQ among exclusively breastfed children. The association between water fluoride concentration and FSIQ must be interpreted with caution, however, because the association became non-significant when two outliers were removed. We observed an even stronger association between water fluoride and PIQ (non-verbal intelligence). A 0.5 mg/L increase in water fluoride level predicted a decrement in PIQ in both the formula-fed (9.3-points) and the breastfed groups (6.2-points). Adjusting for fetal exposure or removing two extreme scores did not appreciably alter these results.

We observed converging results using fluoride intake from formula, which is a continuous, time-weighted exposure estimate. For each 0.5 mg/day of fluoride intake, we found an 8.8-point decrement in PIQ; adjusting for fetal exposure using MUF attenuated the association only slightly (7.6-point decrement in PIQ). MUF was also negatively associated with PIQ (2.4-point decrement for each 0.5 mg/L increase in MUF). The fluoride intake estimate may reflect a more refined measure of exposure in infancy because it captures differences in both water fluoride level and the proportion of time each child was given formula over the first year of life. Yet, our binary classification of whether a child was exclusively breastfed for 6 months may better capture children who are most vulnerable to neurotoxic effects of fluoride because it subsets those exposed to fluoride during the early infancy period when the brain undergoes significant development (Huttenlocher and Dabholkar, 1997; Kostovic, 2006). Taken together, these findings suggest that using optimally fluoridated water (0.7 mg/L) to reconstitute infant formula may diminish the development of intellectual abilities in young children, particularly for non-verbal abilities. The findings also suggest that both prenatal and postnatal fluoride exposure affect the development of non-verbal intelligence to a greater extent than verbal intelligence. Prior studies examining prenatal exposure to fluoride and IQ showed a similar pattern (Bashash et al., 2017; Green et al., 2019).

Consistent with prior studies showing a positive effect of breastfeeding on cognition (Horta et al., 2015), children in the breastfed group had higher FSIQ and VIQ scores relative to the formula-fed group, regardless of fluoridation status (Table 1); higher education and income levels in the breastfed group likely accounts for part of this association (Walfisch et al., 2013). In contrast, the breastfed group did not differ significantly from the formula-fed group with respect to PIQ score. Children who lived in non-fluoridated regions showed higher PIQ scores than children who lived in fluoridated regions, though this difference was significant only for the formula-fed group, perhaps reflecting a higher vulnerability of nonverbal abilities to fluoride exposure in infancy.

Most studies of fluoride exposure from infant formula consumption have focused on risk for later development of dental enamel fluorosis (Brothwell and Limeback, 2003; Hong et al., 2006; Berg et al., 2011). Beyond fluorosis, the safety of fluoride exposure from infant formula has not been rigorously tested, despite warnings of overexposure (Diesendorf and Diesendorf, 1979). A recent study showed that up to 59% of infants younger than four months exceed the upper limit (0.1 mg/kg/day) (Institutes of Medicine, 1997) when optimally fluoridated water is used to reconstitute infant formula (Harriehausen et al., 2019); 33% and 14.3% of six- and nine-month old infants exceeded the upper limit threshold, respectively. Conversely, breastfed infants receive very low fluoride intake (generally less than 0.01 mg/L), even in communities with fluoridated water (Dabeka et al., 1986; Ekstrand, 1981; Fomon et al., 2000). Our estimate of fluoride intake (0.34 mg F/day) among formula-fed infants who live in a fluoridated region is an

underestimate of actual fluoride intake because we did not include fluoride from other sources, such as the fluoride found in the formula or foods; thus, the association between fluoride intake and IQ scores among formula-fed infants may be stronger than the association obtained in our analysis.

Our results, which showed that higher fluoride exposure in infancy was associated with diminished IQ scores in young children, are consistent with two longitudinal birth cohort studies. In one study involving 299 mother–child pairs living in Mexico City, there was a decrement of 3.2 IQ points in preschool aged children for every 0.5 mg/L of MUF level during pregnancy (Bashash et al., 2017). In the other study, which we conducted using the same Canadian cohort, we reported a decrement of 2.2 IQ points among preschool aged boys for every 0.5 mg/L of MUF level during pregnancy (Green et al., 2019). When MUF was included as a covariate in the current study, the association between MUF and FSIQ was not significant (see Table 2, note a). This discrepancy arises because (1) Green et al. (2019) did not include fluoride exposure in infancy as a covariate and (2) Green et al. (2019) estimated sex-specific MUF effects whereas the current study estimated an overall MUF effect.

The beneficial effects of fluoride predominantly occur at the tooth surface, after teeth have erupted (Limeback, 1999). Fluoride contributes to the prevention of dental caries primarily when it is topically applied to teeth, such as brushing with fluoridated toothpaste (Featherstone, 2001; Limeback, 1999; NRC, 2006; Pizzo et al., 2007; Warren and Levy, 2003). Because fluoride is not essential for growth and development (Scientific Committee on Health and Environmental Risks (SCHER), 2011), there is no recommended intake level of fluoride during fetal development or in the first six months of life before teeth have erupted. Accordingly, the Canadian Pediatric Society recommends administering supplemental fluoride (i.e. systemic exposure) only when primary teeth begin to erupt (American Dental Association) (at approximately 6 months) and only if the child is susceptible to high caries activity and is not exposed to other fluoride-based interventions, such as toothbrushing or water fluoridation (Godel, 2002).

The American Dental Association (Berg et al., 2011; American Dental Association, 2018) advises parents to use optimally fluoridated drinking water to reconstitute concentrate infant formulas, while being cognizant of the potential risk of mild enamel fluorosis development. This recommendation is echoed by the Centers for Disease Control and Prevention (Community Water Fluoridation. Infant Formula) as well as the U.S. Department of Health and Human Services (2015). The Canadian Dental Association (2019) recommends using water with low fluoride concentration (or ready-to-feed formula) when the fluoride level in drinking water is above the optimal level. In addition to tap water, which is reportedly used by 93% of caregivers who feed formula to infants (Brothwell and Limeback, 2003), “nursery” water (which may contain up to 0.7 mg F/L) is marketed for reconstituting formula and sold in Canada and the United States. The availability of fluoridated nursery water gives the false impression that fluoride exposure during early infancy is beneficial prior to teeth eruption.

Formula-fed infants who reside in fluoridated areas have a 70-fold higher intake of fluoride than exclusively breastfed infants (Ekstrand, 1981; Zohoori et al., 2018; United States Environmental Protection Agency, 2010). Formula-fed infants also retain more fluoride than breastfed infants (Zohoori et al., 2018; Ekstrand and Hardell, 1984) because infants have a limited capacity to excrete fluoride before renal function reaches its full capacity at about two years of age (National Research Council (NRC), 2006; Zohoori et al., 2018). Fluoride absorption also depends on the presence of other nutrients (Health Canada, 2010); when fluoride intake is exclusively from reconstituted formula, the bioavailability of fluoride is 65%, whereas a varied diet reduces fluoride absorption in tissues and bone to about 47% (Ekstrand and Ehrnebo, 1979). These factors place formula-fed infants at an even higher risk of fluoride toxicity.

Our study has some limitations. First, infant formulas vary in fluoride content. Ready-to-use formulas typically have less fluoride than powdered formula (Dabeka and McKenzie, 1987; Fomon et al., 2000); information about formula type was only available for 100 of 198 (50.5%) participants in the formula group; of those, 75% reported using powdered formula, which is the most common type of formula used by the general population (Infant Feeding Practices Survey II; Fomon et al., 2000). Variability in fluoride content is also seen across different types of powdered formula (United States Environmental Protection Agency, 2010; Harriehausen et al., 2019; Mahvi et al., 2010). Additionally, soy-based formula reconstituted with distilled water has more fluoride (0.24–0.30 mg/L depending on whether it is ready-to-feed or concentrated) than milk-based powdered formulas (0.12–0.17 mg/L) (Harriehausen et al., 2019; Van Winkle et al., 1995). Although we lacked data on brand of formula, we have no reason to expect that use of powdered versus ready-to-feed or soy- versus milk-based formula would differ by fluoridation status. Moreover, our effects were primarily based on water fluoride content, which is the major source of fluoride (Buzalaf et al., 2001). Second, we did not have specific information on the type of water (bottled versus tap) used to reconstitute formula. However, mothers typically report using tap water for reconstituting formula (Van Winkle et al., 1995) and we only included children of women who reported drinking tap water in our analyses. Third, there is potential for non-differential misclassification of the feeding status variable because mothers may have been confused by the definition of exclusive breastfeeding on the questionnaire or the responses may have been affected by recall or response bias. As with any survey, women could be confused by the question, but given the demographic of the sample – highly educated, English speaking, and non-teenage mothers – confusion seems less likely. Fourth, our method of estimating infant fluoride intake has not been validated. Finally, children were tested between 3 and 4 years of age and we have no information regarding other possible sources of fluoride that occurred between post-weaning and the age of testing. Thus, other sources of fluoride (e.g. dental products) or more frequent brushing, might differ between participants who lived in fluoridated versus non-fluoridated communities or among those in the breastfeeding versus formula-feeding group. To control for these potential differences, we included maternal education in all models. In addition, the design of our study compares water fluoride level and IQ scores in the formula-fed children using the breast-fed children as a control.

In summary, fluoride intake among infants younger than 6 months may exceed the tolerable upper limits if they are fed exclusively with formula reconstitued with fluoridated tap water. After adjusting for fetal exposure, we found that fluoride exposure during infancy predicts diminished non-verbal intelligence in children. In the absence of any benefit from fluoride consumption in the first six months, it is prudent to limit fluoride exposure by using non-fluoridated water or water with lower fluoride content as a formula diluent.

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Contributors statement

Dr Till conceptualized and designed the study, drafted the initial manuscript, and reviewed and revised the manuscript. Ms Green designed the study, curated the data, carried out the initial data analysis,

reviewed and revised the manuscript. Drs Flora and Hornung supervised data analysis, reviewed and revised the manuscript. Ms. Farmus assisted with data analysis, reviewed and revised the manuscript. Dr Martinez-Mier reviewed and revised the manuscript and supervised the analysis of maternal urinary fluoride. Mr Blazer collected the water fluoride data from water treatment plants and reviewed the manuscript. Drs Ayotte and Muckle assisted with initial data collection, and critically reviewed and revised the manuscript. Dr Lanphear conceptualized the study, and critically reviewed and revised the manuscript. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

Declaration of Competing Interest

The authors declared that there is no conflict of interest.

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Appendix A. Supplementary material

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Table S1. *Number of study participants and exclusions*

Characteristic	N	Feeding Status: Breastfed ≥6 mo.	Feeding Status: Formula-fed
All mother-child pairs with infant feeding and IQ data	591	296	295
Excluded: subjects with no water fluoride data or reported drinking well water or other		81	82
Subjects with water fluoride data available and reported drinking tap water	413	207	206
Excluded: subjects with missing covariate data		7	8
Analytic sample: water fluoride and covariate data	398	200	198
Excluded: subjects without maternal urinary fluoride values		27	21
Secondary analysis (with maternal urinary fluoride) using analytic sample	350	173	177

Table S2. *Comparison of analytic sample to other MIREC samples*

	Original MIREC cohort	Analytic sample	Participants excluded due to missing water fluoride and/or covariate data
n	1945	398	203
Mean (SD) age (years) of mother at enrollment	32.18 (5.06)	32.65 (4.61)	32.21 (4.62)
Caucasian (%)	85.63	88.2	91.4
Married or Common law (%)	95.2	96.2	95.3
Married to biological father (%)	75.9	73.9	73.7
Born in Canada (%)	81.38	79.4	87.8
Maternal Education (%)			
High school or less	8.7	4.8	5.6
Some college	5.2	3.8	4.1
College diploma	23.5	20.6	31.8
University degree	62.6	70.9	58.5
Employed at time of pregnancy (%)	85.4	88.9	86.3
Net income household > $70,000 (%)	69.6	72.1	72.3

Abbreviations: SD = standard deviation
Note: Differences between the analytic sample, original MIREC cohort, and excluded participants were all considered small (i.e. Cohen's effect size *h* of ≤0.20).

Table S3.

Adjusted difference in IQ scores at 3-4 years of age per 0.5 mg/L water fluoride concentration and 0.5 mg fluoride intake from formula per day without extreme IQ outliers.

Exposure variable	FSIQ		PIQ		VIQ	
	N	B (95% CI)	N	B (95% CI)	N	B (95% CI)
Model 1						
Water Fl (mg/L)	396		391		395	
Formula-fed		-3.14 (-6.99, 0.71)		-8.23 (-12.70, -3.77)*		2.07 (-1.60, 5.74)
Breastfed		-1.38 (-4.97, 2.22)		-6.22 (-10.41, -2.04)*		3.03 (-0.41, 6.46)
Water Fl (mg/L) adjusted for MUF[a]	349		344		348	
Formula-fed		-2.82 (-7.00, 1.35)		-7.31 (-12.20, -2.43)*		1.65 (-2.35, 5.65)
Breastfed		-1.69 (-5.58, 2.19)		-6.29 (-10.86, -1.72)*		2.42 (-1.31, 6.14)
Model 2						
Fluoride intake from formula	396	-1.12 (-6.17, 2.93)	391	-7.88 (-14.18, -3.34)*	395	4.08 (-0.26, 8.42)
Fluoride intake from formula adjusted for MUF[b]	349	-1.20 (-6.24, 3.85)	344	-7.01 (-12.98, -1.03)*	348	3.77 (-1.06, 8.60)

*p <.05

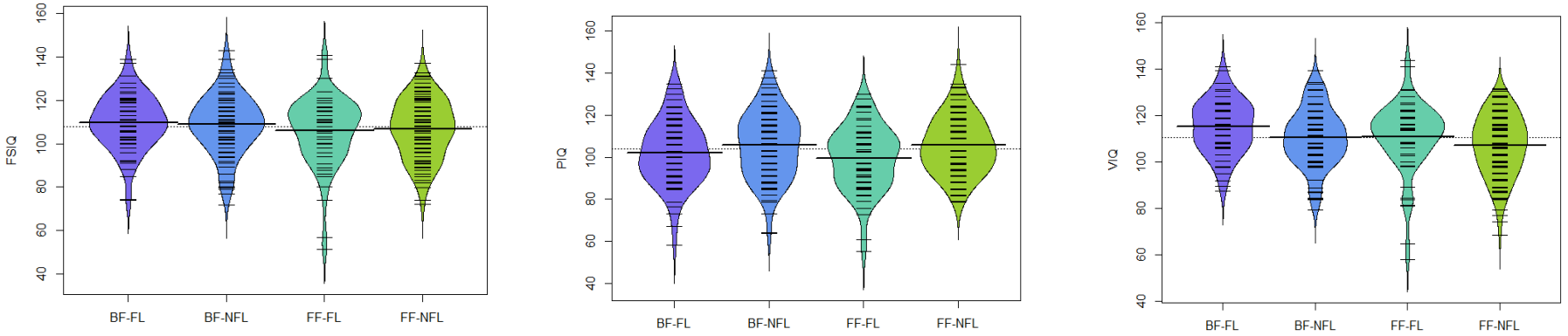
Abbreviations: Fl = fluoride; MUF = maternal urinary fluoride. Regression model adjusted for maternal education (binary), maternal race (binary), child's age at IQ testing (continuous), child's sex, HOME total score (continuous), and second-hand smoke status in the child's house (yes, no)

[a] MUF was not significantly associated with FSIQ score (B = -0.54, 95% CI: -3.04, 0.90, p = .28), PIQ score (B = -1.31, 95% CI: -3.61, 1.00, p = .27), or VIQ score (B = -0.34, 95% CI: -2.22, 1.55, p=.73). Regression coefficients represent the predicted IQ difference per 0.5 mg/L MUF; effect for both sexes is reported.

[b] MUF is significantly associated with PIQ score (B = -2.38, 95% CI: -4.60, -0.16, p = .04), but not FSIQ score (B = -1.49, 95% CI: -3.37, 0.39, p = .12) or VIQ score (B = -0.10, 95% CI: -1.89, 1.70, p = .92). Regression coefficients represent the predicted IQ difference per 0.5 mg/L MUF; effect for both sexes is reported.

Supplemental Figure 1.

IQ scores shown as a function of feeding status (BF vs. FF) and fluoridation status (fluoridated vs. non-fluoridated). In the bean plot, long black lines represent the mean value and the short black lines represent individual data points.



Abbreviations: FSIQ = Full Scale IQ score; PIQ = Performance IQ; VIQ = Verbal IQ; BF-F = Breastfed and fluoridated region (purple); BF-NFL = Breastfed and non-fluoridated region (blue); FF-F = Formula fed and fluoridated region (turquoise); FF-NFL = Formula fed and non-fluoridated region (green)