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

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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

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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:

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

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:

$$MUF_{CRE_1(mg/g)} = MUF_i / CRE_i \tag{2}$$

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:

$$MUF_{CRE_2(mg/L)} = (MUF_i / CRE_i) \times CRE_{avg} \tag{3}$$

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.

	MUF_{SG}			MUF_{CRE_1}		
Variables	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

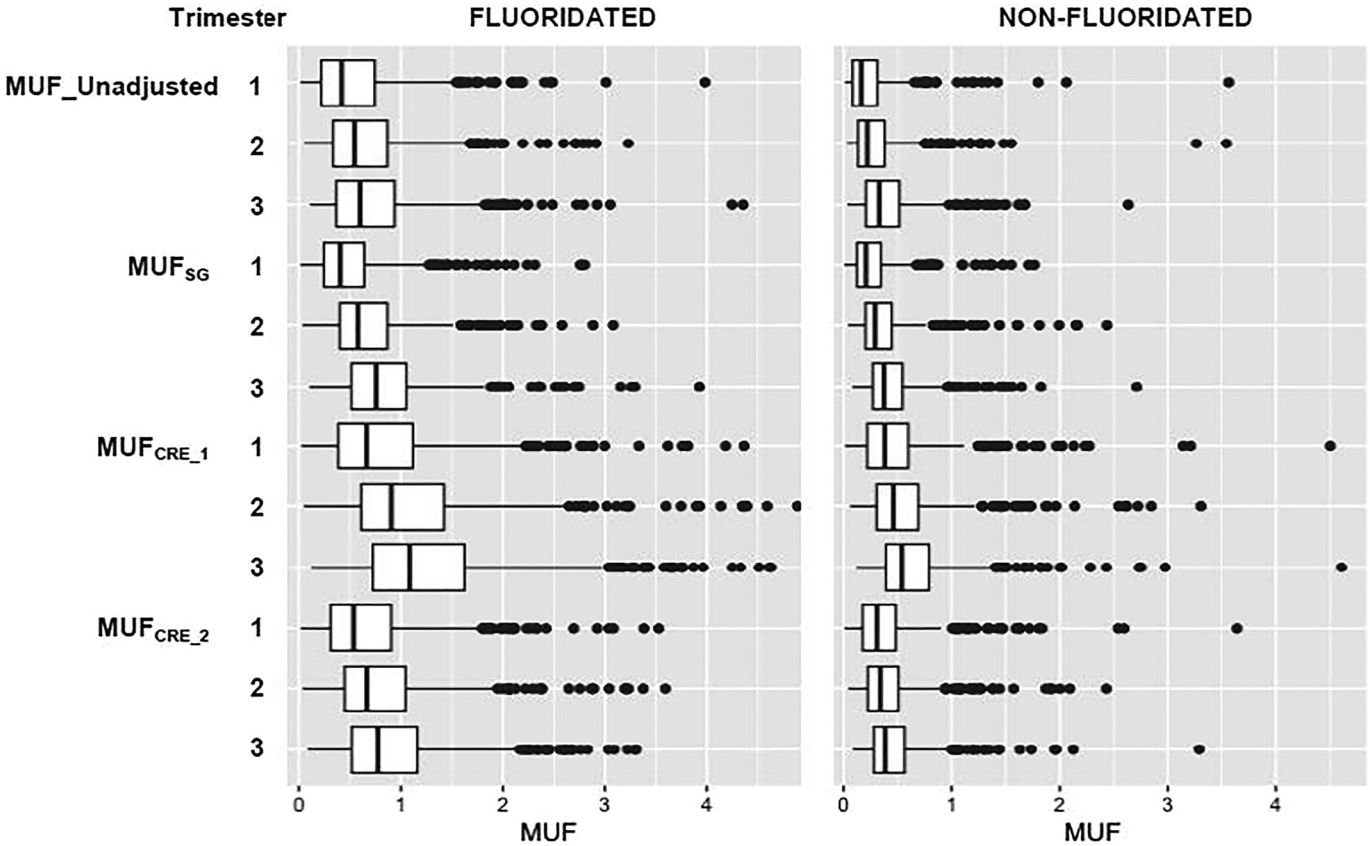

Figure 1. Fluoride concentrations by trimester in the urine of pregnant women from the MIREC cohort living in fluoridated versus nonfluoridated communities. MUF (maternal urinary fluoride) levels are shown unadjusted and adjusted for specific gravity (MUF_{SG}; Equation 1) and creatinine using two different 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

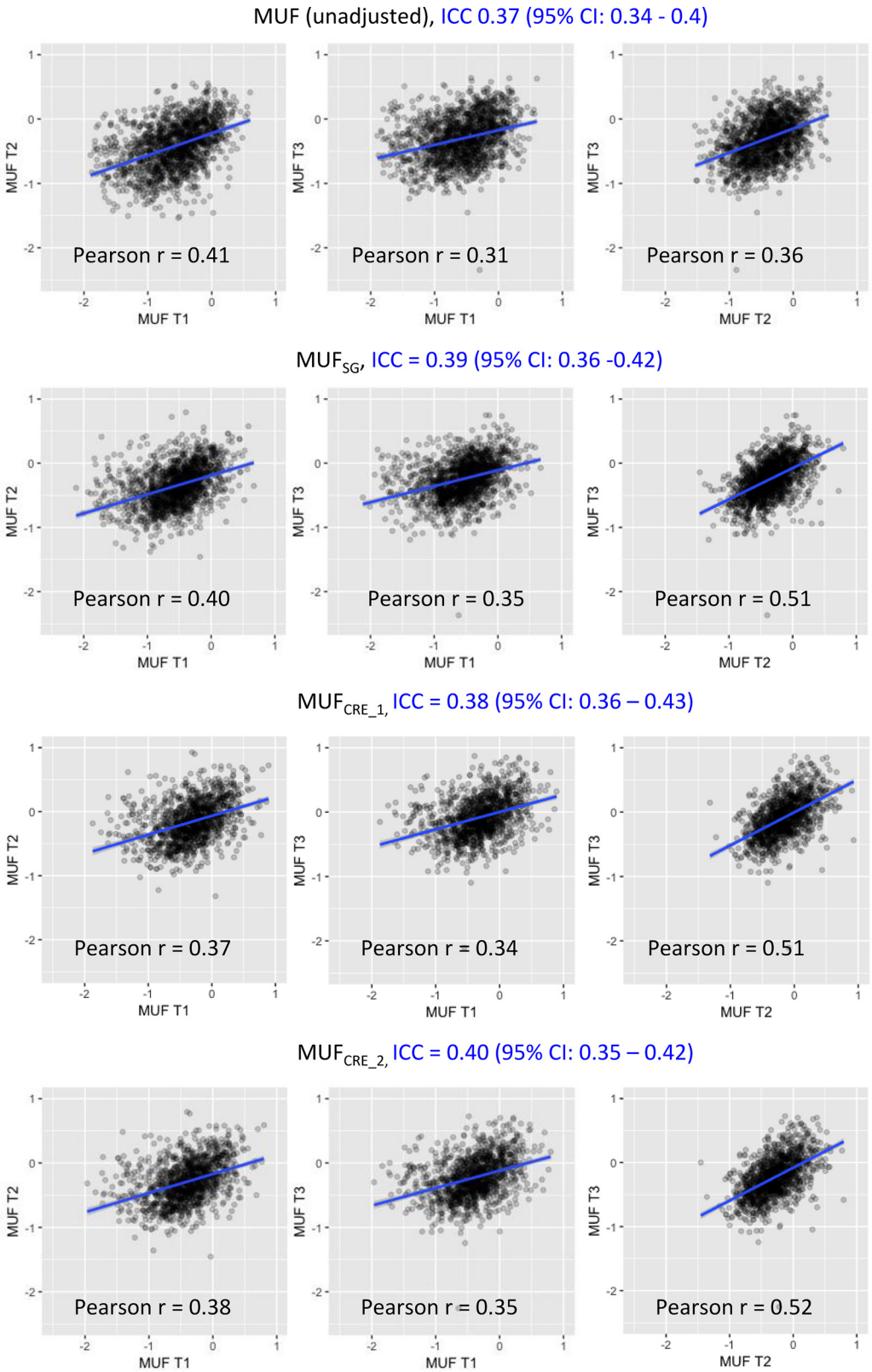

Figure 2. Pearson *r* correlations between pairs of trimesters (T1, T2, T3) and intraclass correlation coefficients (ICCs) across trimesters [with 95% confidence interval (CI)] for $\log_{10}$-transformed maternal urinary fluoride (MUF) levels without adjustment, with adjustment for specific gravity (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

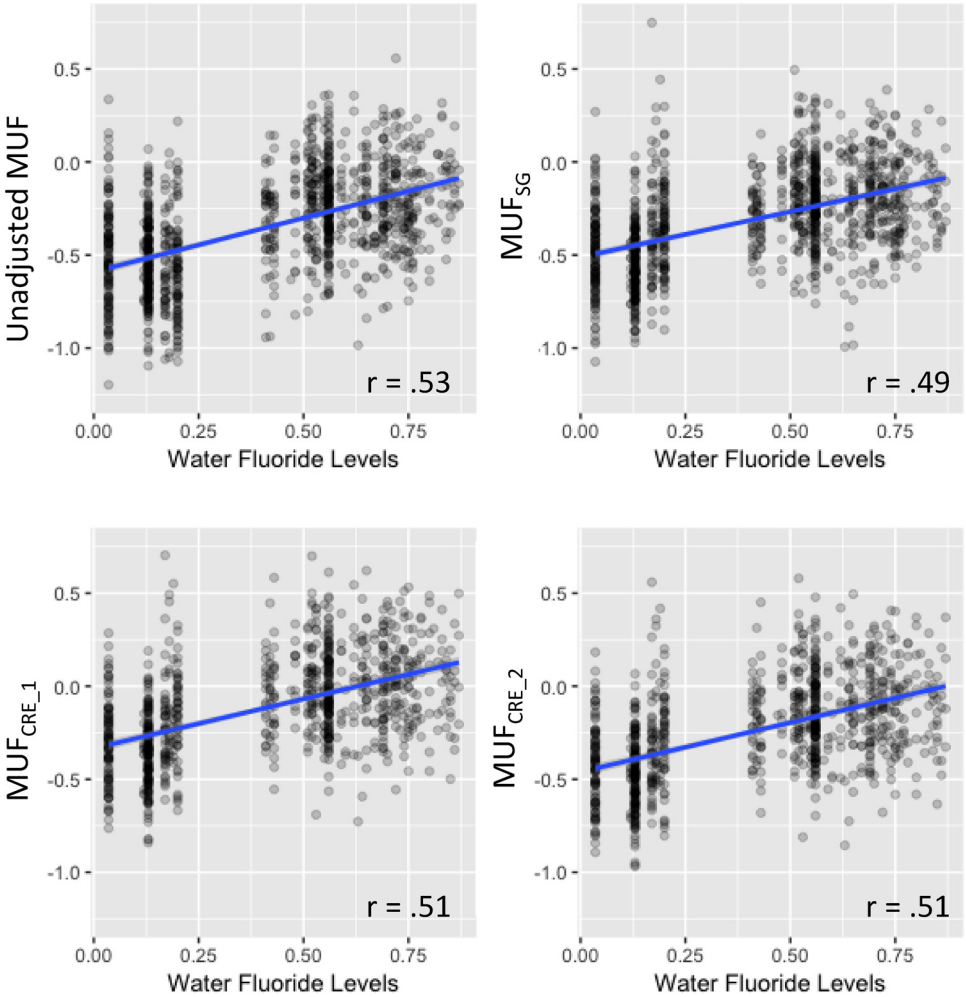

Figure 3. Log_{10}-transformed maternal urinary fluoride (MUF) exposure levels as a function of water treatment plant fluoride levels. MUF levels are shown unadjusted and adjusted for specific gravity (MUF_{SG}) and creatinine using two different methods (MUF_{CRE_1} and MUF_{CRE_2}). Individual data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