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Maternal fluoride exposure, fertility and birth outcomes: The MIREC cohort

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### Abstract

**Objective:** Fluoride exposure  $>1.5$  mg/L from water has been associated with adverse pregnancy and birth outcomes. Little is known, however, about the effect of fluoride at levels consistent with water fluoridation (i.e., 0.7 mg/L) on pregnancy and birth outcomes. We examined the relationship between maternal fluoride exposure, fertility, and birth outcomes in a Canadian pregnancy cohort living in areas where municipal drinking water fluoride concentrations ranged from 0.04 to 0.87 mg/L.

**Methods:** Using data from the Maternal-Infant Research on Environmental Chemicals (MIREC) study, we estimated fluoride exposure during pregnancy using three different metrics: 1) maternal urinary fluoride concentrations standardized for specific gravity ( $\text{MUF}_{\text{SG}}$ ) and averaged across all three trimesters ( $N = 1566$ ), 2) water fluoride concentration ( $N = 1370$ ), and 3) fluoride intake based on self-reported consumption of water, coffee, and tea, adjusted for body weight ( $N = 1192$ ). Data on fertility, birth weight, gestational age, preterm birth, and small-for-gestational age (SGA) were assessed. We used multiple linear regression to examine associations between fluoride exposure, birth weight and gestational age, and logistic regression to examine associations with fertility, preterm birth, and SGA, adjusted for relevant covariates.

**Results:** Median (IQR)  $\text{MUF}_{\text{SG}}$  was 0.50 (0.33-0.76) mg/L, median water fluoride was 0.52 (0.17-0.64) mg/L, and median fluoride intake was 0.008 (0.003-0.013) mg/kg/day.  $\text{MUF}_{\text{SG}}$ , water fluoride concentrations, and fluoride intake were not significantly associated with fertility, birth weight, gestational age, preterm birth, or SGA. Fetal sex did not modify any of the associations.

**Conclusion:** Fluoride exposure during pregnancy was not associated with fertility or birth outcomes in this Canadian cohort.

**Keywords:** Fluoride, pregnancy, birth outcomes, fertility, drinking water

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**Conflict of Interest:**

No conflicts of interest to disclose.

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## Introduction

Exposure to toxic chemicals during gestation has been associated with adverse birth outcomes, including preterm birth (< 37 weeks' gestation), low birth weight (LBW; < 2500 g), and decreased fetal growth, also termed small-for-gestational age (SGA; Berkowitz et al., 2006; Lam et al., 2014; Latini et al., 2003; National Toxicology Program, 2012; Stieb et al., 2012; U.S. Environmental Protection Agency, 2013). Complications related to these adverse birth outcomes are a leading cause of infant mortality (Ely & Driscoll, 2020; Statistics Canada, 2021). Infants born preterm or LBW are at increased risk of various developmental and health related issues, including acute respiratory and immunologic problems, as well as long-term motor, cognitive, behavioural, and social-emotional deficits (Bélanger et al., 2018; Bhutta et al., 2002; Hall et al., 2008; Lemons et al., 2001; U.S. Environmental Protection Agency, 2013). Exposure to toxic chemicals prior to conception has also been associated with reduced fertility (Buck Louis, 2014; Chevrier et al., 2013; Cole et al., 2005; Fei et al., 2009; Whitworth et al., 2012).

Fluoridated water contributes the largest source of fluoride exposure in adolescents and adults (U.S. Environmental Protection Agency, 2010). Fluoride can occur naturally in some freshwater or be added to public water supplies at a level of 0.7 mg/L for protection against dental caries. In some parts of the world, naturally occurring fluoride levels can far exceed the recommended upper limit of 1.5 mg/L (World Health Organization, 2004).

High levels of fluoride exposure in pregnant women living in Africa and India have been associated with greater risk of miscarriage and stillbirth (Goyal et al., 2020), as well as preterm and LBW infants (Diouf et al., 2012; Sastry et al., 2011). These observed associations may be explained, in part, by the increased risk of anemia that has been linked to high-level fluoride exposure (Goyal et al., 2020; Susheela et al., 2016; Susheela et al., 2010). High water fluoride

concentrations have also been associated with reductions in annual fertility rate in ecological studies (Freni, 1994; Yousefi et al., 2017). In experimental studies, long-term exposure to sodium fluoride (NaF) in mice and rats has been linked to reductions in fertility, number of viable fetuses, concentration of reproductive hormones, total number of follicles, and sperm quality (Chaithra et al., 2020; Darmani et al., 2001; Elbetieha et al., 2000; Gupta et al., 2007; Pushpalatha et al., 2005; Yin et al., 2015; Zhou et al., 2013b).

Recent epidemiological studies conducted in communities with fluoridation have associated fluoride exposure in pregnancy with increased risk of neurotoxicity in the offspring (Green et al., 2019; Bashash et al., 2017); however, little is known about the effect of exposure to these concentrations of fluoride on fertility or birth outcomes. Some studies have suggested that lower-level fluoride exposure (~0.7 mg/L) for pregnant women may protect against the adverse effects of maternal periodontal disease on birth outcomes. Specifically, an experimental study conducted in mice subjected to intrauterine inflammation during gestation (a sequela of maternal periodontal disease; Jia et al., 2019) reported that exposure to low levels of fluoride was associated with reduced prevalence of preterm birth. An ecologic study (Zhang et al., 2019) showed that dental cleaning, and dental cleaning in tandem with community water fluoridation (CWF) were associated with reduced prevalence of preterm birth; yet there was no association with CWF alone. While an increased risk of adverse birth outcomes has been observed among women with oral health diseases other than caries (i.e., periodontal disease; Xiong et al., 2007), no associations have been identified between dental caries in pregnancy and birth outcomes, including preterm birth (Wagle et al. 2018).

Considering the ubiquity of fluoride exposure and the large social, health, and economic burdens of infertility and preterm birth (Behrman & Butler, 2007), we examined the relationship

between maternal fluoride exposure and fertility as well as birth outcomes, including birth weight, gestational age, preterm birth, and SGA. We measured fluoride concentrations in urine and tap water, and fluoride intake was estimated through beverage consumption in a large sample of pregnant women living in 10 cities across Canada, seven of which have CWF. Given that this is the first cohort study to examine the relationship between fluoride exposure, fertility and birth outcomes among pregnant women living in communities with and without CWF, we do not propose specific hypotheses.

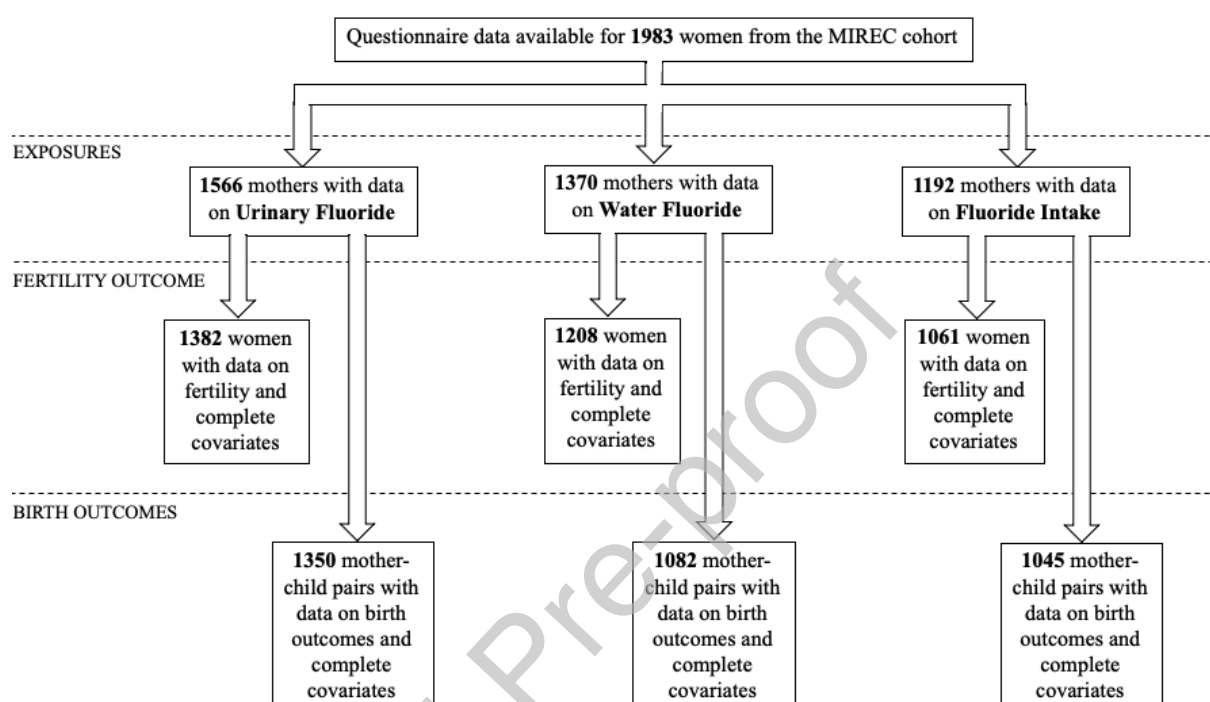
## Methods

### Study Population

Between 2008 and 2011, the Maternal-Infant Research on Environmental Chemicals (MIREC) study recruited 2001 pregnant women from 10 cities across Canada. Women were recruited if they could communicate in English or French, were 18 years of age or older, and were within the first 14 weeks of gestation. Women were excluded if there were known fetal abnormalities, medical complications, or illicit drug use during pregnancy. For the fertility outcome sample, women were excluded if their male partner reported infertility. For the birth outcomes sample, women were excluded if they did not have singleton, live births and one mother-infant pair was excluded due to an extremely small birth weight (~1110 g). Additional details are provided by Arbuckle et al. (2013).

Of the 2001 women recruited, 1983 had available questionnaire data; 1566 of these women had three urinary fluoride measures of which fertility and birth outcomes with complete covariates were available for 1382 (88.3%) and 1350 (86.2%), respectively; 1370 women had water fluoride concentration of which fertility and birth outcomes with complete covariates were available for 1208 (88.2%) and 1082 (79.0%), respectively; and 1192 women had fluoride intake

measured of which fertility and birth outcomes with complete covariates were available for 1061 (89.0%) and 1045 (87.7%), respectively (see Figure 1 for our population flow chart and Supplemental Figure 1 for complete covariates).



*Figure 1.* Population flow chart. Eligible participants recruited from the Maternal-Infant Research on Environmental Chemicals (MIREC) study.

Participants completed a questionnaire during the first and third trimester of pregnancy. Sociodemographic (e.g., maternal age, level of education, income, ethnicity, and marital status) and behavioural information (e.g., beverage consumption and smoking) were collected. Pre-pregnancy body mass index (BMI) was determined by dividing self-reported weight (kg) by measured height squared (m<sup>2</sup>).

The MIREC study received ethics approval from all recruitment sites and this study received ethics approval from Health Canada and York University. All women in MIREC provided written informed consent.



## Measures

### *Maternal Urinary Fluoride (MUF) Concentration*

We collected urine spot samples at each trimester of pregnancy (see Till et al., 2018). Urine was collected in Nalgene containers and then aliquoted into smaller cryovials. Samples were stored and shipped at appropriate temperatures. To strengthen reliability, women were only included in the analysis if they had all three spot samples. Urine samples were analyzed for fluoride at the Indiana University School of Dentistry using a modification of the hexamethyldisiloxane (Sigma Chemical Co., USA) microdiffusion method with ion-selective electrode (Martínez-Mier et al., 2011). The limit of detection for urinary analyses was 0.02 mg/L; precision and validity of the analyses used are reported in Martínez-Mier et al. (2011).

To account for variability in urinary dilution, each trimester MUF value (mg/L) was standardized for specific gravity (SG), prior to calculating the average MUF concentration, using the following formula (Till et al., 2018):

$$\text{MUF}_{\text{SG}} = \text{MUF}_i \times (\text{SG}_M - 1) / (\text{SG}_i - 1)$$

Where  $\text{MUF}_{\text{SG}}$  is the SG-standardized fluoride concentration (mg/L),  $\text{MUF}_i$  is the observed fluoride concentration (mg/L),  $\text{SG}_i$  is the SG of the individual urine sample, and  $\text{SG}_M$  is the median SG for the cohort.

After standardizing for SG, one average MUF concentration was excluded because the adjusted value exceeded the highest concentration standard (5 mg/L) and there was less certainty of it being a representative exposure value.

### *Water Fluoride*

Water fluoride concentration was determined for women who reported drinking tap water during pregnancy, by matching participants' postal codes to municipal water treatment plants.

Water treatment plants measured fluoride levels daily if fluoride was added to public drinking water, and weekly or monthly if fluoride was not added to public water (Till et al., 2018). To estimate water fluoride concentration for each woman, we calculated geometric means across pregnancy.

### *Maternal Fluoride Intake*

Information on women's body weight and consumption of tap water, tea, and coffee in their first and third trimesters were collected through the self-report questionnaire. We estimated maternal fluoride intake (mg/kg/day) adjusted for body weight in trimester one and three separately by multiplying water fluoride concentration (mg/L) by total volume (L) consumed of water, tea, and coffee; we then added the additional fluoride content that would be expected from each cup of black tea or green tea consumed. We used 0.326 mg as an estimate of fluoride intake in a 200-mL cup of black tea and 0.260 mg as an estimate of fluoride intake in a 200-mL cup of green tea (Krishnankutty et al., 2021). The final estimate of maternal fluoride intake (mg/kg/day) was derived by taking the average of the two estimates for trimesters one and three. Overall, maternal fluoride intake was calculated using the following formula:

$$\frac{(\text{WaterF}_{T1} * \text{TotalCups}_{T1}) + \text{BlackTeaF}_{T1} + \text{GreenTeaF}_{T1}}{\text{BW}_{T1}} + \frac{(\text{WaterF}_{T3} * \text{TotalCups}_{T3}) + \text{BlackTeaF}_{T3} + \text{GreenTeaF}_{T3}}{\text{BW}_{T3}}$$

2

Where WaterF is the amount of fluoride in a 200mL cup based on each women's individual water fluoride concentration, TotalCups is the total volume of water, coffee, and tea consumed, BlackTeaF is the fluoride intake in a 200-mL cup of black tea, GreenTeaF is the fluoride intake in a 200-mL cup of green tea, and BW is maternal body weight in kilograms. The subscript T1 represents trimester 1 data, and the subscript T3 represents trimester 3 data.

### **Outcome Measures**

In the MIREC cohort, fertility was assessed through answers to the following question:

“How many months did it take you to get pregnant with this pregnancy?” Infertility was defined as a time to conception of 12 months or longer. This measure is consistent with the World Health Organization’s definition of infertility as “a failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse” (Zegers-Hochschild et al., 2009). Birth weight was extracted from medical charts at delivery and was measured in grams. SGA was assessed using sex-specific, Canadian-based reference charts assessing birth weight for gestational age (Kramer et al., 2001). Infants were categorized as SGA if their birth weight was less than the 10th percentile for gestational age (Kramer et al., 2001). Gestational age was determined based on last menstrual period (LMP) or ultrasound established dates. LMP was used unless LMP and ultrasound established dates differed by more than seven days; in which case ultrasound dates were used (Kieler et al., 1993). Preterm birth was defined as a gestational age at delivery of less than 37 weeks.

### Statistical Analyses

We used linear regression models to estimate the associations between our three measures of fluoride exposure ( $MUF_{SG}$ , water fluoride, and fluoride intake) and birth weight and gestational age. We used logistic regression models to estimate the associations between our three measures of fluoride exposure ( $MUF_{SG}$ , water fluoride, and fluoride intake) and increased odds of dichotomous outcomes: infertility, SGA, and preterm birth. In supplementary analyses, chi-square tests for categorical covariates and t-tests for continuous covariates were used to test for sampling differences. Given that  $MUF_{SG}$  is the gold standard for measuring fluoride exposure, we specifically tested for differences between the sample with  $MUF_{SG}$  and outcome data and the original group of women included in the MIREC cohort.

Potential covariates were identified *a priori* based on biologically plausible and reported associations with fluoride, fertility, gestational age, and birth weight (Buzalaf et al., 2015; Buzalaf & Whitford, 2011; Cogswell & Yip, 1995; Kelly-Weeder & Cox, 2007; Stephen & Chandra, 2006; Till et al., 2018) and were conceptualized in directed acyclic graphs (DAGs; see Supplemental Figure 1). Based on the relationships outlined within our DAGs, a covariate was retained in a model if its *P* value was less than .20 and its inclusion changed the regression coefficient associated with fluoride exposure measures by more than 10%.

For our analysis of fertility, the covariates considered included pre-pregnancy BMI, ethnicity (white, other), maternal age, income (less than \$100,000, \$100,000 or more), level of education (bachelor's degree or higher, trade school diploma or lower), exposure to secondhand smoke (yes, no), smoking in first trimester (yes, no), and city of residence.

For our analyses of birth outcomes, the covariates considered included pre-pregnancy BMI, infant sex, ethnicity (white, other), parity (zero, one, two or more), marital status (in a relationship, single), maternal age, income (less than \$100,000, \$100,000 or more), level of education (bachelor's degree or higher, trade school diploma or lower), alcohol intake during pregnancy (yes, no), exposure to secondhand smoke (yes, no), smoking in first trimester (yes, no), and city of residence.

Models estimating odds of SGA and preterm birth were adjusted for the same set of covariates as those used in the analyses of birth weight and gestational age. Given findings that males may be more sensitive to prenatal fluoride exposure (Green et al., 2020), we also examined sex-specific associations in all birth outcome models by testing the interaction between child sex and each fluoride measure; however, no interactions were observed (i.e., all *p* values > .20).

Regression diagnostics confirmed that there were no issues with collinearity in any of the models (variance inflation factor  $<4$  for all covariates). Plots of residuals against fitted values did not suggest any assumption violations. Sensitivity analyses run without influential observations, as measured by studentized residuals, leverages, Cook's distance and DFITS, provided no substantial differences. Including quadratic effects of MUF<sub>SG</sub>, water fluoride, or fluoride intake did not significantly improve the regression models.

Analyses were conducted using STATA version 16.1 (STATA corporation). The *P* value level of significance was .05, and all tests were 2-tailed. All coefficients are reported for every 1 mg/L increase in MUF<sub>SG</sub> and water fluoride concentration, and for every 0.01 mg/kg increase in fluoride intake per day.

## Results

Demographic characteristics of the subsamples with data on MUF<sub>SG</sub>, water fluoride, and fluoride intake and fertility and birth outcomes can be found in Tables 1 and 2, respectively. The 1382 women with data on MUF<sub>SG</sub>, fertility and complete covariates did not significantly differ from the original sample of 1983 women on most of the demographic characteristics except for the percentage of smokers in trimester 1 (Supplemental Table 1). Similarly, the 1350 mother-child dyads with MUF<sub>SG</sub>, singleton, live births and complete covariate data did not differ significantly from the original sample of 1828 women with singleton, live births on many of the demographic characteristics except for the mean gestational age and percentage of women with a graduate school education (Supplemental Table 2).

Approximately 12% of the women took 12 months or longer to become pregnant. Infants had a mean birth weight of 3478 g (SD = 471.8; range: 1765-5070) and a mean gestational age of 39.47 weeks (SD = 1.41; range: 33.30-42.40). Approximately 5% of women delivered infants

who were preterm or SGA. Among fetal growth outcomes, birth weight and gestational age were moderately correlated ( $r = .46$ ).

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**Table 1**

Demographic characteristics of the subsamples with data on fertility, complete covariates and MUF<sub>SG</sub> (N = 1382), water fluoride (N = 1208), or fluoride intake (N = 1061). Continuous variables are reported as mean ( $\pm$  SD), and categorical variables are reported as n (%).

Characteristics	Exposure Predictor for Fertility Sample:		
	MUF <sub>SG</sub> (N = 1382)	Water Fluoride (N = 1208)	Fluoride Intake (N = 1061)
<i>Outcomes</i>			
Infertility			
Yes	159 (11.51)	141 (11.67)	124 (11.69)
No	1223 (88.49)	1067 (88.33)	937 (88.31)
Time to conception (months)	5.18 ( $\pm$ 10.47)	5.07 ( $\pm$ 9.88)	5.11 ( $\pm$ 9.97)
<i>Covariates</i>			
Ethnicity			
White	1202 (86.98)	1016 (84.11)	893 (84.17)
Other	180 (13.02)	192 (15.89)	168 (15.83)
Maternal age (yr)	32.41 ( $\pm$ 4.85)	32.62 ( $\pm$ 5.07)	32.58 ( $\pm$ 5.05)
Pre-pregnancy BMI (kg/m <sup>2</sup> )	24.80 ( $\pm$ 5.38)	24.56 ( $\pm$ 5.22)	24.53 ( $\pm$ 5.19)
Household income (CAD)			
<100 000	807 (58.39)	689 (57.04)	609 (57.40)
$\geq$ 100 000	575 (41.61)	519 (42.96)	452 (42.60)
Level of Education			
College degree or less	478 (34.59)	386 (31.95)	333 (31.39)
Graduate school	904 (65.41)	822 (68.05)	728 (68.61)
Smoked in trimester 1			
Yes	58 (4.20)	53 (4.39)	43 (4.05)
No	1324 (95.80)	1155 (95.61)	1018 (95.95)
Second hand smoke in trimester 1			
Yes	74 (5.35)	63 (5.22)	57 (5.37)
No	1308 (94.65)	1145 (94.78)	1004 (94.63)

Abbreviations: MUF<sub>SG</sub> = maternal urinary fluoride standardized for specific gravity; BMI = body mass index

**Table 2**

Demographic characteristics of the subsamples with data on singleton, live births, complete covariates and MUF<sub>SG</sub> (N = 1350), water fluoride (N = 1082), or fluoride intake (N = 1045).

Continuous variables are reported as mean ( $\pm$  SD), and categorical variables are reported as n (%).

Characteristics	<u>Exposure Predictor for Birth Outcomes Sample:</u>		
	MUF <sub>SG</sub> (N = 1350)	Water Fluoride (N = 1082)	Fluoride Intake (N = 1045)
<i>Outcomes</i>			
Birth weight (g)	3479 ( $\pm$ 468.2) <sup>a</sup>	3473 ( $\pm$ 480.4) <sup>b</sup>	3468 ( $\pm$ 480.3) <sup>c</sup>
Gestational age (wks)	39.48 ( $\pm$ 1.39)	39.48 ( $\pm$ 1.43)	39.48 ( $\pm$ 1.44)
Small-for-gestational age			
Yes	70 (5.20)	62 (5.75)	62 (5.96)
No	1275 (94.8)	1016 (94.25)	979 (94.04)
Preterm birth			
<37	61 (4.52)	58 (5.36)	56 (5.36)
$\geq$ 37	1289 (95.48)	1024 (94.64)	989 (94.64)
<i>Covariates</i>			
Infant sex			
Boy	706 (52.30)	579 (53.51)	559 (53.49)
Girl	644 (47.70)	503 (46.49)	486 (46.51)
Ethnicity			
White	1174 (86.96)	915 (84.57)	880 (84.21)
Other	176 (13.04)	167 (15.43)	165 (15.79)
Maternal age (yr)	32.39 ( $\pm$ 4.86)	32.56 ( $\pm$ 5.01)	32.54 ( $\pm$ 5.04)
Pre-pregnancy BMI (kg/m <sup>2</sup> )	24.84 ( $\pm$ 5.43)	24.64 ( $\pm$ 5.27)	24.58 ( $\pm$ 5.19)
Parity			
0	612 (45.33)	509 (47.04)	498 (47.66)
1	537 (39.78)	420 (38.82)	406 (38.85)
2+	201 (14.89)	153 (14.14)	141 (13.49)
Marital status			
Married or common-law	1297 (96.07)	1031 (95.29)	994 (95.12)
Single	53 (3.93)	51 (4.71)	51 (4.88)



Household income (CAD)	791 (58.59)	626 (57.86)	602 (57.61)
<100 000	559 (41.41)	456 (42.14)	443 (42.39)
≥100 000			
Level of Education			
College degree or less	471 (34.89)	342 (31.61)	331 (31.67)
Graduate school	879 (65.11)	740 (68.39)	714 (68.33)
Drinks alcohol			
Yes	250 (18.52)	206 (19.04)	199 (19.04)
No	1100 (81.48)	876 (80.96)	846 (80.96)
Smoked in trimester 1			
Yes	57 (4.22)	44 (4.07)	43 (4.11)
No	1293 (95.78)	1038 (95.93)	1002 (95.89)
Second hand smoke in trimester 1			
Yes	72 (5.33)	59 (5.45)	57 (5.45)
No	1278 (94.67)	1023 (94.55)	988 (94.55)

Abbreviations:  $MUF_{SG}$  = maternal urinary fluoride standardized for specific gravity;  $BMI$  = body mass index

<sup>a</sup>N = 1345, <sup>b</sup>N = 1078, <sup>c</sup>N = 1041

### *Fluoride Measurements*

In both our fertility and birth outcomes samples, the median MUF<sub>SG</sub> concentration was 0.50 mg/L (range: 0.05-3.33; IQR: 0.33-0.76 mg/L). Similarly, the median water fluoride concentration was 0.52 mg/L (range: 0.04-0.87; IQR: 0.17-0.64 mg/L), and the median estimated fluoride intake was 0.008 mg per kg of body weight per day (range: 0.000-0.043; IQR 0.003-0.013 mg/kg/day). MUF<sub>SG</sub> was moderately correlated with both water fluoride concentration ( $r = .35$ ;  $p < .001$ ) and fluoride intake ( $r = .47$ ;  $p < .001$ ); likewise, water fluoride concentration was highly correlated with fluoride intake ( $r = .68$ ;  $p < .001$ ).

### *Fluoride Exposure and Birth Weight and Gestational Age*

There was a significant positive association between MUF<sub>SG</sub> and birth weight in the unadjusted model ( $B = 78.97$ ; 95% CI: 15.13, 142.81;  $p = .015$ ); however, in covariate-adjusted models, no significant associations were observed between MUF<sub>SG</sub> and birth weight or gestational age (Table 2). Similarly, no significant associations were detected between water fluoride concentration or fluoride intake and birth weight or gestational age in either unadjusted or covariate-adjusted models (Table 2).

### *Fluoride Exposure and Preterm Birth, SGA, and Infertility*

No significant associations were observed between MUF<sub>SG</sub>, water fluoride concentration, or fluoride intake and the risk of preterm birth, SGA, or infertility in either the unadjusted or covariate-adjusted models (Table 2).

**Table 2.**

Unadjusted and adjusted effect estimates of association between fluoride exposure variables and birth weight, gestational age, SGA, preterm birth, and infertility

Outcome	N	MUF <sub>SG</sub>	N	Water Fluoride	N	Fluoride Intake
<b>Birth Weight</b>						
Unadjusted B (95% CI)	1345	78.97 (15.13, 142.81)*	1078	35.07 (-78.99, 149.14)	1041	-10.24 (-51.35, 30.87)
Adjusted B (95% CI)	1345	54.66 (-14.11, 123.44) <sup>a</sup>	1078	14.87 (-97.66, 127.40) <sup>b</sup>	1041	-46.88 (-101.45, 7.71) <sup>c</sup>
<b>Gestational Age</b>						
Unadjusted B (95% CI)	1350	0.10 (-0.09, 0.29)	1082	0.00 (-0.34, 0.34)	1045	0.09 (-0.03, 0.21)
Adjusted B (95% CI)	1350	0.08 (-0.13, 0.28) <sup>d</sup>	1082	0.01 (-0.32, 0.35) <sup>e</sup>	1045	0.14 (-0.02, 0.31) <sup>f</sup>
<b>SGA</b>						
Unadjusted OR (95% CI)	1345	0.80 (0.41, 1.55)	1078	0.58 (0.21, 1.60)	1041	0.68 (0.45, 1.04)
Adjusted OR (95% CI)	1328	0.89 (0.43, 1.84) <sup>g</sup>	1078	0.66 (0.24, 1.82) <sup>h</sup>	1025	0.61 (0.34, 1.11) <sup>i</sup>
<b>Preterm Birth</b>						
Unadjusted OR (95% CI)	1350	0.99 (0.51, 1.91)	1082	1.27 (0.44, 3.65)	1045	0.86 (0.58, 1.29)
Adjusted OR (95% CI)	1350	0.97 (0.47, 2.00) <sup>j</sup>	1082	1.38 (0.48, 3.94) <sup>k</sup>	1045	0.70 (0.40, 1.23) <sup>l</sup>
<b>Infertility</b>						
Unadjusted OR (95% CI)	1382	1.10 (0.72, 1.65)	1208	0.62 (0.31, 1.23)	1061	0.78 (0.59, 1.04)
Adjusted OR (95% CI)	1382	0.99 (0.63, 1.57) <sup>m</sup>	1208	0.70 (0.35, 1.37) <sup>n</sup>	1061	0.85 (0.56, 1.29) <sup>o</sup>

\*  $p < .05$ ; MUF<sub>SG</sub> = maternal urinary fluoride standardized for specific gravity; CI = confidence interval;

\*5 participants with MUF<sub>SG</sub> data and 4 with water fluoride and fluoride intake data were missing data on birth weight/SGA

<sup>a</sup>Model adjusted for city, parity, and infant sex

<sup>b</sup>Model adjusted for pre pregnancy BMI, parity, marital status, and infant sex

<sup>c</sup>Model adjusted for city, income, alcohol consumption, infant sex, and ethnicity

<sup>d</sup>Model adjusted for maternal age, city, income, and parity

<sup>e</sup>Model adjusted for maternal age, maternal level of education, pre-pregnancy BMI, parity, and marital status

<sup>f</sup>Model adjusted for city

<sup>g</sup>Model adjusted for maternal age, city, parity, marital status, and infant sex

<sup>h</sup>Model adjusted for maternal age, maternal level of education, pre pregnancy BMI, parity, marital status, and infant sex

<sup>i</sup>Model adjusted for city, income, infant sex, ethnicity, and alcohol consumption

<sup>j</sup>Model adjusted for maternal age, city, parity, and income

<sup>k</sup>Model adjusted for maternal age, maternal level of education, pre pregnancy BMI, parity, and marital status

<sup>l</sup>Model adjusted for city

<sup>m</sup>Model adjusted for maternal age, city, smoke, second hand smoke, and maternal level of education

<sup>n</sup>Model adjusted for maternal age, smoke, second hand smoke, and maternal level of education

<sup>o</sup>Model adjusted for maternal age, city, smoke, second hand smoke, and pre pregnancy BMI

## Discussion

To our knowledge, this is the first cohort study to examine the relationship between maternal fluoride exposure and both fertility and birth outcomes in women living in regions with and without water fluoridation. The majority of women in MIREC were exposed to water fluoride levels lower than 0.7 mg/L. Fluoride measured in women's urine and in tap water, as well as maternal fluoride intake estimated via consumption of water, tea, and coffee, was not significantly associated with fertility, birth weight, gestational age, preterm birth, or SGA. Non-significant findings were coupled with small effect sizes, despite reporting coefficients for every 1 unit increase in fluoride exposure (i.e., 1 mg/L), which represents a higher level of exposure for this group of women.

We did not find evidence of an association between fluoride exposure in pregnancy and risk of female infertility. In contrast, one ecological study based in the U.S. reported that counties with higher levels of fluoride in drinking water had lower total fertility rates among women aged 10 to 49 years (Freni, 1994). Another ecological study based in Iran found that women aged 10 to 49 years living in areas with high water fluoride levels (~10 mg/L) were less fertile and had higher rates of both infertility and abortion without known etiology when compared to women exposed to relatively lower levels of fluoride (~1.5 mg/L; Yousefi et al., 2017). Experimental studies have also demonstrated that NaF toxicity decreases the rate of successful pregnancy, inhibits the synthesis and secretion of reproductive hormones, and causes structural damage to the ovaries and uterus (Al-shammari, 2019; Al-Hiyasat et al., 2000; Darmani et al., 2001; Thakare & Dhurvey, 2016; Zhou et al., 2013a, 2013b). It is possible that fluoride exposure levels in the current sample were too low to impact fertility in females; however, more research is warranted on this topic given that lower levels of fluoride exposure

may interact with specific genes to impact reproductive hormones (Zhou et al., 2016). Measures of women's reproductive hormone levels may be a more reliable estimate of female fertility and may also be more sensitive to detecting an effect than a retrospective self-report about the amount of time it took to get pregnant (Cooney et al., 2009).

Future studies may also want to consider the effects of fluoride exposure on the male reproductive system as a potential contributor to fluoride-induced infertility. In experimental studies, significant reductions in the number of mature Leydig and Sertoli cells, weight of testes, serum concentration of testosterone, and sperm count, motility, density, and viability have been observed among NaF-treated mice and rats (Chaithra et al., 2020; Elbetieha et al., 2000; Gupta et al., 2007; Pushpalatha et al., 2005). Importantly, direct associations have been established between these histological alterations and infertility, even when those males were mated with untreated, healthy females (Chaithra et al., 2020; Elbetieha et al., 2000).

To date, few studies have examined the association between fluoride exposure and birth outcomes, especially among women living in areas with levels of fluoride consistent with water fluoridation. Of these, some have found increased risk of adverse birth outcomes in areas where fluoride levels in drinking water are high ( $> 1.5$  mg/L; Diouf et al., 2012; Goyal et al., 2020; Sastry et al., 2011; Susheela et al., 2010), whereas others have found that fluoride exposure may offer protection against adverse birth outcomes (Aghaei et al., 2015; Jia et al., 2019; Zhang et al., 2019). Inconsistency in results from the present study and those from pre-existing studies may be attributed to differences in characteristics of the study populations, methodology, quality of the exposure matrix, and levels of fluoride exposure. Specifically, some studies reporting that high-level fluoride is associated with birth outcomes have relied on correlational analyses, failed to control for relevant confounders (Aghaei et al., 2015; Sastry et al., 2011), and, therefore, are

subject to confounding bias (Skelly et al., 2012). Past studies examining fluoride and birth outcomes have evaluated fluoride exposure using measures of dental fluorosis (Diouf et al., 2012), drinking water fluoride concentration (Aghaei et al., 2015; Zhang et al., 2019), whether an individual gets dental cleaning (potentially exposing them to fluoride by fluoridated prophylaxis pastes; Zhang et al., 2019), and serum fluoride concentration (Sastry et al., 2011).

### **Strengths and Limitations**

Strengths of this study include the use of a large pregnancy cohort with robust measures of fluoride exposure. Fluoride exposure was assessed using three different methods that resulted in a better individualized assessment of exposure levels, and included a fluoride biomarker (urinary fluoride), water fluoride concentration, and an estimate of fluoride intake from beverage consumption. Measuring fluoride in urine and from tea consumption (used to estimate fluoride intake) allowed us to assess additional sources of fluoride beyond that from drinking water. Moreover, our statistical analyses controlled for a wide array of potential confounding factors.

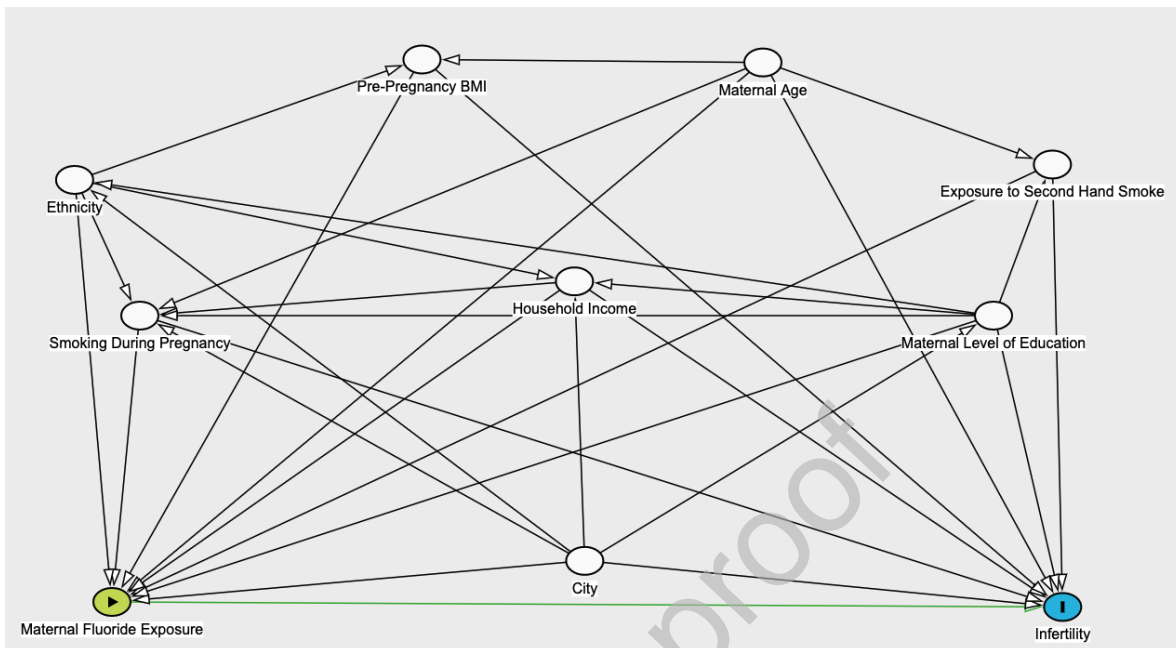
Our study also has some limitations. Compared to the general Canadian population, women in the MIREC cohort tend to be older, predominantly Caucasian, have higher household incomes and education levels, and are more likely to be married/common law and less likely to smoke (Arbuckle et al., 2013). Many of these sociodemographic factors have been shown to be protective against LBW and preterm birth (Hidalgo-Lopezosa et al., 2019). Indeed, the prevalence of preterm birth and SGA among our sample was only 5%, which is lower than the national average of around 8% (Public Health Agency of Canada, 2013). Future studies are needed to determine if the obtained results are generalizable to other, more diverse populations. An additional limitation is that we used spot urine samples without control for behaviours that could contribute to acute changes in fluoride concentration, such as consumption of fluoride-free

bottled water prior to urine collection. Effects of this limitation were minimized by averaging urine fluoride across all three trimesters of pregnancy and adjusting for urinary dilution. Finally, we used urine samples collected in pregnancy and water fluoride concentrations matched in time to the pregnancy period as a proxy for preconception fluoride exposure. While some studies report consistent fluoride metabolism among nonpregnant and pregnant women (Maheshwari et al., 1983, 1981), others have reported lower urinary excretion of fluoride in pregnant women compared to nonpregnant women, that is likely due to increased fetal uptake (Gedalia et al., 1959; Opydo-Szymaczek & Borysewicz-Lewicka, 2005). Considering these inconsistencies, future studies should aim to obtain urine samples prior to conception when examining the association between fluoride exposure and fertility outcomes.

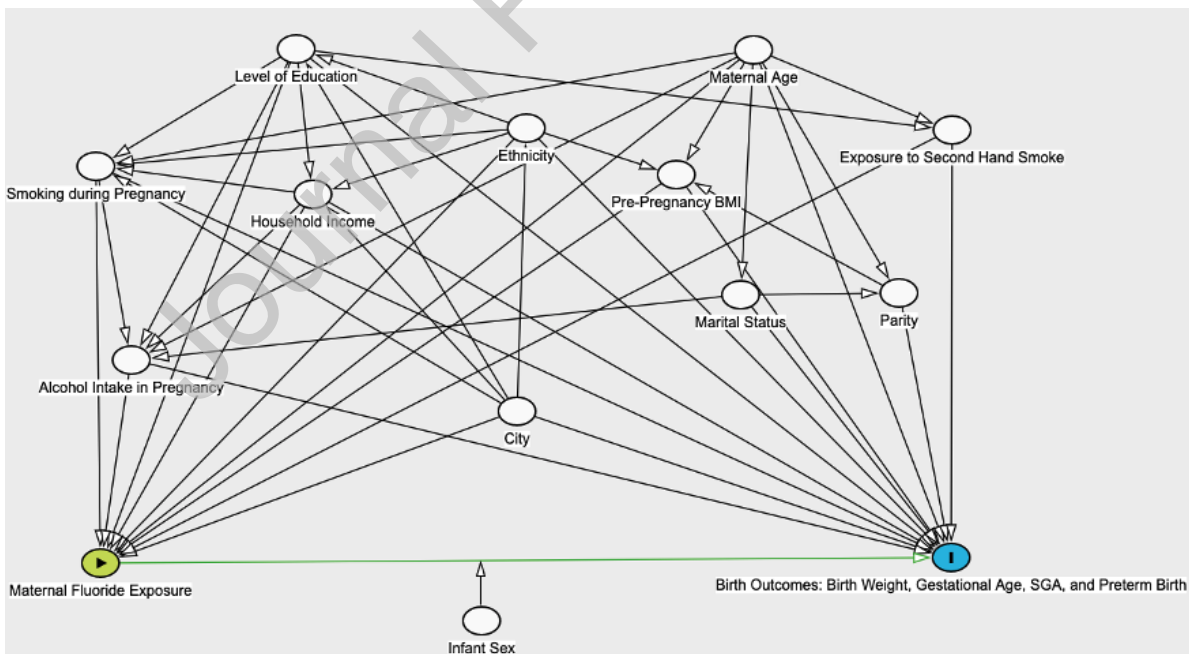
## **Conclusion**

In this large Canadian pregnancy and birth cohort, fluoride exposure during pregnancy was not significantly associated with fertility or birth outcomes after controlling for important covariates. Given the ubiquity of fluoride exposure among pregnant women, prospective cohort studies in other populations are warranted to validate the current findings.

## Supplemental Figures and Tables



*Supplemental Figure 1a.* Directed acyclic graph depicting the relationship between various sociodemographic variables, prenatal fluoride exposure, and infertility.



*Supplemental Figure 1b.* Directed acyclic graph depicting the relationship between various sociodemographic variables, prenatal fluoride exposure, and birth outcomes.



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**Supplemental Table 1**

Demographic characteristics of the full sample (N = 1983) and the sample with data on MUF<sub>SG</sub>, fertility, and complete covariates (N = 1382). Continuous variables are reported as mean ( $\pm$  SD), and categorical variables are reported as n (%).

Characteristics	Full sample (N = 1983)	Sample with data on MUF <sub>SG</sub> fertility and complete covariates (N =1382)	p-value
<i>Outcomes</i>			
Infertility			
Yes	219 (11.47)	159 (11.51)	.972
No	1691 (88.53)	1223 (88.49)	
Time to conception (months)	5.07 ( $\pm$ 10.21) <sup>a</sup>	5.18 ( $\pm$ 10.47)	.763
<i>Covariates</i>			
Ethnicity			
White	1651 (85.63)	1202 (86.98)	.269
Other	277 (14.37)	180 (13.02)	
Maternal age (yr)	32.18 ( $\pm$ 5.06) <sup>b</sup>	32.41 ( $\pm$ 4.85)	.189
Pre-pregnancy BMI (kg/m <sup>2</sup> )	24.94 ( $\pm$ 5.48) <sup>c</sup>	24.80 ( $\pm$ 5.38)	.471
Household income (CAD)			
<100 000	1104 (59.84)	807 (58.39)	.409
$\geq$ 100 000	741 (40.16)	575 (41.61)	
Level of Education			
College degree or less	723 (37.50)	478 (34.59)	.086
Graduate school	1205 (62.50)	904 (65.41)	
Smoked in trimester 1			
Yes	112 (5.76)	58 (4.20)	.043
No	1831 (94.24)	1324 (95.80)	
Second hand smoke in trimester 1			
Yes	117 (6.08)	74 (5.35)	.377
No	1807 (93.92)	1308 (94.65)	

MUF<sub>SG</sub> = maternal urinary fluoride standardized for specific gravity

<sup>a</sup>N = 1910, <sup>b</sup>N = 1945, <sup>c</sup>N = 1803

**Supplemental Table 2**

Demographic characteristics of the sample with data on singleton, live births (N = 1828) and the sample with singleton, live births and complete covariates (N = 1466). Continuous variables are reported as mean ( $\pm$  SD), and categorical variables are reported as n (%).

Characteristics	Sample with singleton, live births (N = 1828)	Sample with MUF <sub>SG</sub> , singleton, live births and complete covariate data (N = 1350)	p-value
<i>Outcomes</i>			
Birth weight (g)	3455 ( $\pm$ 528.2) <sup>a</sup>	3479 ( $\pm$ 468.2) <sup>b</sup>	.185
Gestational age (wks)	39.35 ( $\pm$ 1.73) <sup>c</sup>	39.48 ( $\pm$ 1.39)	.023
Small-for-gestational age			
Yes	104 (5.73)	70 (5.20)	.524
No	1712 (94.27)	1275 (94.8)	
Preterm birth			
<37	110 (6.03)	61 (4.52)	.062
$\geq$ 37	1714 (93.97)	1289 (95.48)	
<i>Covariates</i>			
Infant sex			
Boy	964 (52.91)	706 (52.30)	.733
Girl	858 (46.09)	644 (47.70)	
Ethnicity			
White	1554 (85.17)	1174 (86.96)	.313
Other	259 (14.29)	176 (13.04)	
Maternal age (yr)	32.16 ( $\pm$ 5.06)	32.39 ( $\pm$ 4.86)	.198
Pre-pregnancy BMI (kg/m <sup>2</sup> )	24.93 ( $\pm$ 5.45) <sup>d</sup>	24.84 ( $\pm$ 5.43)	.650
Parity (n; %)			
0	799 (43.71)	612 (45.33)	.631
1	742 (40.59)	537 (39.78)	
2+	287 (15.70)	201 (14.89)	
Marital status			
Married or common-law	1726 (95.20)	1297 (96.07)	.238
Single	87 (4.80)	53 (3.93)	
Household income (CAD)			
<100 000	1034 (59.53)	791 (58.59)	.600

$\geq 100\ 000$	703 (40.47)	559 (41.41)	
Level of Education			
College degree or less	678 (37.40)	471 (34.89)	<.001
Graduate school	135 (62.60)	879 (65.11)	
Drinks alcohol			
Yes	289 (17.37)	250 (18.52)	.412
No	1375 (82.63)	1100 (81.48)	
Smoked in trimester 1			
Yes	103 (5.64)	57 (4.22)	.071
No	1724 (94.36)	1293 (95.78)	
Second hand smoke in trimester 1			
Yes	117 (6.46)	72 (5.33)	.202
No	1706 (94.24)	1278 (94.67)	

$MUF_{SG}$  = maternal urinary fluoride standardized for specific gravity

<sup>a</sup>N = 1818, <sup>b</sup>N = 1345, <sup>c</sup>N = 1824, <sup>d</sup>N = 1693

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**Meaghan Hall:** Conceptualization, Methodology, Writing – Original Draft, Writing – Review & Editing. **Carly Goodman:** Conceptualization, Methodology, Formal Analysis, Writing – Original Draft, Writing – Review & Editing. **Rivka Green:** Conceptualization, Writing – Review & Editing. **Richard Hornung:** Validation, Writing – Review & Editing. **E. Angeles Martinez-Mier:** Resources, Writing – Review & Editing. **Bruce Lanphear:** Conceptualization, Writing – Review & Editing, Funding Acquisition. **Christine Till:** Conceptualization, Writing – Review & Editing, Supervision, Project Administration, Funding Acquisition.

#### Conflicts of Interest:

No conflicts of interest to disclose.

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