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Fluoride exposure and thyroid hormone levels in pregnancy: The MIREC cohort

Meaghan Hall [a], Rick Hornung [b], Jonathan Chevrier [c], Pierre Ayotte [d], Bruce Lanphear [e], Christine Till [a,*]

[a] Psychology Department, York University, Toronto, ON, Canada
[b] Retired, Consultant to Psychology Department, York University, Toronto, ON, Canada
[c] School of Population and Global Health, McGill University, Montreal, QC, Canada
[d] Department of Social and Preventive Medicine, Faculty of Medicine, Université Laval, Québec City, QC, Canada
[e] Faculty of Health Sciences, Simon Fraser University, Burnaby, BC, Canada

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ABSTRACT

Background: Fluoride exposure may increase the risk of hypothyroidism, but results from previous studies are inconsistent at low-level fluoride exposure (i.e., ≤ 0.7 mg/L). Human studies of fluoride and thyroid hormone levels in pregnancy are scarce.

Objectives: We examined associations between fluoride exposure and maternal thyroid hormone levels in a Canadian pregnancy cohort, with consideration for fetal sex-specific effects.

Methods: We measured fluoride concentrations in drinking water and spot urine samples collected during each trimester from 1876 pregnant women enrolled in the Maternal-Infant Research on Environmental Chemicals (MIREC) study. We also measured maternal thyroid stimulating hormone (TSH), free thyroxine (FT4), and total thyroxine (TT4) levels during the first trimester of pregnancy. We used linear and non-linear regression models to estimate associations between fluoride exposure and levels of TSH, FT4, and TT4. We explored effect modification by fetal sex and considered maternal iodine status as a potential confounder.

Results: A 1 mg/L increase in urinary fluoride was associated with a 0.30 (95 %CI: 0.08, 0.51) logarithmic unit (i.e., 35.0 %) increase in TSH among women pregnant with females, but not males (B = 0.02; 95 %CI: −0.16, 0.19). Relative to women with urinary fluoride concentrations in the first quartile (0.05–0.32 mg/L), those with levels in the third quartile (0.49–0.75 mg/L) had higher FT4 and TT4 (i.e., inverted J-shaped associations), but the association was not statistically significant after adjustment for covariates ($p = 0.06$). Water fluoride concentration showed a U-shaped association with maternal FT4, whereby women with water fluoride concentrations in the second (0.13–0.52 mg/L) and third (0.52–0.62 mg/L) quartiles had significantly lower FT4 compared to those with levels in the first quartile (0.04–0.13 mg/L). Adjustment for maternal iodine status did not change the results.

Discussion: Fluoride exposure was associated with alterations in maternal thyroid hormone levels, the magnitude of which appeared to vary by fetal sex. Given the importance of maternal thyroid hormones for fetal neurodevelopment, replication of findings is warranted.

1. Introduction

Fluoride is a naturally occurring element that is known for its ability to prevent tooth decay (Centers for Disease Control and Prevention, 2019). In some parts of the world, fluoride is added to drinking water, constituting the largest source of exposure for children and adults living in fluoridated communities (Centers for Disease Control and Prevention, 2019; United States Environmental Protection Agency, 2010). In Canada, 0.7 mg fluoride per liter of water is the recommended concentration for dental health while minimizing the occurrence of fluorosis (Health Canada, 2010). Other sources of exposure include dental products, such as fluoridated toothpaste, and black tea (Centers for Disease Control and

* Corresponding author at: 4700 Keele Street, 125 Behavioural Science Building, Toronto, ON M3J 1P3.
E-mail addresses: mkhall@yorku.ca (M. Hall), rwhornung@yahoo.com (R. Hornung), jonathan.chevrier@mcgill.ca (J. Chevrier), pierre.ayotte@inspq.qc.ca (P. Ayotte), bruce_lanphear@sfu.ca (B. Lanphear), ctill@yorku.ca (C. Till).

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Prevention, 2019; Krishnankutty, 2022).

Fluoride has been shown to disrupt the thyroid system, but few studies have examined thyroid functioning in people living in areas with community water fluoridation. Some studies of children and non-pregnant adults in Asia have reported associations between higher drinking water- and urine-fluoride concentrations and elevated serum thyroid stimulating hormone (TSH), lower serum free and total thyroxine (T4) and triiodothyronine (T3) concentrations, and increased thyroid gland volume, all of which have been observed in those with hypothyroidism (Khandare et al., 2018; Kheradpisheh, 2018; Wang, 2020; Du, 2021). Yet, other studies have reported opposite results, linking higher water-fluoride levels to elevated serum total T4 and T3 levels (Yasmin et al., 2013). Variability in findings may be attributed to differences in study design, methodological rigor, level and duration of fluoride exposure, as well as age at exposure. An ecologic study conducted in England found a higher prevalence of hypothyroidism in areas with higher levels of fluoride in drinking water (Peckham et al., 2015). In experimental studies, lower free T4 (FT4) and T3 (FT3) were observed in Wistar rat offspring whose mothers were exposed to higher doses of fluoride (i.e., 20 mg/kg (Banji et al., 2013) of body weight and > 100 ppm (Basha et al., 2011) in gestation. Similar findings were reported in adult Wistar rats at lower, prolonged fluoride exposure levels (Jiang et al., 2016).

Thyroid disruption is of particular concern in pregnancy because the developing fetus relies exclusively on maternal thyroid hormones during the first 10–12 weeks of gestation, and to a lesser extent throughout the second and third trimesters (de Escobar et al., 2004; Morreale de Escobar et al., 2000). As such, maternal hypothyroidism during pregnancy has been associated with adverse effects on offspring development, including preterm birth, increased risk of neurodevelopmental disorders, and lower intelligence quotient (IQ) (Andersen et al., 2013; Andersen et al., 2018; Chevrier, 2011). A prospective birth cohort study conducted in Denmark (i.e., Danish National Birth Cohort) found that elevated maternal TSH (≥10 mIU/L) and low FT4 (<10 pmol/L) were associated with an 8 to 13-point reduction in child verbal IQ (Andersen et al., 2018). Similar findings were reported in a recent *meta*-analysis (Levie, 2018). Importantly, even mild reductions in maternal thyroid hormone levels during gestation have been associated with lower child IQ (Moog, 2017).

Until recently, little was known about the potential impact of fluoride exposure on maternal thyroid function in pregnancy, especially in areas with optimally fluoridated water (Griebel-Thompson, 2022; Kampouri, 2022). In a previous study of this same cohort, we found a significant association between maternal fluoride exposure and hypothyroidism in pregnancy; a 0.5 mg/L increase in drinking water fluoride concentration was associated with a 65 % increase in the odds of having a diagnosis or meeting criteria for primary hypothyroidism (Hall, 2023). We also found that boys born to women with hypothyroidism had significantly lower Full-Scale IQ scores (Hall, 2023). These results suggest that maternal thyroid disruption may play a role in fluoride-induced developmental neurotoxicity observed in previous studies (Bashash, 2017; Green, 2019; Cantoral, 2021).

Some (Green, 2019; Cantoral, 2021) but not all (Bashash, 2017; Ibarluzea, 2022) studies examining the developmental neurotoxicity of fluoride have reported that boys were adversely impacted by prenatal, but not postnatal, exposure. More broadly, a growing body of evidence suggests that the male brain may be more vulnerable to neurotoxicants than the female brain (Green et al., 2020; Kern, 2017; Goodman, 2023). Women's thyroid hormone levels in pregnancy have also been observed to differ by child sex. More specifically, pregnant women who have a male fetus were found to be more likely to exhibit elevated TSH (Sitoris, 2022; Wang, 2019). Taken together, these findings suggest that sex differences may exist in the association between fluoride exposure and maternal thyroid hormone levels in pregnancy.

We evaluated the association between fluoride exposure and maternal TSH, FT4, and TT4 levels of pregnant women living in areas with optimally fluoridated water. Considering findings from our previous study linking fluoride exposure to hypothyroidism, we hypothesized that greater fluoride exposure during pregnancy would be associated with higher maternal TSH and lower maternal FT4 levels. We also investigated whether sex-specific differences exist in the association between fluoride and thyroid dysfunction. Finally, we considered the potential for confounding by maternal iodine status given that iodine is required for thyroid hormone production (Krassas et al., 2010) and iodine deficiency may exacerbate the impact of thyroid-disrupting chemicals (Demeneix, 2019).

2. Methods

2.1. Participants

Pregnant women were enrolled in the Maternal-Infant Research on Environmental Chemicals (MIREC) Study (Arbuckle, 2013) between 2008 and 2011 from ten cities across Canada, seven of which add fluoride to drinking water (Toronto, Hamilton, Ottawa, Sudbury, Halifax, Edmonton, Winnipeg) and three of which do not (Vancouver, Montreal, Kingston). Women were eligible to participate if they were ≥ 18 years of age, able to communicate in English or French, and < 14 weeks' gestation. Participants were considered ineligible if they had known fetal abnormalities, medical complications, or reported drug use. Of 2001 women recruited, 1983 consented to participate. Of these, 1885 (95.1 %) provided plasma samples in trimester one. We excluded those missing data on fetal sex (n = 9), for a final study sample of 1876 pregnant women.

The current study received approval from the research ethics boards at Health Canada and York University. All participants provided written informed consent at time of enrollment in MIREC.

2.2. Maternal fluoride exposure

2.2.1. Maternal urinary fluoride (MUF; mg/L)

We analyzed MUF concentration in spot urine samples collected in each trimester of pregnancy (at mean [SD] = 11.6 [1.6], 19.1 [2.3], and 33.1[1.5] weeks' gestation, respectively), using a modification of the hexamethyldisiloxane (HMDS; Sigma Chemical Co., USA) micro-diffusion method with ion-selective electrode by the Indiana University School of Dentistry (Martínez-Mier, 2011). The limit of detection (LoD) was 0.02 mg/L; trimester-specific concentrations below the LoD (n = 23 or < 0.005 % of all urine spot samples) were replaced with the value of 0.02 mg/L. Each MUF concentration was standardized for urine specific gravity (SG) to account for variability due to urinary dilution using the following equation: $MUFSG = MUF \times [(SGM - 1) \div (SGi - 1)]$, where MUF_{SG} is the SG-adjusted fluoride concentration (mg/L), SG_i is the observed SG concentration for the individual urine sample, and SG_M is the median SG for the cohort (Duty et al., 2005). We derived the average dilution-adjusted MUF_{SG} concentration by taking the average across all three trimesters for each woman. We removed one averaged MUF_{SG} -value (>5 mg/L) because of uncertainty that it reflected an individual's true exposure. As described previously (Hall, 2023), this high concentration was driven by one trimester-specific value of 16 mg/L, which was inconsistent with the other trimester values that were close to zero.

2.2.2. Water fluoride (mg/L)

We solicited municipal drinking water reports for the ten cities in the MIREC cohort study. These reports listed water fluoride concentrations that were measured daily for cities that add fluoride to public water supplies, and weekly or monthly for cities that do not add fluoride to public water (Till, 2018). Using the first three letters of their postal code, participants' residences were matched with boundary regions serviced by each Water Treatment Plant. Average water fluoride concentration (i.e., geometric mean; mg/L) was estimated for each woman who reported drinking tap water in pregnancy by averaging water fluoride

concentrations across each woman's pregnancy; thus, each woman has a water fluoride concentration that is matched in time to the levels of fluoride found in tap water for the duration of her pregnancy. Further details can be found in Till et al (Till, 2018).

2.3. Maternal thyroid hormones

We analyzed thyroid hormones (i.e., TSH, FT4, and TT4) and antibodies (i.e., anti-thyroglobulin [Tg] and anti-thyroid peroxidase [TPO]) in first trimester maternal plasma collected at mean [SD] = 11.6 [1.6] weeks' gestation. Plasma FT4 and TT4 were measured using gold standard equilibrium dialysis isotope dilution mass spectrometry (ED-ID-MS) and isotope dilution high performance liquid chromatography mass spectrometry (ID-HPLC-MS), respectively, by the accredited Toxicology Laboratory at the Institut National de Santé Publique du Québec (INSPQ). Plasma TSH, anti-Tg, and anti-TPO were quantified using commercial immunoassays by an accredited biochemistry laboratory at the Institut Universitaire de Cardiologie et de Pneumologie de Québec (IUCPQ). TSH concentrations (n = 7) below the LoD (0.0025 μIU/mL) were given a value of $LoD/\sqrt{2}$, which is a validated method for estimation of the average concentration from data containing nondetectable values (Hornung and Reed, 1990).

2.4. Measures of maternal iodine status

We measured thyroglobulin (Tg) concentration in plasma collected in trimester one as an indicator of long-term iodine status. We also measured urinary iodine concentration (UIC) in two spot samples collected during the first and second trimesters. We standardized UIC for SG (i.e., UIC_{SG}) and also with creatinine (UIC/Cr; μg/g) to account for variability in urinary dilution, and then averaged across both trimesters.

2.5. Statistical analysis

We examined the distribution and descriptive statistics for all demographics, maternal fluoride exposure and thyroid hormone variables. We used Spearman's correlation coefficients to examine associations between MUF_{SG} and water fluoride concentrations, and thyroid hormone levels. We used multiple linear regression to test the association between maternal fluoride exposure (MUF_{SG} concentration and water fluoride concentration) and thyroid hormone (TSH, TT4, and FT4) levels. Given the right-skewed distribution of TSH and FT4, natural log transformation was used to approximate a normal distribution for both variables. Change in log TSH associated with MUF_{SG} concentration was interpreted as a percent increase or decrease (i.e., [(e^(B coefficient log TSH) – 1) * 100]) for every 1 mg/L increase in MUF_{SG}, which corresponds to the approximate difference in MUF_{SG} concentrations between women at the 10th and 95th percentiles. We also explored quadratic models to test for non-linearity (or departure from log-linearity) in these associations. We observed some non-linear relationships, which we probed by rerunning models with MUF_{SG} and water fluoride concentrations divided into quartiles. Women in the first quartile (i.e., lowest exposure levels) served as the reference group. Where there was evidence of a non-linear association (i.e., significant differences in thyroid hormone levels between quartiles of fluoride exposure), results from the linear regression model were not reported; instead, we estimated regression coefficients and 95 % confidence intervals for MUF_{SG} and water fluoride levels modeled in quartiles.

We adjusted all models for potential confounding variables: maternal age, level of education (dichotomized as bachelor's degree or higher), pre-pregnancy body mass index (BMI), and race (White or Other), and precision covariates associated with thyroid hormone measurement: parity, anti-Tg and anti-TPO levels, gestational age at time of blood sampling, second-hand smoke exposure, and fetal sex (assigned at birth). All covariates were included in models based on a directed acyclic graph (Supplemental Fig. 1). We also adjusted for study site when MUF_{SG} was used as the independent variable because thyroid health outcomes could vary across the study sites. We did not control for study site in our water fluoride models because site is collinear with fluoride levels in municipal drinking water.

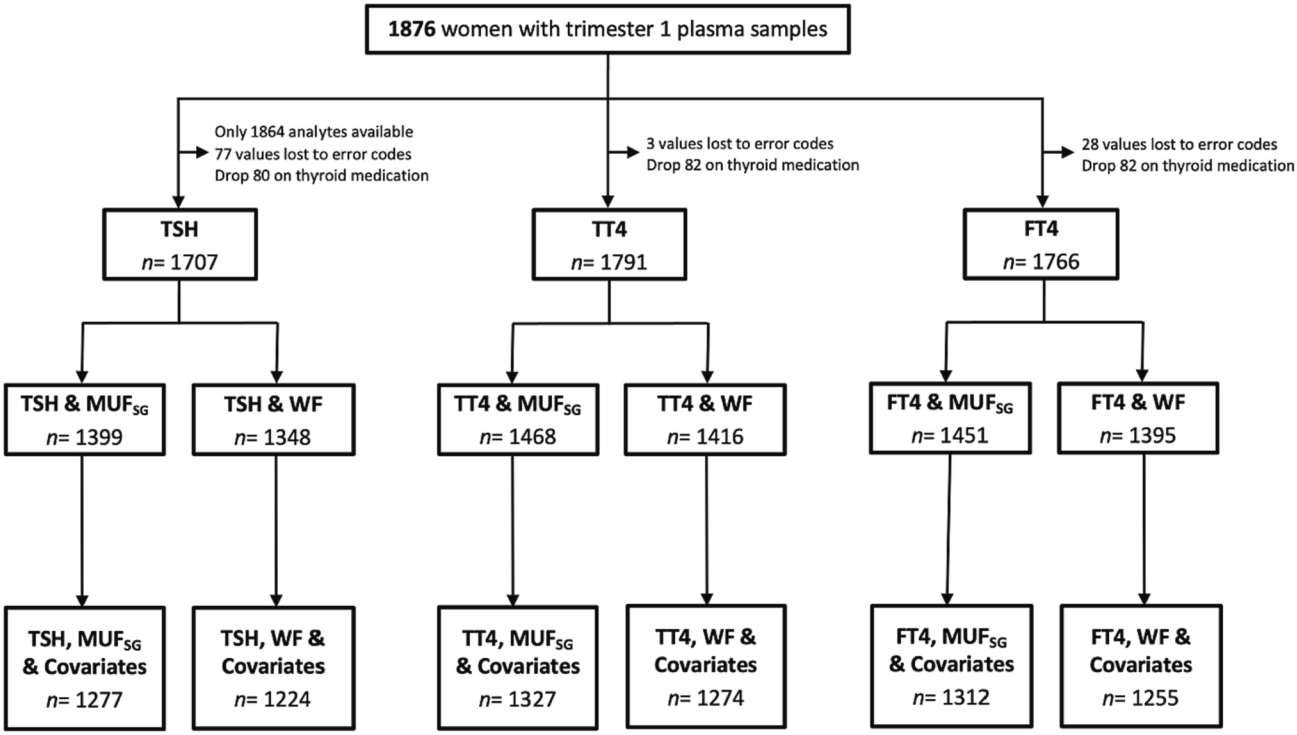

Fig. 1. Study sample flow chart. *Note.* One woman with a MUF_{SG} concentrations > 5 mg/L was excluded from all models. MUF_{SG} = maternal urinary fluoride, standardized for specific gravity; WF = water fluoride; TSH = thyroid stimulating hormone; TT4 = total thyroxine; FT4 = free thyroxine.

We only included women who reported drinking tap water during pregnancy in models involving water fluoride concentration. Women who reported taking medication to treat a thyroid disorder at the time of study enrolment were excluded from all analyses. Additionally, we tested for effect modification by fetal sex in all models by including a sex*exposure interaction term. If the interaction term was significant ($p < .05$), we estimated the sex-specific slopes. As a sensitivity analysis, we reran models with a significant main effect with Tg, a biomarker of long-term iodine nutrition (Dineva, 2023), UIC_{SG}, and UIC/Cr added as covariates to evaluate potential confounding by maternal iodine status.

We used STATA version 18.0 (STATA corporation) for all statistical analyses. Two-sided p values ≤ 0.05 were considered to indicate statistical significance.

3. Results

3.1. Model diagnostics

Regression diagnostics (i.e., Cook-Weisberg test) indicated that the models of MUF_{SG} and water fluoride concentrations with maternal TSH (log transformed) violated the assumption of variance homogeneity (i.e., variance of the residuals was not equal across the levels of fluoride exposure), but the deviations were small (Cooks' d values < 0.1). There were no other assumption violations, issues with model fit, collinearity, influential cases, or outliers in any of the models.

3.2. Participant characteristics

We studied a total of 1876 women with blood plasma samples collected in trimester one; 1707 (91.0 %), 1791 (95.5 %), and 1766 (94.1 %) of the women had data on TSH, TT4, and FT4, respectively, and were not taking thyroid medication. Fig. 1 shows the sample flow chart for thyroid hormone subgroups with water fluoride, MUF_{SG}, and covariate data.

On average, women were 32.2 years (SD = 5.0) old at time of first visit; 86 % were White, 95 % were married, 62 % had a university education or higher, and 54 % had male infants (Table 1). Maternal FT4 and TT4 were normally distributed, while maternal TSH remained skewed following log transformation. Means (Table 1) and interquartile ranges for TSH (0.73–1.74 μIU/mL), TT4 (93–120 ng/mL), and FT4 (12–15 pg/mL) were consistent with reference values for pregnant women. Mean MUF_{SG} concentration was 0.59 mg/L (SD = 0.39; range: 0.05 to 3.33 mg/L) and concentrations at the 25th, 50th, and 75th percentiles were 0.32, 0.49, and 0.75 mg/L, respectively. Mean water fluoride concentration was 0.42 mg/L (SD = 0.25; range: 0.04 to 0.87 mg/L) and concentrations at the 25th, 50th, and 75th percentiles were 0.13, 0.52, and 0.62 mg/L, respectively; 61 % of the sample lived in an area with community water fluoridation. As predicted, water fluoride concentration was moderately associated with MUF_{SG} concentration ($r = 0.49$, $p < .01$). Maternal FT4 was moderately associated with TT4 ($r = 0.33$, $p < .01$), while FT4 ($r = -0.18$, $p < .01$) and TT4 ($r = -0.10$, $p < .01$) were weakly correlated with TSH.

3.3. Maternal fluoride exposure and thyroid hormone levels

In a covariate-adjusted multiple linear regression analysis, we found a non-significant ($p = .08$) positive association between MUF_{SG} and TSH; a 1 mg/L increase in MUF_{SG} was associated with a 0.13 logarithmic unit (i.e., 13.9 %) increase in maternal TSH (Fig. 2A; Table 2). There was an inverted J-shaped association between MUF_{SG} and maternal FT4 (Fig. 2B). Compared with women with MUF_{SG} concentrations in the first quartile (0.05–0.32 mg/L), those with levels in the third quartile (0.49–0.75 mg/L) had non-significantly ($p = 0.055$) higher FT4 (Table 3). A similar trend was observed between MUF_{SG} concentration and maternal TT4 (Fig. 2C; Table 3).

Water fluoride concentration showed a U-shaped association with maternal FT4 (Fig. 3). Relative to women with water fluoride concentrations in the first quartile (0.04–0.13 mg/L), those with levels in the third (0.52–0.62 mg/L) quartile had significantly lower FT4 (Table 3). No statistically significant association was observed between water fluoride concentration and maternal TSH or TT4 (Table 2; Table 3).

Table 1
Demographic characteristics of women with TSH, TT4, and FT4 data.

	TSH	TT4	FT4
N	1707	1791	1766
Maternal age (years; mean; SD)	32.1 (5.1)	32.1 (5.0)	32.1 (5.1)
Race (*n*; %)			
White	1465 (85.8)	1536 (85.8)	1517 (85.9)
Other	242 (14.2)	255 (14.2)	249 (14.1)
Marital status (n; %)			
Married or common law	1624 (95.1)	1702 (95.0)	1677 (95.0)
Single	83 (4.9)	89 (5.0)	89 (5.0)
Level of education (*n*; %)			
College diploma or less	644 (37.7)	679 (37.9)	673 (38.1)
University degree	1063 (62.3)	1112 (62.1)	1093 (61.9)
Household income (*n*; %)			
< 100,000	985 (60.4)	1034 (60.4)	1020 (60.4)
≥ 100,000	646 (39.6)	678 (39.6)	668 (39.6)
City (*n*; %)			
Fluoridated[a]	1041 (61.0)	1091 (60.9)	1074 (60.8)
Non-fluoridated[b]	666 (39.0)	700 (39.1)	692 (39.2)
Second-hand smoke in trimester 1 (*n*; %)			
Yes	107 (6.3)	110 (6.2)	109 (6.2)
No	1599 (93.7)	1680 (93.8)	1656 (93.8)
Pre-pregnancy BMI (kg/m^2; mean; SD)	24.8 (5.4)	24.8 (5.4)	24.8 (5.4)
Parity (*n*; %)			
0	760 (44.5)	799 (44.6)	790 (44.7)
1	686 (40.2)	719 (40.2)	707 (40.0)
2+	261 (15.3)	273 (15.2)	269 (15.3)
Gestational age (weeks; mean; SD)[c]	11.6 (1.5)	11.6 (1.5)	11.6 (1.5)
Maternal fluoride exposure			
MUF_{SG} (mg/L; mean; SD)	0.59 (0.41)	0.59 (0.41)	0.59 (0.41)
Water fluoride (mg/L; mean; SD)	0.42 (0.25)	0.42 (0.25)	0.42 (0.25)
Maternal thyroid hormones			
TSH (log; mean; SD)	−0.01 (0.96)	−0.01 (0.96)	−0.00 (0.95)
TSH (μIU/mL; mean; SD)	1.3 (0.98)	1.3 (0.98)	1.3 (0.98)
FT4 (log; mean; SD)	2.6 (0.20)	2.6 (0.20)	2.6 (0.20)
FT4 (pg/mL; mean; SD)	13.6 (4.5)	13.6 (4.4)	13.6 (4.4)
TT4 (ng/mL; mean; SD)	106.2 (21.3)	106.3 (21.4)	106.2 (21.2)
Maternal thyroid antibodies			
Anti-Tg (IU/mL; mean; SD)	10.4 (50.4)	10.2 (49.5)	10.1 (49.8)
Anti-TPO (IU/mL; mean; SD)	20.6 (86.9)	20.8 (88.6)	20.0 (86.7)
Anti-TPO + (≥5.61 IU/mL; *n*; %)	216 (12.8)	222 (12.7)	214 (12.4)
Maternal iodine status			
Plasma Tg (ng/mL; median; IQR)	13.7 (13.1)	13.8 (13.2)	13.7 (13.0)
UIC unadjusted (μg/L; median; IQR)	182.3 (179.4)	182.3 (177.8)	183.5 (177.2)
UIC_{SG} (μg/L; median; IQR)	191.7 (128.5)	191.9 (129.8)	191.1 (128.3)
UIC/Cr (μg/g; median; IQR)	299.0 (216.6)	300.2 (219.5)	299.4 (219.3)
Child sex (*n*; %)			
Male	899 (54.0)	948 (54.2)	931 (54.0)
Female	765 (46.0)	800 (45.8)	792 (46.0)

Due to missing data, percentage totals for subgroups may not sum to the total sample population; percentages are reported based on total sample in each subgroup with available data.
Abbreviations: SD = standard deviation; MUF_{SG} = maternal urinary fluoride, adjusted for specific gravity; TSH = thyroid stimulating hormone; FT4 = free thyroxine; TT4 = total thyroxine; Tg = thyroglobulin; TPO = thyroid peroxidase; UIC_{SG} = urinary iodine
concentration, adjusted for specific gravity; urinary iodine concentration, adjusted for creatinine.
Means represent geometric means.
[a] Edmonton, Winnipeg, Toronto, Hamilton, Sudbury, Ottawa, Halifax.
[b] Vancouver, Kingston, Montreal.
[c] Gestational age at time of maternal blood collection in trimester one (T1).

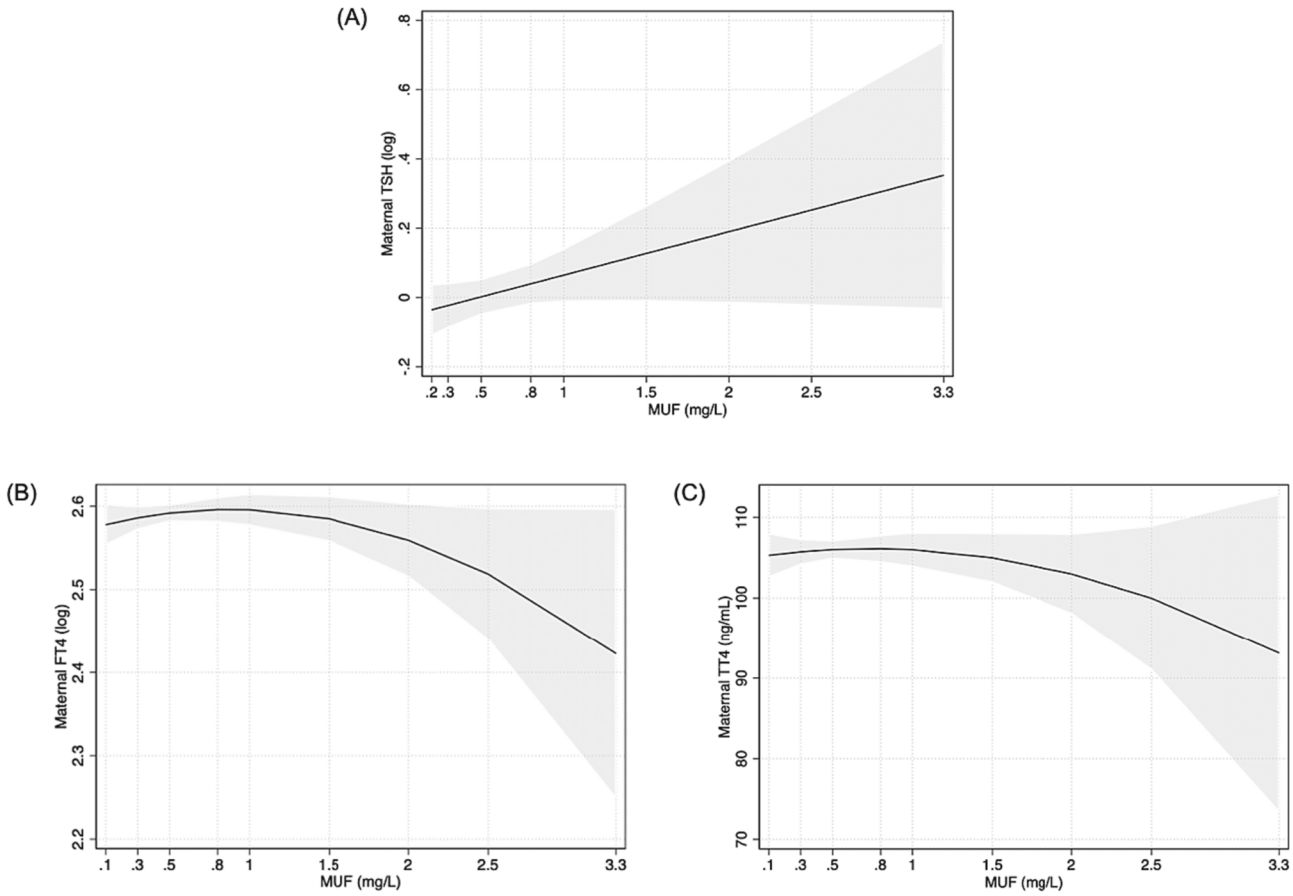

Fig. 2. Associations between MUF_{SG} concentration and (A) maternal TSH, (B) FT4, and (C) TT4 levels. *Note.* The depicted associations were adjusted for covariates. MUF_{SG} = maternal urinary fluoride, standardized for specific gravity; TSH = thyroid stimulating hormone; FT4 = free thyroxine; TT4 = total thyroxine.

Table 2
Linear associations between MUF_{SG} and water fluoride concentrations, and TSH, FT4, and TT4 levels in pregnant women participating in the MIREC study.

	Unadjusted[a]				Adjusted[b]			
	n	B	95 % CI	*p*	*n*	B	95 % CI	*p*
TSH (log)								
MUF_{SG} (mg/L)	1399	0.09	−0.04,0.21	0.17	1277	0.13	−0.02,0.27	0.08
Water fluoride (mg/L)	1348	−0.08	−0.28,0.12	0.43	1224	−0.17	−0.38,0.04	0.10
FT4 (log)								
MUF_{SG} (mg/L)	1451	−0.02	−0.04,0.01	0.18	1312	−0.01	−0.04,0.02	0.50
Water fluoride (mg/L)	1395	−0.04	−0.08,0.00	0.05	1255	−0.04	−0.08,-0.00	0.04
TT4 (ng/mL)								
MUF_{SG} (mg/L)	1468	−0.72	−3.49,2.06	0.61	1327	−1.16	−4.26,1.95	0.47
Water fluoride (mg/L)	1416	4.92	0.55,9.28	0.03	1274	3.88	−0.53,8.29	0.09

Abbreviations: CI = confidence interval; TSH = thyroid stimulating hormone; MUF_{SG} = maternal urinary fluoride, standardized for specific gravity; FT4 = free thyroxine; TT4 = total thyroxine.

[a] Multiple linear regression models of associations between MUF_{SG} and water fluoride concentration, and maternal TSH, FT4, and TT4 levels, *not adjusted* for covariates.

[b] Multiple linear regression models of associations between MUF_{SG} and water fluoride concentration, and maternal TSH, FT4, and TT4 levels, *adjusted* for maternal age, level of education, pre-pregnancy BMI, race, parity, anti-Tg and anti-TPO levels, gestational age at time of blood sampling, second-hand smoke exposure, and fetal sex; study site was also adjusted in models involving MUF_{SG}.

3.4. Effect modification by fetal sex

The interaction between MUF_{SG} concentration and fetal sex in predicting maternal TSH levels was statistically significant (*p* interaction term = 0.04). Among pregnant women who had a female fetus, a 1 mg/L increase in MUF_{SG} was associated with a 0.30 (SE = 0.11; 95 % CI: 0.08, 0.51; *p* =.01) logarithmic unit (i.e., 35.0 %) increase in TSH (Fig. 4). In contrast, MUF_{SG} was not significantly associated with TSH among pregnant women with males (B = 0.02; SE = 0.09; 95 % CI: −0.16, 0.19; *p* =.84; Fig. 4). No evidence of effect modification by fetal sex was observed for the associations between MUF_{SG} concentration and maternal FT4 or TT4, or between water fluoride concentration and maternal TSH, FT4, or TT4.

3.5. Sensitivity analysis

When maternal Tg, UIC_{SG}, and UIC/Cr were added as covariates, we did not observe any substantial differences in the observed associations

Table 3
Quartile regression effect estimates (95% CI) for the association between urinary and water fluoride concentration, and TSH, FT4, and TT4 levels in pregnant women participating in the MIREC study.

	Unadjusted[a]				Adjusted[b]			
	n	B	95 % CI	*p*	*n*	B	95 % CI	*p*
TSH (log)								
MUF (mg/L)	1399				1277			
Q2		−0.04	−0.17,0.10	0.62		−0.06	−0.21,0.09	0.44
Q3		−0.11	−0.25,0.02	0.10		−0.10	−0.26,0.06	0.22
Q4		0.04	−0.10,0.18	0.58		0.05	−0.11,0.22	0.52
Water fluoride (mg/L)	1348				1224			
Q2		0.08	−0.06,0.22	0.28		0.07	−0.08,0.22	0.38
Q3		0.01	−0.13,0.16	0.88		−0.08	−0.23,0.08	0.31
Q4		−0.03	−0.17,0.12	0.71		−0.08	−0.23,0.07	0.31
FT4 (log)								
MUF (mg/L)	1451				1312			
Q2		0.02	−0.01,0.04	0.27		0.02	−0.01,0.05	0.19
Q3		0.01	−0.02,0.04	0.55		0.03	−0.00,0.06	0.06
Q4		−0.01	−0.04,0.02	0.55		0.01	−0.02,0.04	0.59
Water fluoride (mg/L)	1395				1255			
Q2		−0.03	−0.06,-0.01	0.01		−0.03	−0.06,0.00	0.06
Q3		−0.04	−0.06,-0.01	0.01		−0.04	−0.07,-0.01	0.01
Q4		−0.03	−0.05,0.00	0.06		−0.02	−0.05,0.00	0.10
TT4 (ng/mL)								
MUF (mg/L)	1468				1327			
Q2		1.43	−1.62,4.47	0.36		1.30	−1.92,4.52	0.43
Q3		4.39	1.34,7.44	0.01		3.33	−0.13,6.79	0.06
Q4		0.50	−2.55,3.55	0.75		0.59	−2.97,4.14	0.75
Water fluoride (mg/L)	1416				1274			
Q2		3.76	0.69,6.83	0.02		1.66	−1.51,4.83	0.30
Q3		2.20	−0.97,5.38	0.17		2.09	−1.17,5.36	0.21
Q4		2.96	−0.18,6.10	0.07		2.00	−1.21,5.22	0.22

Abbreviations: CI = confidence interval; TSH = thyroid stimulating hormone; MUF_{SG} = maternal urinary fluoride, standardized for specific gravity; FT4 = free thyroxine; TT4 = total thyroxine.

[a] Multiple linear regression models of associations between MUF_{SG} and water fluoride concentration, divided into quartiles, and maternal TSH, FT4, and TT4 levels, *not adjusted* for covariates.

[b] Multiple linear regression models of associations between MUF_{SG} and water fluoride concentration, divided into quartiles, and maternal TSH, FT4, and TT4 levels, *adjusted* for maternal age, level of education, pre-pregnancy BMI, race, parity, anti-Tg and anti-TPO levels, gestational age at time of blood sampling, second-hand smoke exposure, and fetal sex; study site was also adjusted in models involving MUF_{SG}.

Q2: corresponds to results for women with MUF_{SG} and water fluoride concentrations in the second quartile (i.e., between the 25th and 50th percentiles), relative to those with levels in the first quartile (i.e., below the 25th percentile).

Q3: corresponds to results for women with MUF_{SG} and water fluoride concentrations in the third quartile (i.e., between the 50th and 75th percentiles), relative to those with levels in the first quartile (i.e., below the 25th percentile).

Q4: corresponds to results for women with MUF_{SG} and water fluoride concentrations in the fourth quartile (i.e., above the 75th percentile), relative to those with levels in the first quartile (i.e., below the 25th percentile).

between maternal MUF_{SG} concentration and water fluoride concentration and thyroid hormones (TSH and FT4) (data not shown). Notably, the interaction between MUF_{SG} concentration and fetal sex in predicting maternal TSH levels remained statistically significant in all models (p interaction term < 0.05).

4. Discussion

In this Canadian pregnancy cohort, we found a significant and positive log-linear association between MUF_{SG} concentration and maternal TSH for women pregnant with female fetuses. We found that a 1 mg/L increase in MUF_{SG} was associated with a 35 % increase in maternal TSH. In our sample, a 1 mg/L increase in MUF_{SG} represents the difference in MUF_{SG} concentrations between women at the 10th and 95th percentiles. We also found a non-linear trend between MUF_{SG} and maternal FT4 and TT4 (i.e., inverted J-shaped associations), such that women with MUF_{SG} concentrations in the third quartile had higher FT4 and TT4 relative to those with concentrations in the first quartile.

In comparison, water fluoride concentration showed a U-shaped association with maternal FT4 (i.e., women with levels in the third quartile had significantly lower FT4 than those with levels in the first quartile) and was not significantly associated with TT4 or TSH levels. These findings should be interpreted with caution, however, given the bi-modal distribution of water fluoride concentration resulting from a gap in water fluoride data (from 0.20 to 0.40 mg/L) between those with lower levels of fluoride living in non-fluoridated cities (values: $\leq$ 0.20 mg/L), and those with higher fluoride levels living in cities with community water fluoridation (values: 0.41 – 0.87 mg/L). As such, there was a greater range of water fluoride concentration in the second quartile (n = 337; range: 0.13 – 0.52 mg/L) relative to the other three quartiles. Accordingly, the observed non-linear association between water fluoride concentration and maternal FT4 may not be interpolated within the range of missing water fluoride data (i.e., between 0.20 and 0.40 mg/L).

Urinary fluoride concentration is an objective biomarker of short-term fluoride exposure that allows for more precise estimates of individuals' fluoride intake from multiple sources (e.g., fluoridated water, high-fluoride foods, black tea, dental products, etc.). Further, MUF_{SG} concentrations were measured during pregnancy, coinciding with measurement of thyroid hormones. As such, MUF_{SG} is likely a more accurate measure of women's contemporaneous exposure than water fluoride, and it would be reasonable to expect a short-term urinary biomarker of fluoride exposure to be more strongly associated with maternal thyroid hormone levels. In contrast, water fluoride concentration is a better measure of cumulative or chronic exposure, which may explain the lack of association observed between water fluoride and maternal thyroid hormone levels in the current study.

In a previous study, we found that water fluoride concentration, and not $MUF_{SG,}$ was significantly associated with increased risk of

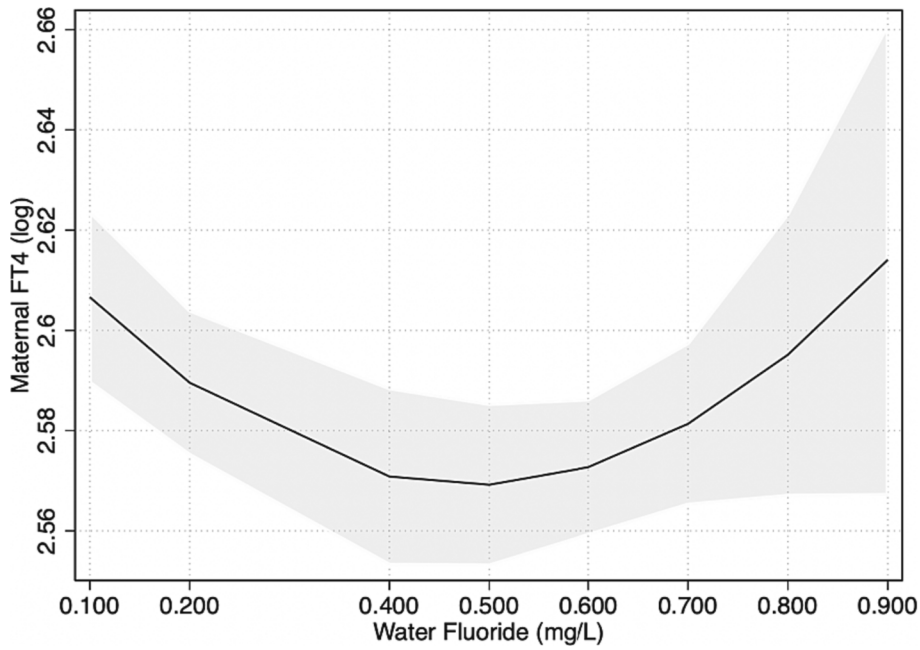

Fig. 3. U-shaped association between water fluoride concentration and maternal FT4 levels. *Note.* The depicted association was adjusted for covariates. FT4 = free thyroxine.

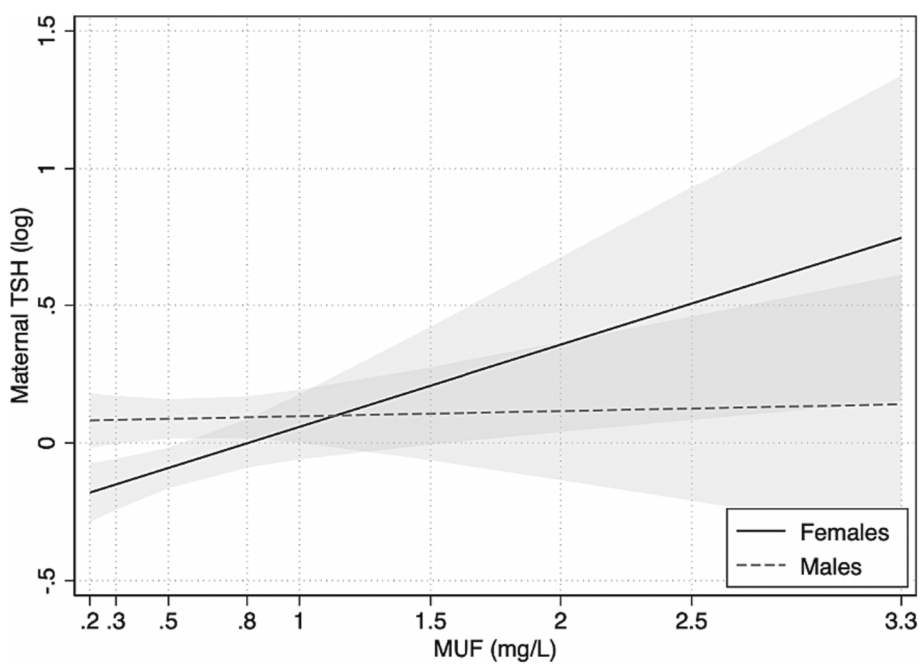

Fig. 4. Association between MUF_{SG} concentration and maternal TSH levels by fetal sex. *Note.* Depicted associations were adjusted for covariates. Females: women pregnant with female fetuses; Males: women pregnant with male fetuses. MUF_{SG} = maternal urinary fluoride, standardized for specific gravity; TSH = thyroid stimulating hormone.

hypothyroidism in pregnancy (Hall, 2023). As mentioned, water fluoride concentration is more likely to be indicative of cumulative or chronic fluoride exposure. If hypothyroidism develops over a longer period, it is plausible that our long-term measure of fluoride exposure (i.e., water fluoride concentration) would be more strongly associated with risk of hypothyroidism.

Our finding that MUF_{SG} concentration was not significantly associated with maternal FT4 or TSH levels (in the total sample) is consistent with results from two studies of pregnant women (Griebel-Thompson, 2022; Kampouri, 2022). One cross-sectional study of 583 pregnant women from Sweden (Kampouri, 2022) also did not observe associations between maternal urinary fluoride levels and thyroid biomarkers, except for a weak positive association with plasma FT3:FT4 ratio. Likewise, another study of 966 pregnant women from the United States (Griebel-Thompson, 2022) did not observe an association between maternal urinary fluoride levels and TSH. Neither of these studies reported whether fetal sex may modify the association between maternal fluoride exposure and thyroid hormone levels.

In our study, MUF_{SG} concentration was only significantly associated with higher TSH levels among pregnant women carrying females. While

little is known about potential sex-specific effects in the association between maternal fluoride exposure and thyroid function in pregnancy, a recent study from China reported sex differences in school-aged children's thyroid gland volumes in response to fluoride exposure (Du, 2021). Specifically, thyroid gland volumes of males were found to be more vulnerable (i.e., larger) in response to fluoride exposure compared to females. Sex-specific effects have also been found in pregnant women and children's thyroid hormone levels in response to other neurotoxicants (Ballesteros, 2017), such as perfluorooctanoic acid (Liang, 2020). Furthermore, sex differences have been established in children's vulnerability to neurotoxicants, with the developing male brain being disproportionally affected (Bashash, 2017; Green, 2019). Considering this and the importance of the maternal thyroid for supporting optimal fetal neurodevelopment, it is possible that the increase in TSH observed among women pregnant with females might indicate a potential protective effect, whereby maternal TSH levels increase in response to fluoride exposure to ensure adequate supply of thyroid hormone to the developing fetus. The biological mechanism to explain why this effect would only be observed among women pregnant with females is unknown, but some evidence suggests that environmental chemicals may induce sex-specific changes in the expression of thyroid related genes in the placenta (Kim, 2019; Leonetti, 2016). More specifically, prenatal exposure to persistent organic pollutants was found to influence the methylation of thyroid transporter genes in the placenta (e.g., deiodinase type 3 [DIO3] and monocarboxylate transporter 8 [MCT8]), in a sexually dimorphic manner (Kim, 2019). Thus, it is also possible that sex-specific changes in the quantity and frequency of thyroid hormone transport from mother to fetus may explain the fluoride-associated increase in TSH observed among women pregnant with females. Given the complexity of the hypothalamic-pituitary-thyroid axis, we cannot dismiss this elevation in TSH as a potential indicator of maternal thyroid dysfunction; accordingly, it is also possible that the developing female may be more resilient to disturbances in maternal thyroid function in pregnancy. Considering this is the first study to investigate sex differences in the association between fluoride exposure and maternal thyroid hormone levels in pregnancy, and that the mechanism remains unknown, future research in this area will be important for replicating these findings.

Controlling for maternal urinary iodine status in all models of fluoride exposure and maternal thyroid hormone levels did not change the results. Iodine is an essential nutrient for thyroid hormone synthesis and plays an important role in determining the magnitude of fluoride's effect on the thyroid. Specifically, iodine has been found to modify the association between urinary fluoride concentration and TSH levels among pregnant women (Griebel-Thompson, 2022) and non-pregnant adults (Malin et al., 2018), such that this association was only significant among those who were classified as iodine insufficient. Effect modification was not tested directly in the current study due to limited statistical power given nearly all women in the MIREC cohort were classified as iodine sufficient in a prior study (Krzeczkowski, 2023) based on our estimate of daily iodine intake. Given reports that fluoride may interact with iodine to exert adverse effects on the thyroid (Buckalew, 2020; Waugh, 2019) and increase risk of poorer neurodevelopmental outcome (Goodman, 2022) in offspring, further research in this area is warranted.

4.1. Strengths and limitations

Strengths of this study include the use of a large Canadian pregnancy cohort with individual assessments of fluoride exposure and thyroid hormones analysed using gold-standard methods. We were also able to adjust for several potential confounding variables in our statistical analyses, including maternal urinary iodine, an essential nutrient for thyroid hormone production. However, we only had urine iodine measurements from two spot samples, which is not optimal given that urinary iodine can vary considerably. Further, women in the MIREC cohort tend to be older, more educated, more likely to be married or common law, primarily White, and more likely to report prenatal vitamin use (Arbuckle, 2013), which may not be representative of the broader Canadian population of pregnant women. In this study, we excluded women who reported having a thyroid disorder or taking medication to treat a thyroid disorder; however, it is possible that some women may have failed to self-report having a thyroid disorder, which would be an additional limitation. Moreover, by excluding women who were being treated with medication for hypothyroidism, we may have reduced the sensitivity of our analyses to detect a significant association between both fluoride exposure measures and maternal thyroid hormone levels. Importantly, however, we have already explored the association between fluoride exposure and maternal hypothyroidism in a previous study (Hall, 2023). Another limitation is that we did not control for thyroid-binding globulins (i.e., proteins that bind T4 to form TT4), which may increase measurement error in TT4 given that these levels have been shown to fluctuate over the course of pregnancy; this may have impacted the level of precision with which the associations between fluoride exposure and TT4 were estimated. Similarly, we were also unable to control for biomarkers of other nutrients like selenium, which have been shown to play an important role in T4 to T3 activation by iodothyronine deiodinases (Arthur et al., 1992). Future studies in this area may also want to obtain measures of maternal T3 as it may be more sensitive to fluoride. An additional limitation pertaining to our measure of MUF_{SG} concentration, is that fluoride was measured in spot samples instead of 24-hour urine samples or first morning voids, preventing us from being able to control for behaviours that could contribute to fluctuations in urinary fluoride concentration given the short half-life of fluoride (approximately 5 h). We attempted to mitigate the effects of this limitation by averaging urinary fluoride across all three trimesters.

4.2. Conclusions

In this Canadian pregnancy cohort, higher levels of fluoride exposure were associated with alterations in maternal thyroid hormone levels, the magnitude of which varied by fetal sex. Our findings make an important contribution to the growing body of evidence suggesting that higher levels of fluoride exposure in pregnancy may have adverse effects on maternal thyroid function. The implications of this work are of public health significance when considering the vital role of the maternal thyroid in supporting optimal fetal growth and neurodevelopment. Future studies in this area are warranted to replicate the current findings.

CRediT authorship contribution statement

Meaghan Hall: Writing – review & editing, Writing – original draft, Visualization, Methodology, Formal analysis, Data curation, Conceptualization. **Rick Hornung:** Methodology, Validation, Writing – review & editing. **Jonathan Chevrier:** Writing – review & editing, Validation, Methodology, Investigation. **Pierre Ayotte:** Writing – review & editing, Investigation, Funding acquisition. **Bruce Lanphear:** Writing – review & editing, Investigation, Funding acquisition, Conceptualization. **Christine Till:** Writing – review & editing, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Rick Hornung reports financial support was provided by York University for statistical consulting fees paid from R01 ES030365. Dr. Lanphear served as a non-retained expert witness in the federal fluoride case to describe the results of the fluoride studies using the MIREC cohort (Food & Water Watch, et al. vs. U.S. Environmental Protection Agency, United States District Court for the Northern District of California at San

Francisco). He received no payment for his service. All other authors declare that they have no known competing or financial interests or personal relationships that could have appeared to influence the work in this paper.

Data availability

The authors do not have permission to share data.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2024.108442.

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