Critical Windows of Fluoride Neurotoxicity in Canadian Children

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

PII: S0013-9351(21)00609-5

DOI: https://doi.org/10.1016/j.envres.2021.111315

Reference: YENRS 111315

To appear in: *Environmental Research*

Received Date: 19 February 2021

Revised Date: 28 April 2021

Accepted Date: 7 May 2021

Please cite this article as: Farmus, L., Till, C., Green, R., Hornung, R., Martinez-Mier, E.A., Ayotte, P., Muckle, G., Lanphear, B., Flora, D., Critical Windows of Fluoride Neurotoxicity in Canadian Children, *Environmental Research*, https://doi.org/10.1016/j.envres.2021.111315.

Credit Author Statement

Linda Farmus: Data curation, formal analysis, methodology, software, visualization, writing original draft, writing- review and editing

Christine Till: Conceptualization, Data curation, formal analysis, funding acquisition, methodology, supervision, visualization, writing- review and editing

Rivka Green: Data curation, formal analysis, writing- review and editing

Richard Hornung: methodology, writing- review and editing

E. Angeles Martinez-Mier: writing- review and editing

Pierre Ayotte: writing- review and editing

Gina Muckle: writing- review and editing

Bruce Lanphear: Conceptualization, funding acquisition, writing- review and editing

David Flora: Conceptualization, formal analysis, methodology, software, supervision, writing- review and editing

Critical Windows of Fluoride Neurotoxicity in Canadian Children

Linda Farmus[1], MA, Christine Till[1], PhD, Rivka Green[1], MA, Richard Hornung, PhD, E. Angeles Martinez-Mier[2], DDS, PhD, Pierre Ayotte[3,4], PhD, Gina Muckle[3,5], PhD, Bruce Lanphear[6,7], MD, David Flora[1], PhD

Affiliations: [1] Faculty of Health, York University, Ontario, Canada

[2] Department of Cariology, Operative Dentistry and Dental Public Health, Indiana University School of Dentistry, Indiana, USA

[3] Centre de Recherche du CHU de Québec, Université Laval, Québec, Canada

[4] Department of Social and Preventive Medicine, Laval University, Quebec, Canada.

[5] School of Psychology, Laval University, Quebec, Canada

[6] Faculty of Health Sciences, Simon Fraser University, British Columbia, Canada

[7] Child & Family Research Institute, BC Children's Hospital, University of British Columbia, British Columbia, Canada

Address correspondence to: Christine Till, Department of Psychology, York University 4700 Keele Street, M3J 1P3 Toronto, ON Canada , ctill@yorku.ca Tel: 416-736-2100 x 20776

Short title: Critical windows of fluoride neurotoxicity

Funding Source: This study was funded by a grant from the National Institute of Environmental Health Science (NIEHS) (grant #R21ES027044). The MIREC Study was supported by the Chemicals Management Plan at Health Canada, the Ontario Ministry of the Environment, and the Canadian Institutes for Health Research (grant # MOP-81285).

Financial Disclosure: The authors have no financial disclosures.

Conflict of Interest: The authors have no conflicts of interest relevant to this article to disclose.

Abbreviations: CI = confidence interval; HOME = Home Observation for Measurement of the Environment; FDR = false discovery rate; FSIQ = Full Scale IQ; PIQ= Performance IQ; VIQ = Performance IQ; IFI = infant fluoride intake; MIREC = Maternal-Infant Research on Environmental Chemicals; MUF = maternal urinary fluoride; SD = standard deviation.

Abstract

Background: Fluoride has been associated with IQ deficits during early brain development, but the period in which children are most sensitive is unknown.

Objective: We assessed effects of fluoride on IQ scores across prenatal and postnatal exposure windows.

Methods: We used repeated-exposures from 596 mother-child pairs in the Maternal-Infant Research on Environmental Chemicals pregnancy and birth cohort. Fluoride was measured in urine (mg/L) collected from women during pregnancy and in their children between 1.9 and 4.4 years; urinary fluoride was adjusted for specific gravity. We estimated infant fluoride exposure (mg/day) using water fluoride concentration and duration of formula-feeding over the first year of life. Intelligence was assessed at 3 to 4 years using the Wechsler Preschool and Primary Scale of Intelligence-III. We used generalized estimating equations to examine the associations between fluoride exposures and IQ, adjusting for covariates. We report results based on standardized exposures given their varying units of measurement.

Results: The association between fluoride and performance IQ (PIQ) significantly differed across prenatal, infancy, and childhood exposure windows collapsing across child sex (p = .001). The strongest association between fluoride and PIQ was during the prenatal window, B = -2.36, 95% CI: -3.63, -1.08; the association was also significant during infancy, B = -2.11, 95% CI: -3.45, -0.76, but weaker in childhood, B = -1.51, 95% CI: -2.90, -0.12. Within sex, the association between fluoride and PIQ significantly differed across the three exposure windows (p = .01); among boys, the strongest association was during the prenatal window, B = -3.01, 95% CI: -4.60, -1.42, whereas among girls, the strongest association was during infancy, B = -2.71, 95% CI: -4.59, -0.83. Full-scale IQ estimates were weaker than PIQ estimates for every window. Fluoride was not significantly associated with Verbal IQ across any exposure window.

Conclusion: Associations between fluoride exposure and PIQ differed based on timing of exposure. The prenatal window may be critical for boys, whereas infancy may be a critical window for girls.

Critical Windows of Fluoride Neurotoxicity in Canadian Children

Fluoride has been associated with IQ deficits at water fluoride concentrations >1.2 mg/L (Choi, Sun, Zhang, & Grandjean, 2012; Dong, Yao, Chen, Li, & Shi, 2018; Grandjean, 2019; National Toxicology Program, 2020; Seraj et al., 2012; Xiang et al., 2003; Valdez Jiménez et al., 2017). Early-life exposure to optimal levels (i.e., 0.7 mg/L) of fluoride – as defined by levels sufficient to protect against tooth decay and minimize against dental fluorosis – has also been associated with diminished cognitive abilities in prospective studies of children (Bashash et al., 2017; Green et al., 2019; Till et al., 2020). Drinking water is a main source of fluoride for pregnant women (Till et al., 2018) and young children (dela Cruz, Rozier, & Bawden, 2008; Green et al., 2020) living in communities with water fluoridation (US EPA, 2010). Therefore, associations between fluoride in pregnancy and child outcomes may be conflated by continuous exposure to fluoride over the lifespan. Few human studies have examined the developmental period of greatest vulnerability to fluoride neurotoxicity (Xu et al., 2020).

Identifying critical windows of vulnerability to fluoride during early brain development is important because the timing of exposure may result in a greater risk of potentially permanent adverse outcomes (Hornung, Lanphear, & Dietrich, 2009; Selevan, Kimmel, & Mendola, 2000). During fetal development, the brain is particularly vulnerable to environmental toxicants (Lanphear, 2015). Still, the brain continues to undergo an orderly sequence of neuronal developmental processes (e.g., synaptogenesis, myelination), and the period of heightened vulnerability may extend for many months after birth (Rice & Barone, 2000). Thus, sensitivity to neurotoxicants may continue into infancy.

The susceptibility of infants to fluoride from drinking water is further amplified by their higher level of water intake than adults on a per body-weight basis (Snodgrass, 1992) and lower ability to detoxify exogenous compounds than adults. In particular, formula-fed infants, whose

formula is made with fluoridated water, have an approximate 70-fold higher fluoride intake than exclusively breastfed infants (Ekstrand, 1981; Zohoori et al., 2018; US EPA, 2010). Thus, level and timing of fluoride exposure are critical for determining the window of greatest vulnerability for neurodevelopmental outcomes.

We examined the impact of fluoride exposure on children's intelligence quotient (IQ) scores as a function of exposure timing and sex in the same cohort. Previous studies have used ordinary least-squares linear regression to covary exposures at timepoints other than those of substantive interest. For example, Bashash et al. (2017) estimated prenatal effects while controlling postnatal effects and Till et al. (2020) estimated neonatal effects while controlling prenatal effects. This approach, however, cannot fully account for non-independent observations due to measurements at different timepoints being nested within mother-infant pairs nor make formal comparison of associations across timepoints (Buckley, Hamra, & Braun, 2019).

To overcome these limitations, we adapted an approach from Sanchez, Hu, Litman, and Tellez-Rojo (2011) using generalized estimating equations (GEE) for repeated exposure variables and a single outcome measure of IQ score. Each fluoride exposure measure is treated as a window (i.e., a particular exposure time). We incorporated interactions to estimate sex-specific associations with IQ based on our prior finding that boys may be more susceptible to prenatal fluoride exposure than girls (Green et al., 2019), a recent review of sex effects in animal and human fluoride studies (Green, Rubenstein, Popoli, Capulong, & Till, 2020), and literature on other neurotoxins suggesting interactions between sex and exposure timing (Comfort & Re, 2017; Kern et al., 2017; Torres-Rojas & Jones, 2018).

Methods

Study Participants

We used data from the Maternal-Infant Research on Environmental Chemical (MIREC) longitudinal cohort, which recruited 2001 pregnant women between 2008 and 2011. Women were recruited from prenatal clinics if they were at least 18 years old, less than 14 weeks gestation, and spoke English or French. Exclusion criteria included fetal abnormalities, medical complications, and illicit drug use during pregnancy; further details have been previously described (Arbuckle, Fraser, & Fisher, 2013; Green et al., 2019; Till et al., 2020). Our sample included 601 mother-child dyads who completed the follow-up phase of the study (MIREC-Child Development Plus) when children's neurodevelopmental testing was conducted at 3 to 4 years of age. Data from five mother-child dyads were excluded due to the mothers' declining prenatal and birth data collection (i.e., trimester fluoride exposures, demographic information, covariates, and offspring date of birth), leaving N = 596 mother-child dyads for our full analytic sample (Figure 1). Other mother-child pairs missing some data on fluoride exposure, outcomes, or covariates were retained due to the flexibility of GEE to incorporate missing data. On outcomes and covariates, no more than 4.6% of data was missing (range 0 to 4.6, M = 1.08). Dyads lived in one of six cities that either adhere to community water fluoridation (i.e., Toronto, Halifax, and Hamilton) or do not (i.e., Montreal, Vancouver, and Kingston). About half of all dyads (44%) lived in fluoridated cities.

Fluoride Exposure Measures

Maternal Urinary Fluoride (MUF). We used MUF (see Till et al., 2018) as a measure of prenatal fluoride exposure. The MIREC study collected spot urine samples in each trimester. To

account for urine dilution, concentrations for fluoride were adjusted by specific gravity (SG) with

$$P_c = P_i\left[\frac{SG_m - 1}{SG_i - 1}\right],$$

where P_c is the SG-adjusted fluoride concentration, P_i is the observed fluoride concentration, SG_i is the specific gravity of the i^{th} urine sample, and SG_m is the median SG for the cohort (Duty et al., 2005). Of the 593 women with at least one valid measure of MUF, 526 (88.3%) had a urine sample collected for all three trimesters. Our prenatal fluoride exposure variable was calculated by averaging the trimester-specific MUF measures. We calculated average MUF levels only when valid samples were available for all three trimesters to strengthen reliability of the measure (Till et al., 2018). Because GEE can incorporate missing data, we retained the 67 women (of the N = 596 dyads) for whom an average MUF value was missing; for these participants, trimester MUF measures were used in preliminary trimester analyses, and we included their data on covariates or exposures assessed at other time points in the primary analysis. Urinary fluoride concentrations were analyzed using a modification of the hexamethydisiloxane (Sigma Chemical Co., USA) micro-diffusion procedure (Martinez-Mier et al., 2011).

Infant fluoride intake (IFI). Following Till et al. (2020), we estimated IFI over the first year of the child's life using the following equation:

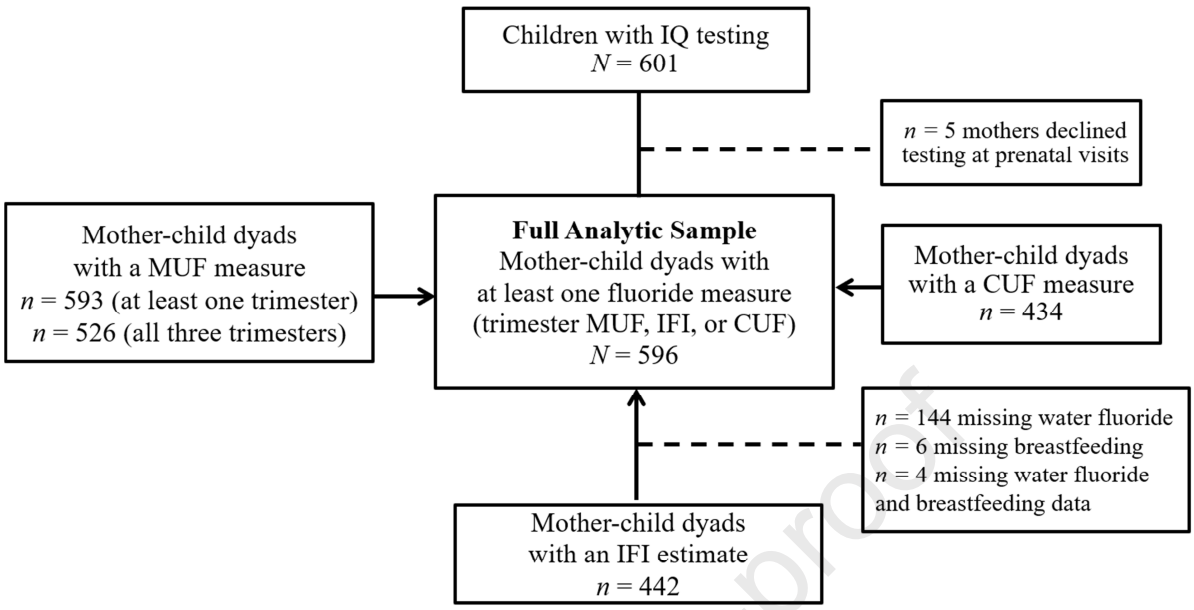
IFI = water fluoride (mg/L) × 0.8 L/day × (1-(exclusive breastfeeding / 11.99)),

where *water fluoride (mg/L)* is the average water fluoride concentration in the community during the first six months of the infant's life, *0.8 L/day* is the approximate amount of water used to reconstitute powdered formula at 3 months of age (Carignan et al., 2015), and *1-(exclusive breastfeeding /11.99)* is the proportion of time over the first year of life that the infant was not exclusively breastfed. Water fluoride levels were based on reports from water treatment plants

associated with postal codes matching each mother's residence during the third trimester of her pregnancy. The number of months of exclusive breastfeeding was recoded so that mothers who reported exclusive breastfeeding between 12 and 24 months were assigned a value of 12. Thus, formula-fed infants living in areas with community water fluoridation had IFI values near 1 and exclusively breastfed infants had values near 0. Infants receive very low concentrations of fluoride through breastmilk due to the limited transfer of fluoride from plasma to breast milk (Ekstrand et al, 1984). Mean fluoride concentration in breast milk is <0.02 ug/mL, with similar levels found among mothers living in fluoridated and non-fluoridated areas (Zohoori et al. 2018). The type of infant formula used was not reported and thus fluoride from infant formula could not be added to the derivation (see Till et al., 2020). IFI values were available for 440 mother-child dyads (Figure 1).

Child urinary fluoride (CUF). We measured CUF as an estimate of childhood exposure using a spot urine sample taken when children were between 1.86 and 4.40 years old (M = 3.25, SD = 0.54), also adjusted for specific gravity (n = 437) (Figure 1).

Figure 1. Flowchart of mother-child dyads with neurodevelopmental testing.



Note. MUF = maternal urinary fluoride; IFI = infant fluoride intake; CUF = child urinary fluoride.

Child Intellectual Abilities

Trained research assistants assessed children's intellectual abilities at the age of 3 to 4 years using the Wechsler Preschool and Primary Scale of Intelligence-III (WPPSI-III; Canadian norms; Wechsler, 2002). Outcomes included Performance IQ (PIQ), a measure of nonverbal reasoning, Verbal IQ (VIQ), a measure of verbal reasoning and comprehension, and Full-Scale IQ (FSIQ), a measure of overall intellectual ability. Examiners administered the WPPSI between 2012 and 2015 prior to proposing our fluoride research; examiners are therefore considered blinded to exposure status.

Covariates

We selected covariates consistent with prior work examining fluoride exposure and child intellectual abilities (Green et al., 2019; Till et al., 2020). Covariates included maternal education (dichotomized as Bachelor's degree or higher; yes/no), maternal race (Caucasian/non-

Caucasian), mother-reported exposure to second-hand smoke (yes/no) while pregnant, and a continuous measure of quality of home environment using the Home Observation for Measurement of the Environment (HOME) - Revised Edition (Caldwell & Bradley, 1984) at the 3-4 year-old home visit. We also included child age (in months) at urine sampling to control for age-related differences in CUF. We did not include child age at IQ testing as the WPPSI-III is age-normed in 2-month intervals. We also did not include city as a covariate in our GEE model based on its redundancy with water fluoride that is used to calculate IFI. There was no collinearity among the covariates or exposures included.

Statistical Analysis

Using the GEE method of Sanchez et al. (2011), we constructed a model to estimate associations between the fluoride exposure variables and IQ scores while adjusting for covariates. To support our decision to combine trimester exposures into a single prenatal measure, we tested whether the associations between MUF and IQ outcomes (FSIQ, PIQ, and VIQ) differed across trimesters of pregnancy by using each trimester as a separate exposure period. For our trimester-specific analysis, we included women with at least one valid MUF value (n = 593).

Our primary model included all exposure variables (MUF, IFI, and CUF) as predictors of either FSIQ, PIQ, or VIQ. We first present results of a fluoride by time interaction with girls and boys combined (i.e., comparisons of fluoride exposure windows for the overall sample). Each analysis also produces a test of the three-way interaction between fluoride exposure, time, and child sex, which leads to separate fluoride by time interactions for each sex without stratifying

the sample. In addition to testing this three-way interaction, we also tested the exposure by time two-way interaction within each sex regardless of the significance of the three-way interaction.

In sensitivity analyses, we removed mother-child dyads if the child's FSIQ score fell in the intellectual impairment range (i.e. score < 70 in three cases) or if removal of a mother-child dyad would change coefficients of exposure variables by at least 0.40 standard deviations according to DFBETAS indices (i.e., the difference in magnitude of an estimated coefficient with and without an observation, scaled by the standard error calculated without the deleted observation; Belsley, Kuh, & Welsch, 1980). Table 5 presents results of GEE analyses for our primary model after excluding influential dyads.

Given the large number of comparisons, we corrected *p* values for multiple comparisons using the false discovery rate (FDR) method of Benjamini and Hochberg (1995). A two-tailed FDR correction was implemented using a corrected *p* value of Q = 0.05 across the family of 27 coefficients tested in each of our main analyses, sensitivity analyses, and supplemental analyses. We also applied FDR correction to the tests of whether effect estimates differ across exposure windows.

Diagnostic plots of fitted values against residuals did not reveal violation of the assumptions of linearity or constant variance, and residuals were approximately normally distributed. Analyses were conducted in SAS (version 9.4; SAS Institute Inc.). Statistical significance was set at $\alpha = .05$ for a two-tailed test.

Results

Mothers were on average 32.4 years old (SD = 5.1) when they gave birth, predominantly Caucasian (89%), well-educated (66.7% had at least a bachelor's degree), and very few (2.7%) reported exposure to second-hand smoke during the first trimester of pregnancy (Table 1). Of the

593 mother-child pairs with at least one MUF value, the mean child age at intellectual testing was 3.4 years (SD = 0.3); girls comprised 51.1% of the sample (*n* = 303). The average Full Scale IQ (FSIQ) score was 106.6 (SD = 13.7) for the study sample, which is consistent for a predominantly middle-to-upper class and educated group. Table 2 shows the descriptive statistics and correlations among fluoride exposure variables.

Table 1. Characteristics of study participants for the full analytic sample and for samples with complete data on fluoride exposure windows (Mean (SD) / %).

	Samples				
Characteristic	**Full Analysis** ***N* = 596**	**Trimester** ***n* = 593**	**MUF** ***n* = 526**	**IFI** ***n* = 442**	**CUF** ***n* = 43**
Maternal Characteristics					
Years of age at delivery	32.4 (5.1)	32.4 (5.1)	32.4 (5.1)	32.5 (4.9)	32.5 (5.3
Net household income >$70K CAD	73.1	73.2	74.0	72.7	74.4
Maternal education					
Trade school/high school	33.3	33.4	32.1	30.7	30.0
Bachelor's degree or higher	66.7	66.6	67.9	69.3	70.0
Married/common-law at testing	96.1	96.1	96.8	95.9	96.6
Smoked in trimester 1	2.7	2.7	2.5	2.5	3.0
Child characteristics					
Years of age at IQ testing	3.4 (0.3)	3.4 (0.3)	3.4 (0.3)	3.4 (0.3)	3.5 (0.3)
Female sex	51.2	51.1	51.7	50.7	51.3
HOME total score	47.2 (4.6)	47.2 (4.6)	47.2 (4.7)	47.4 (4.5)	47.2 (4.8
Second-hand smoke in home	3.5	3.5	3.8	3.0	3.0
Gestational age in weeks	39.1 (1.8)	39.1 (1.7)	39.1 (1.6)	39.1 (1.8)	38.9 (2.4
Birth weight (kg)	3.5 (0.5)	3.5 (0.5)	3.5 (0.5)	3.4 (0.5)	3.5 (0.5)
Full Scale IQ	106.6 (13.7)	106.6 (13.8)	106.9 (13.5)	107.6 (13.9)	107.2 (13.
Verbal IQ	109.2 (13.7)	109.2 (13.6)	109.5 (13.3)	109.8 (13.6)	110.1 (13.

Performance IQ	102.7 (14.9)	102.7 (14.9)	102.7 (14.7)	103.8 (14.9)	102.7 (14.

Table 2. Summary statistics and correlations among fluoride exposure variables.

					Pearson correlations					
					MUF					
	N	Median	*M (*SD)	Range	T1	T2	T3	Average	IFI	CUF
MUF (mg/L)										
T1	578	0.31	0.44 (0.46)	0.01–4.29	--					
T2	566	0.37	0.51 (0.48)	0.03–5.28	.36	--				
T3	552	0.49	0.65 (0.53)	0.08–5.56	.36	.37	--			
Average	526	0.44	0.53 (0.37)	0.06–2.48	.74	.76	.77	--		
IFI (mg F)	442	0.09	0.14 (0.13)	0.00–0.61	.17	.16	.30	.28	--	
CUF (mg/L)	434	0.39	0.51 (0.39)	0.05–2.89	.18	.12	.14	.22	.25	--

Note. All urinary fluoride values are adjusted based on specific gravity.
Abbreviations: CUF=child urinary fluoride; IFI=infant fluoride intake; MUF=Maternal urinary fluoride; Average= averaged over three trimesters; SD=standard deviation.

1. Overall effects of exposure windows

The association between MUF and IQ scores did not differ significantly across trimesters [FSIQ: $\chi^2(3) = 1.99$, $p = .57$, PIQ: $\chi^2(3) = 1.08$, $p = .78$, or VIQ: $\chi^2(3) = 2.21$, $p = .53$] (Table S1). Thus, for the remaining analyses, we used average MUF as a single prenatal exposure. We compared average MUF, IFI, and CUF effects to examine the unique associations of prenatal, infancy, and childhood exposures on IQ scores. Table 3 shows the associations between *standardized* fluoride exposures and unstandardized IQ scores whereas Table 4 shows the *unstandardized* coefficients per 0.5 mg/L MUF and CUF and per 0.1 mg IFI/day (to facilitate comparison of average MUF, IFI, and CUF associations with IQ scores). The standardized coefficient indicates the change in the dependent variable (i.e. age-normed IQ score) per one

standard deviation (SD) in the fluoride exposure variable; thus, a standardized coefficient of -1.9 means that the IQ score decreases by 1.9 points per one SD in the exposure variable. An unstandardized coefficient represents the amount by which the dependent variable changes per one unit change in the fluoride exposure (i.e. 1 mg/L) variable keeping other variables constant. Combining across boys and girls, the two-way interaction between fluoride and time was statistically significant for PIQ ($\chi^2(3) = 18.78$, $p < .001$) and VIQ ($\chi^2(3) = 8.28$ $p = .04$), but not for FSIQ ($\chi^2(3) = 4.36$, $p = .23$). Controlling the FDR, there were significant negative effects of standardized fluoride exposures for overall MUF and overall IFI on PIQ (B = -2.36, 95% CI: -3.63, -1.08; B = -2.11, 95% CI: -3.45, -0.76, respectively). The associations between standardized fluoride exposures and IQ scores are visualized in Figure 2.

2. Effects of exposure windows by sex

The three-way interaction between fluoride, child sex, and time was not statistically significant for FSIQ ($\chi^2(2) = 2.74$, $p = .25$), PIQ ($\chi^2(2) = 2.72$, $p = .26$), or VIQ ($\chi^2(2) = 1.92$, $p = .38$). However, among boys, the association between fluoride and PIQ significantly differed across windows ($\chi^2(3) = 11.92$, $p = .01$), but not for FSIQ ($\chi^2(3) = 5.83$, $p = .12$) or VIQ ($\chi^2(3) = 5.80$, $p = .12$) (Table 3). Similarly, among girls, the effect of fluoride exposure significantly differed across windows for PIQ ($\chi^2(3) = 11.69$, $p = .01$), but not for FSIQ ($\chi^2(3) = 1.15$, $p = .77$) or VIQ ($\chi^2(3) = 3.63$, $p = .30$) (Table 3). Probing the time (i.e. exposure windows) interaction within boys and girls, significant effects of standardized fluoride exposures after controlling the FDR were as follows: among boys, MUF had stronger negative associations with FSIQ (B = -1.86, 95% CI: -3.22, -0.49) and PIQ (B = -3.01, 95% CI: -4.60, -1.42) than IFI and CUF. Among girls, IFI had a stronger association with PIQ (B = -2.71, 95% CI: -4.59, -0.83) than MUF and CUF (Figure 2).

Table 3. Effects of standardized average maternal urinary fluoride (MUF), infant fluoride intake (IFI) and child urinary fluoride (CUF) on age-normed IQ scores using GEE. *B* (95% CI) reported.

	Males (n = 291)	Females (n = 305)	Overall (N = 596)
FSIQ			
MUF	**-1.86 (-3.22, -0.49)**	-0.23 (-2.06, 1.60)	-1.28 (-2.37, -0.18)
IFI	-0.01 (-1.67, 1.65)	-0.72 (-2.34, 0.89)	-0.38 (-1.53, 0.78)
CUF	0.07 (-1.66, 1.80)	-0.41 (-2.07, 1.24)	-0.18 (-1.38, 1.02)
p_{int}	.12	.77	.23
PIQ			
MUF	**-3.01 (-4.60, -1.42)**	-1.18 (-3.32, 0.96)	**-2.36 (-3.63, -1.08)**
IFI	-1.45 (-3.40, 0.49)	**-2.71 (-4.59, -0.83)**	**-2.11 (-3.45, -0.76)**
CUF	-1.49 (-3.50, 0.53)	-1.53 (-3.45, 0.39)	-1.51 (-2.90, -0.12)
p_{int}	.01	.01	<.001
VIQ			
MUF	-0.25 (-1.57, 1.07)	0.87 (-0.91, 2.64)	0.15 (-0.91, 1.20)
IFI	1.22 (-0.39, 2.83)	1.31 (-0.25, 2.87)	1.27 (0.15, 2.39)
CUF	1.61 (-0.06, 3.29)	0.63 (-0.98, 2.23)	1.10 (-0.06, 2.26)
p_{int}	.12	.30	.04

Note. N = 596. Covariates include maternal education, maternal race, total HOME score, age at urine sampling, and prenatal second-hand smoke. p_{int} refers to the interaction between exposure timing and fluoride level. Estimates in bold are significant, $p < .05$ (p values corrected for multiple comparisons using the Benjamini-Hochberg FDR method).

272

Figure 2. Standardized associations between fluoride exposure windows and IQ outcomes using GEE. *Note*. Dots represent point estimates and tails represent 95% confidence intervals.

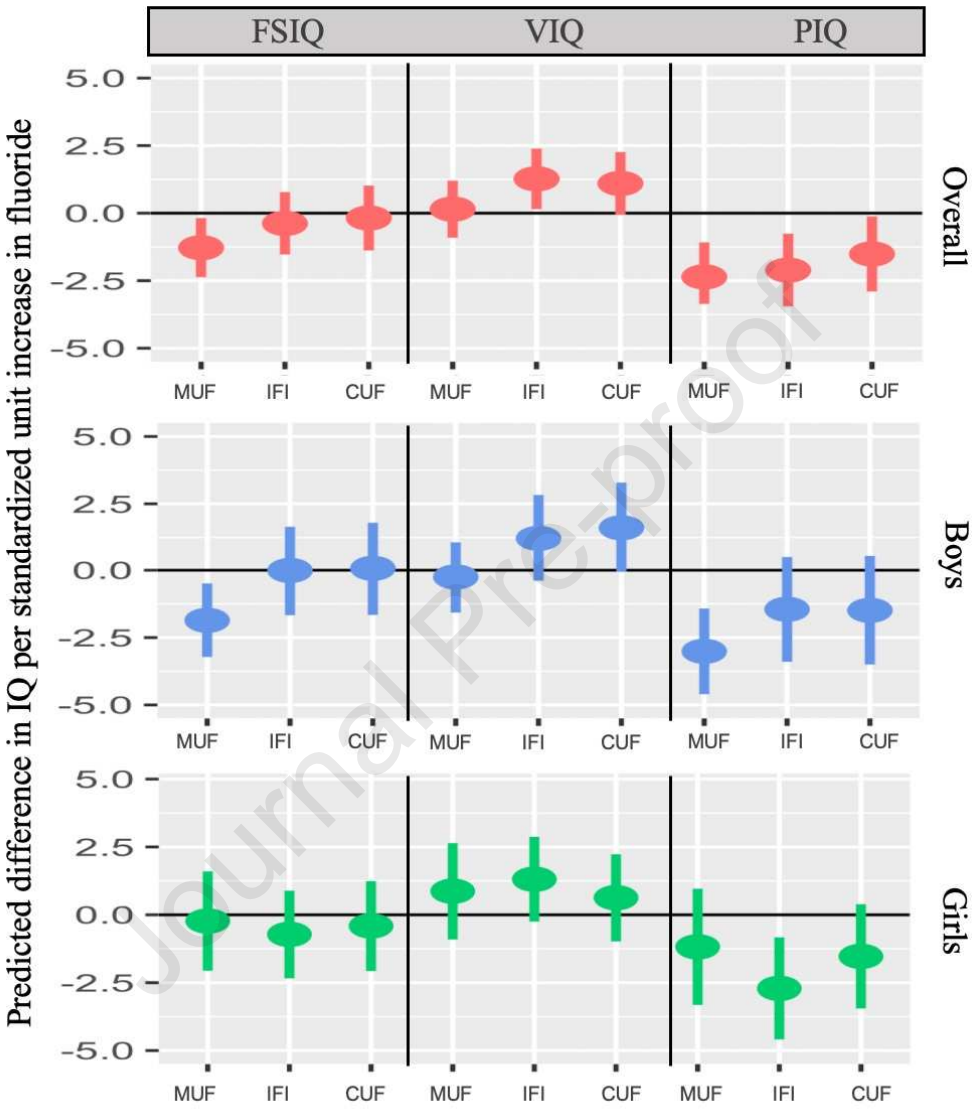


Table 4. Effect of 0.5 mg/L of average maternal urinary fluoride (MUF)*, 0.1 mg/day of estimated infant fluoride intake (IFI)* and 0.5 mg/L of child urinary fluoride (CUF)* on IQ scores using GEE. Unstandardized *B* (95% CI) reported.

	Males (n = 291) [a,b]	Females (n = 305) [a,b]	Overall Participants (N = 596)
FSIQ			
MUF	**-2.48 (-4.30, -0.66)**	-0.31 (-2.76, 2.14)	-1.71 (-3.17, -0.24)
IFI	-0.01 (-1.25, 1.24)	-0.54 (-1.75, 0.66)	-0.28 (-1.15, 0.58)
CUF	0.09 (-2.10, 2.28)	-0.52 (-2.62, 1.58)	-0.23 (-1.75, 1.29)
p_{int}	.12	.77	.23
PIQ			
MUF	**-4.02 (-6.15, -1.89)**	-1.58 (-4.43, 1.28)	**-3.15 (-4.85, -1.44)**
IFI	-1.09 (-2.54, 0.37)	**-2.03 (-3.43, -0.63)**	**-1.58 (-2.59, -0.57)**
CUF	-1.89 (-4.44, 0.67)	-1.94 (-4.37, 0.50)	-1.91 (-3.68, -0.15)
p_{int}	.01	.01	< .001
VIQ			
MUF	-0.34 (-2.10, 1.43)	1.16 (-1.22, 3.53)	0.20 (-1.22, 1.61)
IFI	0.92 (-0.29, 2.12)	0.98 (-0.19, 2.15)	0.95 (0.11, 1.79)
CUF	2.05 (-0.08, 4.16)	0.79 (-1.24, 2.82)	1.39 (-0.08, 2.86)
p_{int}	.12	.30	.04

Note. The overall N = 596 includes mother-child pairs with at least one measure of MUF, IFI, or CUF. Covariates include maternal education, maternal race, total HOME score, age at urine sampling, and prenatal second-hand smoke. p_{int} refers to the p-value for the interaction between exposure timing and fluoride level. Bolded estimates are significant, $p < .05$ (p values corrected for multiple comparisons using the Benjamini-Hochberg FDR method).
*MUF is presented in 0.5 mg/L units based on the mean MUF = 0.53 mg/L; IFI is presented in 0.1 mg/day units based on the mean IFI = 0.14 mg/day; CUF is presented in 0.5 mg/L units based on the mean CUF = 0.51 mg/L
[a] Males: IFI n = 218; Females: IFI n = 214
[b] Males: CUF n = 211; Females: CUF n = 223

3. Sensitivity analyses

Removal of six mother-child dyads that were influential (as per DFBETA) on the sex-specific estimates of fluoride exposures on FSIQ made the negative association between MUF and FSIQ among boys weaker and non-significant after adjustment for the FDR (B = -1.22, 95% CI: -2.41, -0.04). All other prenatal and postnatal sex-specific and overall effects remained significant (or non-significant) with removal of influential dyads for FSIQ, PIQ, or VIQ, adjusted for the FDR (Table 5).

Table 5. Sensitivity analysis for the effects of standardized average maternal urinary fluoride (MUF), infant fluoride intake (IFI) and child urinary fluoride (CUF) on age-normed IQ scores after excluding influential dyads. *B* (95% CI) reported.

	Males (n = 288[a])	Females (n = 302[b])	Overall (N = 590[c])
FSIQ			
MUF	**-1.22 (-2.41, -0.04)**	-1.00 (-2.84, 0.84)	-1.14 (-2.25, -0.04)
IFI	0.10 (-1.55, 1.75)	-1.58 (-3.17, 0.01)	-0.76 (-1.89, 0.38)
CUF	0.40 (-1.14, 1.95)	-0.00 (-1.61, 1.61)	0.18 (-1.01, 1.38)
p_{int}	.19	.12	.08
PIQ			
MUF	**-2.39 (-4.05, -0.73)**	-2.00 (-4.19, 0.20)	**-2.24 (-3.56, -0.92)**
IFI	-1.38 (-3.32, 0.55)	**-3.59 (-5.48, -1.70)**	**-2.51 (-3.86, -1.16)**
CUF	-1.17 (-3.29, 0.94)	-1.21 (-3.12, 0.71)	-1.19 (-2.61, 0.23)
p_{int}	.01	< .001	< .0001
VIQ			
MUF	0.25 (-1.11, 1.61)	0.33 (-1.47, 2.13)	0.28 (-0.80, 1.36)
IFI	1.35 (-0.24, 2.93)	0.64 (-0.91, 2.19)	0.99 (-0.12, 2.09)
CUF	1.89 (0.16, 3.62)	0.98 (-0.60, 2.55)	1.39 (0.23, 2.56)
p_{int}	.13	.36	.03

Note. Covariates include maternal education, maternal race, total HOME score, age that children provided CUF, and prenatal second-hand smoke. Mother-child dyads were influential if DFBETAS indices were > 0.40 and/or child FSIQ < 70. p_{int} refers to the interaction between exposure timing and fluoride level. Influence analyses were conducted simultaneously for boys, girls, and overall effects for each outcome. Bolded estimates are significant, $p < .05$ (p values corrected for multiple comparisons using the Benjamini-Hochberg FDR method).

[a] FSIQ n = 287.

[b] PIQ n = 301.

[c] FSIQ and PIQ n = 589.

285

286

Discussion

We used data from a prospective pregnancy and birth cohort to compare the associations between fluoride exposures during different developmental windows and preschool aged children's intellectual abilities. The GEE method advances our understanding of early-life fluoride neurotoxicity by formally comparing strength of associations across windows of exposure. The strongest association between fluoride and child IQ was observed between standardized MUF and age-normed PIQ (B = -2.36, 95% CI: -3.63, -1.08); the association was significant during infancy, B= -2.11, 95% CI: -3.45, -0.76), but negligible in childhood. Our results, which show that fetal fluoride exposure is more strongly associated with children's intelligence than postnatal fluoride exposure, are consistent with a Chinese study examining different susceptibility windows of fluoride exposure; lower IQ was found in children whose mothers were exposed to high fluoride levels in drinking water (> 1.0 mg/L) during pregnancy compared to those with high postnatal and low prenatal fluoride exposure (Xu et al., 2020). We did not identify clear differences between the effects of different trimester exposure windows on cognitive outcomes (Supplementary Table 1), and so it may be that the entire prenatal period confers susceptibility.

Critical windows of exposure may also differ by sex; animal and human literature have noted sex differences in response to fluoride exposure (Green et al., 2019; Green et al., 2020; Mullenix Debesten, Schunior, & Kernan, 1995) as well as several other environmental neurotoxicants (Comfort & Re, 2017; Torres-Rojas & Jones, 2018). When we tested sex differences across windows, our results suggested that prenatal fluoride exposure was a critical developmental window for boys for FSIQ and PIQ, whereas infancy was a critical developmental window for girls for PIQ. Specifically, boys showed a 4-point decrement in PIQ per 0.5 mg/L increase in MUF whereas girls showed a 2-point decrement in PIQ per 0.1 mg increase in IFI

(effect estimates are shown based on approximate average values for MUF and IFI in our sample). While the effect of exposure in infancy was greater among girls than boys, the IFI by sex interaction for PIQ was not significant indicating that exposure in infancy is not associated with a statistical difference between boys and girls. After excluding outlying dyads, the adverse association between IFI and PIQ strengthened among girls (from B = -2.0 to B = -3.6), while this association among boys remained about the same (from B = -1.1 to B = -1.4).

Within animal research, a rat experiment similarly demonstrated an interaction between sex and fluoride exposure across developmental windows (Mullenix et al., 1995). Male rat pups were most sensitive to late prenatal exposure whereas female rats were most sensitive to exposure occurring in the postnatal (weanling) period. Exposed adult females also showed a lower threshold for behaviour deficits than exposed adult males. These findings are consistent with some (Baran-Poesine et al., 2013; Bera et al., 2007; Flace et al., 2010) but not all (Bartos et al., 2015; Jiang et al., 2014) rat studies examining sex-specific effects of prenatal exposure to fluoride. Further research is needed to examine sex-specific effects of fluoride neurotoxicity as many of the animal studies conducted to date have been identified as having a high risk of bias (NTP, 2016).

Boys and girls may respond differentially to neurotoxicants. Indeed, studies have shown that boys are often more vulnerable to early-life exposure to neurotoxicants than girls (Brubaker, Dietrich, Lanphear, & Cecil, 2010; Desrochers-Couture et al., 2018, Jedrychowski et al., 2009; Kern et al., 2017; Ris, Dietrich, Succop, & Berger, 2004; Pagalan, 2018; Singh et al., 2018; Torres-Rojas & Jones, 2018). While the biological mechanisms underlying sex-based differences of fluoride neurotoxicity are not well understood, disruption to maternal thyroid or sex hormone levels could potentially contribute to sexually dimorphic effects (Batista & Hensch, 2019).

Fluoride may target the hypothalamic-pituitary-thyroid axis (Malin, Riddell, McCague, & Till, 2018; Bai, Huang, Wang, & Guo, 2020; Du et al., 2020), though we are not aware of any epidemiologic studies that have measured fluoride-induced changes in thyroid and sex steroid hormone levels in pregnancy. In addition, the timing of neurologic development of specific brain regions differs between the sexes (Perera & Herbstman, 2011; Lenroot et al., 2007), which might increase susceptibility of fluoride exposure during a particular developmental window. In the Mullenix et al. (1995) rat study, fluoride concentrations differed by sex in some brain structures (e.g. hippocampus), which could also contribute to sexually dimorphic changes in behaviour. See Green et al. (2020) for further discussion of mechanisms that may contribute to sex-based differences of fluoride neurotoxicity.

The difference in magnitudes and divergence in the direction of some of the associations between verbal and non-verbal intellectual abilities may have several progenitors that reflect these distinct types of cognitive ability. While we would not expect higher fluoride intake in infancy to be beneficial to VIQ, we would expect it to be detrimental to non-verbal (PIQ) intelligence. Fluid (i.e. non-verbal) abilities are more biologically determined whereas crystallized intelligence (i.e. VIQ) is more likely to be shaped by experience (Ashbury et al., 2005; Luster & Dubow, 1992). Past studies have suggested that prenatal and early-life exposure to some neurotoxicants, like lead, is more strongly associated with non-verbal intelligence than verbal intelligence in young children (Bellinger et al. 1991; Dietrich et al. 1991, 1993; Factor-Litvak et al. 1999; Jusko et al., 2008; Wasserman et al. 1997). Consistent with this pattern, our findings showed a decrement of IFI to PIQ (statistically significant *decrease* of 1.6 points per 0.1 mg/day), but not VIQ (non-significant *increase* of 1.0 points per 0.1 mg/day).

Our current results are consistent with and extend our previous findings. The effect of MUF on FSIQ was significant for boys (2.45-point decrement in FSIQ per 0.5 mg/L increase in MUF; Table 2), reproducing our prior work (Green et al., 2019) in which we found a 2.2-point decrement in FSIQ per 0.5 mg/L increase in MUF. We note that the current analysis did not include city in the analysis because fluoride intake from formula (i.e. IFI) is a function of residential water fluoride concentration and was therefore deemed redundant. Our finding of a 2.1-point decrement in PIQ per 0.5 mg/L increase in IFI (B = -2.11. 95% CI: -3.45, -0.76) was also consistent with our prior finding that infancy is a critical period for non-verbal intelligence in boys and girls (Till et al., 2020). Our current results extend our prior work by showing that regardless of child sex or the exclusion of influential dyads, the associations of fluoride on PIQ differs across exposure windows. However, exposures do not significantly associate with IQ outcomes once city is controlled and FDR is applied.

A 2- to 4-point decrement in PIQ may seem like a small difference at the individual level. However, a small shift in the mean of IQ scores at the population level translates to millions of lost IQ points given the ubiquity of fluoride exposure. The impact of such a shift has a disproportionate effect among vulnerable populations who are at the lower end of the population IQ distribution because the loss in productivity per IQ point is not the same across the entire IQ distribution (i.e. a drop in IQ from 80 to 77 is not the same as 120 to 117) (Rose, 1985). Finally, previous benchmark dose analyses for testing lead and fluoride neurotoxicity have selected 1 IQ point as the benchmark response because of the significant societal and economic burdens of reduced IQ (Budtz-Jørgensen et al., 2004).

Strengths of the present study include the relatively large sample with repeated exposure measures during pregnancy, infancy, and early childhood that resulted in precise estimates of

effects, as reflected by narrow confidence intervals. We used the FDR method to guard against false positive conclusions due to multiple comparisons, even though multiplicity control is rarely imposed when evaluating multiple predictors in regression-based models (Cribbie, 2017). We adjusted for numerous potential confounders and avoided problems with collinearity among critical windows of fluoride toxicity by using GEE. Although several epidemiological studies have applied GEE to test critical windows of environmental contaminants on neurobehavioral outcomes (Jackson-Browne et al., 2018; Stacy et al., 2017; Vuong et al., 2017; Zhang et al., 2017), this is the first study to use GEE to model critical windows of fluoride toxicity.

Limitations of our study include modeling marginal effects of fluoride exposures without controlling the effects from other exposure windows or assessing cumulative fluoride exposure, which may be more etiologically relevant. However, it would not be realistic to estimate partial effects that vary one exposure window while fixing other exposures. Another limitation is not having MUF, IFI, or CUF levels on all study participants, although we were able to incorporate cases with incomplete data in the GEE analyses. In any research on single neurotoxicants, simultaneous exposure to other environmental contaminants may confound effect estimates. For instance, trace amounts of aluminum can bind fluoride and affect cellular processes (Li, 2003). Moreover, there is always the possibility of residual confounding. We considered many potential confounders in prior research conducted in the same sample examining the association between MUF and child IQ (Green et al., 2019) and they did not influence our findings. We also controlled for several other chemicals in our prior analyses including lead, mercury, PFOA, arsenic, manganese, and second-hand smoke exposure. Controlling for these chemicals did not affect our estimates appreciably. The demographic characteristics of our sample also constrained our ability to test potential fluoride susceptibility in different subpopulations. For example, fewer

than 3% of women smoked in the first trimester of pregnancy and 89% of the sample was Caucasian, which limited our ability to assess effect modification by smoking or race. Further, MUF concentration averaged across three trimesters was the strongest predictor of IQ scores among boys and was more reliable than IFI and CUF. Fluoride concentrations measured in single spot urine samples (i.e. trimester-specific MUF and CUF concentrations) suffer from measurement error due to the rapid elimination kinetics of fluoride (half-life in urine < 6 hours; Ekstrand, 1983) and lack of control for water/beverage consumption and dental product use prior to urine sampling. IFI may also suffer from measurement error due to using mother's self-report of infant water intake and breastfeeding duration, and our reliance on water fluoride measurements made at water treatment plants as opposed to measuring fluoride directly in household tap water. While we did not have specific information on the type of water used to reconstitute formula (i.e. bottled/filtered versus tap water), we derived IFI only for children of women who reported drinking tap water. However, these possible sources of measurement error are more likely to produce negatively biased effect estimates than positively biased estimates (Budtz-Jorgensen, Keiding, & Grandjean, 2004).

Our findings raise the question of whether a decrease in children's cognitive abilities is worth the benefit that fluoride ingestion provides. To answer this question, we need to consider how and when fluoride works for the developing child and pregnant woman. Fluoride prevents dental decay by being present in the mouth when a decay-inducing acid attack occurs, by precluding minerals from leaving the dental enamel during the attack (prevention of demineralization) and by incorporating into the enamel after the acid attack (promotion of remineralization). These processes only occur after teeth have erupted (CDC, 2001; Ten Cate and Buzalaf, 2019) Fluoride incorporated into enamel before eruption has a minimal effect on

the prevention of dental decay (CDC, 2001; Takahashi et al., 2017). In contrast, there is potential risk of reduced IQ associated with fluoride exposure during fetal and infant development. Consistent with this conclusion, the Center for Disease Control and Prevention does not recommend the use of fluoride supplements during pregnancy (CDC, 2001). If a pregnant woman chooses to decrease her ingestion of fluoridated water, which accounts for 75% of her fluoride intake (CDC, 2001) or not drinking tea or eating foods high in fluoride, common alternatives for minimizing risk of dental decay in pregnancy include reducing sugar intake and using topical fluorides, such as fluoridated toothpastes and varnishes.

Given a heightened sensitivity of the developing brain to environmental toxicants, identifying critical windows of vulnerability to fluoride exposure is essential for promoting child health. Our results suggest the associations of prenatal and postnatal fluoride exposure with cognitive development may be modified by sex, though further replication of this finding is needed. These results indicate that it is important to balance the risks of fluoride exposure during early brain development with its potential to prevent caries, especially for pregnant women and infants.

Supplemental Materials

Table S1. Effect of trimester maternal urinary fluoride exposure (1 mg/L) on IQ scores using GEE. Unstandardized *B* (95% CI) reported

	Males (n = 291)	Females (n = 305)	Overall (N = 596)
FSIQ			
trimester 1	-2.34 (-5.03, 0.35)	0.90 (-2.91, 4.71)	-1.28 (-3.52, 0.96)
trimester 2	-3.05 (-6.42, 0.32)	0.99 (-2.83, 4.81)	-1.29 (-3.90, 1.31)
trimester 3	-1.31 (-3.78, 1.16)	-1.20 (-5.19, 2.80)	-1.28 (-3.49, 0.93)
p_{int}	.22	.63	.57
PIQ			
trimester 1	-2.17 (-5.24, 0.91)	3.12 (-1.23, 7.48)	-0.43 (-3.00, 2.13)
trimester 2	-4.35 (-8.33, -0.36)	3.55 (-0.82, 7.91)	-0.78 (-3.83, 2.27)
trimester 3	-1.27 (-4.09, 1.56)	-1.33 (-5.90, 3.24)	-1.28 (-3.81, 1.25)
p_{int}	.29	.11	.78
VIQ			
trimester 1	-1.93 (-4.54, 0.68)	-1.07 (-4.76, 2.63)	-1.65 (-3.82, 0.52)
trimester 2	-0.83 (-4.10, 2.44)	-1.31 (-5.01, 2.39)	-1.04 (-3.57, 1.49)
trimester 3	-0.84 (-3.24, 1.55)	-0.36 (-4.23, 3.52)	-0.72 (-2.86, 1.42)
p_{int}	.49	.90	.53

Note. N = 596. Covariates include city, maternal education, maternal race, total HOME score, and prenatal second-hand smoke. p_{int} refers to the interaction between exposure timing and fluoride level. *P* values were corrected for multiple comparisons using the Benjamini-Hochberg FDR method. We did not apply FDR to our p_{int} values since none of the windows significantly differed. The three-way interaction between fluoride, child sex, and time was statistically significant for PIQ ($\chi^2(2) = 10.64$, $p = .005$) but not for FSIQ ($\chi^2(2) = 4.11$, $p = .13$) or VIQ ($\chi^2(2) = 0.05$, $p = .97$).

References

Arbuckle, T.E., Fraser, W.D., Fisher, M., Davis, K., Liang, C.L., Lupien, N., Bastien, S., Velez, M.P., Von Dadelszen, P., Hemmings, D.G., Wang, J., Helewa, M., Taback, S., Sermer, M., Foster, W., Ross, G., Fredette, P., Smith, G., Walker, M., Shear, R., Dodds, L., Ettinger, A.S., Weber, J.P., D'Amour, M., Legrand, M., Kumarathasan, P., Vincent, R., Luo, Z.C., Platt, R.W., Mitchell, G., Hidiroglou, N., Cockell, K., Villeneuve, M., Rawn, D.F.K., Dabeka, R., Cao, X.L., Becalski, A., Ratnayake, N., Bondy, G., Jin, X., Wang, Z., Tittlemier, S., Julien, P., Avard, D., Weiler, H., Leblanc, A., Muckle, G., Boivin, M., Dionne, G., Ayotte, P., Lanphear, B., Séguin, J.R., Saint-Amour, D., Dewailly, É., Monnier, P., Koren, G., Ouellet, E., 2013. Cohort profile: the maternal-infant research on environmental chemicals research platform. Paediatr. Perinat. Epidemiol. 27 (4), 415-425. http://doi.org/10.1111/ppe.12061

Asbury, K., Wachs, T.D., Plomin, R., 2005. Environmental moderators of genetic influence on verbal and nonverbal abilities in early childhood. Intelligence. 33 (6), 643-661. https://doi.org/10.1016/j.intell.2005.03.008

Bai, R., Huang, Y., Wang, F., Guo, J., 2020. Associations of fluoride exposure with sex steroid hormones among U.S. children and adolescents, NHANES 2013–2016. Environ. Pollu. 260, 114003. https://doi.org/10.1016/j.envpol.2020.114003

Bashash, M., Thomas, D., Hu, H., Martinez-Mier, E.A., Sanchez, B.N., Basu, N., Peterson, K. E., Ettinger, A.S., Wright, R., Zhang, Z., Liu, Y., Schnaas, L., Mercado-Garcia, A., Téllez-Rojo, M.M., Hernández-Avila, M., 2017. Prenatal fluoride exposure and cognitive outcomes in children at 4 and 6 – 12 years of age in Mexico. Environ. Health Perspect. 125 (9), 1-12. https://doi.org/10.1289/EHP655

Batista, G., Hensch, T.K., 2019. Critical period regulation by thyroid hormones: Potential mechanisms and sex-specific aspects. Front. Mol. Neurosci. 12, 77. https://doi.org/10.3389/fnmol.2019.00077

Bellinger, D., Leviton, A., Sloman, J., Rabinowitz, M., Needleman, H.L., Waternaux, C., 1991. Low-level lead exposure and children's cognitive function in the preschool years. Pediatrics. 87 (2), 219-227

Belsley, D.A., Kuh, E., Welsch, R.E., 1980. Regression Diagnostics: Identifying Influential Data and Sources of Collinearity. Wiley: New York

Benjamini, Y., Hochberg, Y., 1995. Controlling the false-discovery rate: A practical and powerful approach to multiple testing. J. R. Stat. Soc. Series B Stat. Methodol. 57, 289–300. https://doi.org/10.1111/j.2517-6161.1995.tb02031.x

Brubaker, C.J., Dietrich, K.N., Lanphear, B.P., Cecil, K.M., 2010. The influence of age of lead exposure on adult gray matter volume. Neurotoxicology. 31 (3), 259–266. https://doi.org/10.1016/j.neuro.2010.03.004

Buckley, J.P., Hamra, G.B., Braun, J.M., 2019. Statistical approaches for investigating periods of susceptibility in children's environmental health research. Curr. Environ. Health Rep. 6, 1–7. https://doi.org/10.1007/s40572-019-0224-5

Budtz-Jorgensen, E., Keiding, N., Grandjean, P., 2004. Effects of exposure imprecision on estimation of the benchmark dose. Risk Anal. 24 (6), 1689–1696. https://doi.org/10.1111/j.0272-4332.2004.00560.x

Caldwell, B., Bradley, R., 1984. Home Observation for Measurement of the Environment (HOME) – Revised Edition. University of Arkansas, Little Rock., Little Rock.

Choi, A.L., Sun, G., Zhang, Y., Grandjean, P., 2012. Developmental fluoride neurotoxicity: a systematic review and meta-analysis. Environ. Health Perspect. 120 (10), 1362-1368. http://doi.org/10.1289/ehp.1104912

CDC (2001) Recommendations for using fluoride to prevent and control dental caries in the United States. Centers for Disease Control and Prevention. MMWR Recomm Rep 50(RR-14):1-42

Comfort, N., Re, D.B., 2017. Sex-specific neurotoxic effects of organophosphate pesticides across the life course. Curr. Environ. Health Rep. 4 (4), 392–404. http://doi.org/10.1007/s40572-017-0171-y

Cribbie, R.A., 2017. Multiplicity control, school uniforms, and other perplexing debates. Can. J. Behav. Sci. 49 (3), 159–165. http://doi.org/10.1037/cbs0000075

Dela Cruz, G.G., Rozier, R.G., Bawden, J.W., 2008. Fluoride concentration in dentin of exfoliated primary teeth as a biomarker for cumulative fluoride exposure. Caries Res. 42 (6), 419–428. http://doi.org/10.1159/000159605

Desrochers-Couture, M., Oulhote, Y., Arbuckle, T.E., Fraser, W.D., Séguin, J.R., Ouellet, E., Forget-Dubois, N., Ayotte, P., Boivin, M., Lanphear, B.P., Muckle, G., 2018. Prenatal, concurrent, and sex-specific associations between blood lead concentrations and IQ in preschool Canadian children. Environ. Int. 121 (Pt 2), 1235–1242. http://doi.org/10.1016/j.envint.2018.10.04

Dietrich, K.N., Succop, P.A., Berger, O.G., Hammond, P.B., Bornschein, R.L., 1991. Lead exposure and the cognitive development of urban preschool children: the Cincinnati Lead Study cohort at age 4 years. Neurotoxicol. Teratol. 13 (2), 203-211. https://doi.org/10.1016/0892-0362(91)90012-L

Dong, L., Yao, P., Chen, W., Li, P., Shi, X., 2018. An investigation of dental fluorosis and intelligence levels of children in drinking water-related endemic fluorosis areas of Xi'an. Chin. J. Epidemiol. 37 (1), 45-48. https://doi.org/10.3760/cma.j.issn.2095-4255.2018.01.010

Duty, S. M., Ackerman, R. M., Calafat, A. M., & Hauser, R. 2005. Personal care product use predicts urinary concentrations of some phthalate monoesters. Environmental Health

Perspectives. 113(11), 1530-1535. https://doi.org/10.1289/ehp.8083
Ekstrand, J., 1981. No evidence of transfer of fluoride from plasma to breast milk. Br. Med. J. 283 (6294), 761-762.

Ekstrand, J., Ehrnebo, M., 1983. The relationship between plasma fluoride, urinary excretion rate and urine fluoride concentration in man. J. Occ. Med. 25 (10), 745-748 https://doi.org/10.1097/00043764-198310000-00014

Ekstrand J, Hardell LI, S. C. (1984). Fluoride balance studies on infants in a 1-ppm-water-fluoride area. Caries Res, 18, 87–92.

Factor-Litvak, P., Wasserman, G., Kline, J.K., Graziano, J., 1999. The Yugoslavia Prospective Study of environmental lead exposure. Environ. Health Perspect. 107 (1), 9-15. https://doi.org/10.1289/ehp.991079

Grandjean, P., 2019. Developmental fluoride neurotoxicity: an updated review. Environ. Health. 18 (110), 1-17. https://doi.org/10.1186/s12940-019-0551-x

Green, R., Lanphear, B., Hornung, R., Flora, D., Martinez-Mier, E.A., Neufeld, R., Ayotte, P., Muckle, G., Till, C., 2019. Association between maternal fluoride exposure during pregnancy and IQ scores in offspring in Canada. JAMA Pediatr. 173 (10), 940-948. https://doi.org/10.1001/jamapediatrics.2019.1729

Green, R. Rubenstein, J., Popoli, R. Capulong, R., Till, C., 2020. Sex-specific neurotoxic effects of early-life exposure to fluoride: A review of the epidemiologic and animal literature. Curr. Epidemiol. Rep. 1-11. https://doi.org/10.1007/s40471-020-00246-1

Green, R., Till, C., Cantoral, A., Lanphear, B., Martinez-Mier, E.A., Ayotte, P., Wright, R.O., Tellez-Rojo, M.M., Malin, A.J., 2020. Associations between Urinary, Dietary, and Water Fluoride Concentrations among Children in Mexico and Canada. Toxics. 8 (4), 110. https://doi.org/10.3390/toxics8040110

Hornung, R.W., Lanphear, B.P., Dietrich, K.M., 2009. Age of greatest susceptibility to childhood lead exposure: A new statistical approach. Environ. Health Perspect. 117 (8), 1309–1312. https://doi.org/10.1289/ehp.0800426

Horta, B.L., Loret De Mola, C., Victora, C.G., 2015. Breastfeeding and intelligence: A systematic review and meta-analysis. Acta Pediatr. 104 (467), 14–19. https://doi.org/10.1111/apa.13139

Jackson-Browne, M.S., Papandonatos, G.D., Chen, A., Calafat, A.M., Yolton, K., Lanphear, B.P., Braun, J.M., 2018. Identifying vulnerable periods of neurotoxicity to triclosan exposure in children. Environ. Health Perspect. 126 (5), 1-9. https://doi.org/10.1289/EHP2777

Jedrychowski, W., Perera, F.P., Jankowski, J., Mrozek-Budzyn, D., Mroz, E., Flak, E., Edwards, S., Skarupa, A., Lisowska-Miszczyk, I., 2009. Very low prenatal exposure to lead and mental development of children in infancy and early childhood: Krakow prospective cohort study.

Neuroepidemiology. 32 (4), 270–278. https://doi.org/10.1159/000203075

Jusko, T.A., Henderson Jr, C.R., Lanphear, B.P., Cory-Slechta, D.A., Parsons, P.J., Canfield, R.L., (2008). Blood lead concentrations< 10 μg/dL and child intelligence at 6 years of age. Environ. Health Perspect. 116 (2), 243-248. https://doi.org/10.1289/ehp.10424

Kern, J.K., Geier, D.A., Homme, K.G., King, P.G., Bjørklund, G., Chirumbolo, S., Geier, M.R., 2017. Developmental neurotoxicants and the vulnerable male brain: A systematic review of suspected neurotoxicants that disproportionally affect males. Acta Neurobiol. Exp. 77, (4), 269–296.

Li, C., 2003. The biochemistry and physiology of metallic fluoride: action, mechanism, and implications. Crit. Rev. Oral Biol. Med. 14 (2), 100-114. https://doi.org/10.1177/154411130301400204

Luster, T., Dubow, E., 1992. Home environment and maternal intelligence as predictors of verbal intelligence: A comparison of preschool and school-age children. Merrill-Palmer Q. (1982-), 151-175

Malin, A.J., Riddell, J., McCague, H., Till, C., 2018. Fluoride exposure and thyroid function among adults living in Canada: Effect modification by iodine status. Environ. Int. 21 (Pt 1), 667–674. https://doi.org/10.1016/j.envint.2018.09.026

Martinez-Mier, E.A., Cury, J.A., Heilman, J.R., Katz, B.P., Levy, S.M., Li, Y., Maguire, A., Margineda, J., O'Mullane, D., Phantumvanit, P., Soto-Rojas, A.E., Stookey, G.K., Villa, A., Wefel, J.S., Whelton, H., Whitford, G.M., Zero, D.T., Zhang, W., Zohouri, V., 2011. Development of gold standard ion-selective electrode-based methods for fluoride analysis. Caries Res. 45 (1), 3-12. https://doi.org/10.1159/000321657

Mullenix, P.J., Denbesten, P.K., Schunior, A., Kernan, W.J., 1995. Neurotoxicity of sodium fluoride in rats. Neurotoxicol. Teratol. 17 (2), 169-177. https://doi.org/10.1016/0892-0362(94)00070-t

National Toxicology Program, 2020. Revised draft NTP monograph on the systematic review of fluoride exposure and neurodevelopmental and cognitive health effects. National Institute of Environmental Health Sciences (Research Triangle Park, NC).

Perer, F., Herbstman, J., 2011. Prenatal environmental exposures, epigenetics, and disease. Reprod. Toxicol. 31 (3), 363–73. https://doi.org/10.1016/j.reprotox.2010.12.055

Rice, D., Barone Jr., S., 2000. Critical periods of vulnerability for the developing nervous system: Evidence from humans and animal models. Environ. Health Perspect. 10 (Suppl 3), 511-533. https://doi.org/10.1289/ehp.00108s3511

Ris, M.D., Dietrich, K.N., Succop, P.A., Berger, O.G., 2004. Early exposure to lead and neuropsychological outcome in adolescence. J. Int. Neuropsychol. Soc. 10 (2), 261-270.

https://doi.org/10.1017/S1355617704102154

Rose G. Sick individuals and sick populations. *Int J Epidemiol*. 1985;14(1):32-38.
Sanchez, B.N., Hu, H., Litman, H.J., Tellez-Rojo, M.M., 2011. Statistical methods to study timing of vulnerability with sparsely sampled data on environmental toxicants. Environ. Health Perspect. 119 (3), 409-415. https://doi.org/10.1289/ehp.1002453

Seraj, B., Shahrabi, M., Shadfar, M., Ahmadi, R., Fallahzadeh, M., Eslamlu, H.F., Kharazifard, M.J., 2012. Effect of high water fluoride concentration on the intellectual development of children in Makoo/Iran. J. Dent. (Tehran). 9 (3), 221–229. PMID: 23119131

Singh, G., Singh, V., Sobolewski, M., Cory-Slechta, D.A., Schneider, J.S., 2018. Sex-dependent effects of developmental lead exposure on the brain. Front. Genet. 9, 89. https://doi.org/10.3389/fgene.2018.00089

Stacy, S.L., Papandonatos, G.D., Calafat, A.M., Chen, A., Yolton, K., Lanphear, B.P., Braun, J.M., 2017. Early life bisphenol A exposure and neurobehavior at 8 years of age: identifying windows of heightened vulnerability. Environ. Int. 107, 258–265. https://doi.org/10.1016/j.envint.2017.07.021

Takahashi R, et al. (2017) Fluoride supplementation (with tablets, drops, lozenges or chewing gum) in pregnant women for preventing dental caries in the primary teeth of their children. Cochrane Database Syst Rev 10:CD011850 doi:10.1002/14651858.CD011850.pub2

Ten Cate JM, Buzalaf MAR. Fluoride Mode of Action: Once There Was an Observant Dentist . . . J Dent Res. 2019 Jul;98(7):725-730. doi: 10.1177/0022034519831604. PMID: 31219410.
Thissen, D., Steinberg, L., Kuang, D., 2002. Quick and easy implementation of the benjamini-hochberg procedure for controlling the false positive rate in multiple comparisons. J. Educ. Behav. Stat. 27 (1), 77–83. https://doi.org/10.3102/10769986027001077

Till, C., Green, R., Flora, D., Hornung, R., Martinez-Mier, E.A., Blazer, M., Farmus, L., Ayotte, P., Muckle, G., Lanphear, B.P., 2020. Fluoride exposure from infant formula and child IQ in a Canadian birth cohort. Environ. Int. 135, 105315. https://doi.org/10.1016/j.envint.2019.105315

Till, C., Green, R., Grundy, J.G., Hornung, R., Neufeld, R., Martinez-Mier, E.A., Ayotte, P., Muckle, G., Lanphear, B.P., 2018. Community water fluoridation and urinary fluoride concentrations in a national sample of pregnant women in Canada. Environ. Health Perspect. 126 (10), 107001. https://doi.org/10.1289/EHP3546

Torres-Rojas, C., Jones, B.C., 2018. Sex differences in neurotoxicogenetics. Front. Genet., 9 (196), https://doi.org/10.3389/fgene.2018.00196

United States Environmental Protection Agency (EPA), 2010. Fluoride: Relative Source Contribution Analysis. Vol. 820-R-10-0.

Valdez Jiménez, L., López Guzmán, O.D. , Cervantes Flores, M., Costilla-Salazar, R., Calderón Hernández, J., Alcaraz Contreras, Y., Rocha-Amador, D.O., 2017. In utero exposure to fluoride

and cognitive development delay in infants. Neurotoxicology. 59, 65-70. https://doi.org/10.1016/j.neuro.2016.12.011

Vuong, A.M., Braun, J.M., Yolton, K., Xie, C., Webster, G.M., Sjödin, A., Dietrich, K.M., Lanphear, B.P., Chen, A., 2017. Prenatal and postnatal polybrominated diphenyl ether exposure and visual spatial abilities in children. Environ. Res. 153, 83–92. https://doi.org/10.1016/j.envres.2016.11.020

Wasserman, G.A., Liu, X., Lolacono, N.J., Factor-Litvak, P., Kline, J.K., Popovac, D., Morina, N., Musabegovic, A., Vrenezi, N., Capuni-Paracka, S., Preteni-Redjepi, E., Hadzialjevic, S., Slakovich, V., Graziano, J.H., 1997. Lead exposure and intelligence in 7-year-old children: the Yugoslavia Prospective Study. Environ. Health Perspect. 105 (9), 956-962. https://doi.org/10.1289/ehp.97105956

Wechsler, D., 2002.Wechsler Preschool and Primary Scale of Intelligence – Third Edition: Canadian. Toronto, ON, Canada: Pearson Clinical Assessment.

Xiang, Q., Liang, Y., Chen, L., Wang, C., Chen, B., Chen, X., Zhou, M., 2003. Effect of fluoride in drinking water on children's intelligence. Fluoride. 36 (2), 84–94.

Xu, K., An, N., Huang, H., Duan, L., Ma, J., Ding, J., He, T., Zhu, J., Li, Z., Cheng, X., Zhou, G., Ba, Y., 2020. Fluoride exposure and intelligence in school-age children: evidence from different windows of exposure susceptibility. BMC Public Health. 20 (1), 1657. https://doi.org/10.1186/s12889-020-09765-4

Zhang, H., Yolton, K., Webster, G.M., Ye, X., Calafat, A.M., Dietrich, K.N., Xu, Y., Xi, C., Braun, J.M., Lanphear, B.P., Chen, A., 2017. Prenatal and childhood perfluoroalkyl substances exposures and children's reading skills at ages 5 and 8 years. Environ. Int. 111, 224–231. https://doi.org/10.1016/j.envint.2017.11.031

Zohoori, F.V., Omid, N., Sanderson, R.A., Valentine, R.A., Maguire, A., 2018. Fluoride retention in infants living in fluoridated and non-fluoridated areas: Effects of weaning. Br. J. Nutr. 121 (1), 74-81. https://doi.org/10.1017/S0007114518003008

Highlights

- The association between fluoride and performance IQ (PIQ) significantly differed across prenatal, infancy, and childhood exposure windows collapsing across child sex (p = .001).
- The strongest association between fluoride and PIQ was during the prenatal window, B = -2.36, 95% CI: -3.63, -1.08; the association was also significant during infancy, B = -2.11, 95% CI: -3.45, -0.76, but weaker in childhood, B = -1.51, 95% CI: -2.90, -0.12.
- Within sex, the association between fluoride and PIQ significantly differed across the three exposure windows (p = .01); among boys, the strongest association was during the prenatal window, B = -3.01, 95% CI: -4.60, -1.42, whereas among girls, the strongest association was during infancy, B = -2.71, 95% CI: -4.59, -0.83.
- Full-scale IQ estimates were weaker than PIQ estimates for every window. Fluoride was not significantly associated with Verbal IQ across any exposure window.

The authors declare that there are no conflicts of interest to share, either financial or personal.

Declaration of interests

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

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: