

Journal Pre-proof



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>.

This is a PDF file of an article that has undergone enhancements after acceptance, such as the addition of a cover page and metadata, and formatting for readability, but it is not yet the definitive version of record. This version will undergo additional copyediting, typesetting and review before it is published in its final form, but we are providing this version to give early visibility of the article. Please note that, during the production process, errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

© 2021 The Author(s). Published by Elsevier Inc.

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 Farmus¹, MA, Christine Till¹, PhD, Rivka Green¹, MA, Richard Hornung, PhD, E. Angeles Martinez-Mier², DDS, PhD, Pierre Ayotte^{3,4}, PhD, Gina Muckle^{3,5}, PhD, Bruce Lanphear^{6,7}, MD, David Flora¹, 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.

31 **Abstract**

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

34

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

37

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

47

48 Results: The association between fluoride and performance IQ (PIQ) significantly differed across
49 prenatal, infancy, and childhood exposure windows collapsing across child sex ($p = .001$). The
50 strongest association between fluoride and PIQ was during the prenatal window, $B = -2.36$, 95%
51 CI: -3.63, -1.08; the association was also significant during infancy, $B = -2.11$, 95% CI: -3.45, -
52 0.76, but weaker in childhood, $B = -1.51$, 95% CI: -2.90, -0.12. Within sex, the association
53 between fluoride and PIQ significantly differed across the three exposure windows ($p = .01$);
54 among boys, the strongest association was during the prenatal window, $B = -3.01$, 95% CI: -4.60,
55 -1.42, whereas among girls, the strongest association was during infancy, $B = -2.71$, 95% CI: -
56 4.59, -0.83. Full-scale IQ estimates were weaker than PIQ estimates for every window. Fluoride
57 was not significantly associated with Verbal IQ across any exposure window.

58

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

62 **Critical Windows of Fluoride Neurotoxicity in Canadian Children**

63 Fluoride has been associated with IQ deficits at water fluoride concentrations >1.2 mg/L
64 (Choi, Sun, Zhang, & Grandjean, 2012; Dong, Yao, Chen, Li, & Shi, 2018; Grandjean, 2019;
65 National Toxicology Program, 2020; Seraj et al., 2012; Xiang et al., 2003; Valdez Jiménez et al.,
66 2017). Early-life exposure to optimal levels (i.e., 0.7 mg/L) of fluoride – as defined by levels
67 sufficient to protect against tooth decay and minimize against dental fluorosis – has also been
68 associated with diminished cognitive abilities in prospective studies of children (Bashash et al.,
69 2017; Green et al., 2019; Till et al., 2020). Drinking water is a main source of fluoride for
70 pregnant women (Till et al., 2018) and young children (de la Cruz, Rozier, & Bawden, 2008;
71 Green et al., 2020) living in communities with water fluoridation (US EPA, 2010). Therefore,
72 associations between fluoride in pregnancy and child outcomes may be conflated by continuous
73 exposure to fluoride over the lifespan. Few human studies have examined the developmental
74 period of greatest vulnerability to fluoride neurotoxicity (Xu et al., 2020).

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

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

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

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

98 To overcome these limitations, we adapted an approach from Sanchez, Hu, Litman, and
99 Tellez-Rojo (2011) using generalized estimating equations (GEE) for repeated exposure
100 variables and a single outcome measure of IQ score. Each fluoride exposure measure is treated as
101 a window (i.e., a particular exposure time). We incorporated interactions to estimate sex-specific
102 associations with IQ based on our prior finding that boys may be more susceptible to prenatal
103 fluoride exposure than girls (Green et al., 2019), a recent review of sex effects in animal and
104 human fluoride studies (Green, Rubenstein, Popoli, Capulong, & Till, 2020), and literature on
105 other neurotoxins suggesting interactions between sex and exposure timing (Comfort & Re,
106 2017; Kern et al., 2017; Torres-Rojas & Jones, 2018).

107

108

109 **Methods**

110 ***Study Participants***

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

128 ***Fluoride Exposure Measures***

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

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

$$133 \quad P_c = P_i \left[\frac{SG_m - 1}{SG_i - 1} \right],$$

134 where P_c is the SG-adjusted fluoride concentration, P_i is the observed fluoride concentration, SG_i
 135 is the specific gravity of the i^{th} urine sample, and SG_m is the median SG for the cohort (Duty et
 136 al., 2005). Of the 593 women with at least one valid measure of MUF, 526 (88.3%) had a urine
 137 sample collected for all three trimesters. Our prenatal fluoride exposure variable was calculated
 138 by averaging the trimester-specific MUF measures. We calculated average MUF levels only
 139 when valid samples were available for all three trimesters to strengthen reliability of the measure
 140 (Till et al., 2018). Because GEE can incorporate missing data, we retained the 67 women (of the
 141 $N = 596$ dyads) for whom an average MUF value was missing; for these participants, trimester
 142 MUF measures were used in preliminary trimester analyses, and we included their data on
 143 covariates or exposures assessed at other time points in the primary analysis. Urinary fluoride
 144 concentrations were analyzed using a modification of the hexamethydisiloxane (Sigma Chemical
 145 Co., USA) micro-diffusion procedure (Martinez-Mier et al., 2011).

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

$$148 \quad IFI = \text{water fluoride (mg/L)} \times 0.8 \text{ L/day} \times (1 - (\text{exclusive breastfeeding} / 11.99)),$$

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

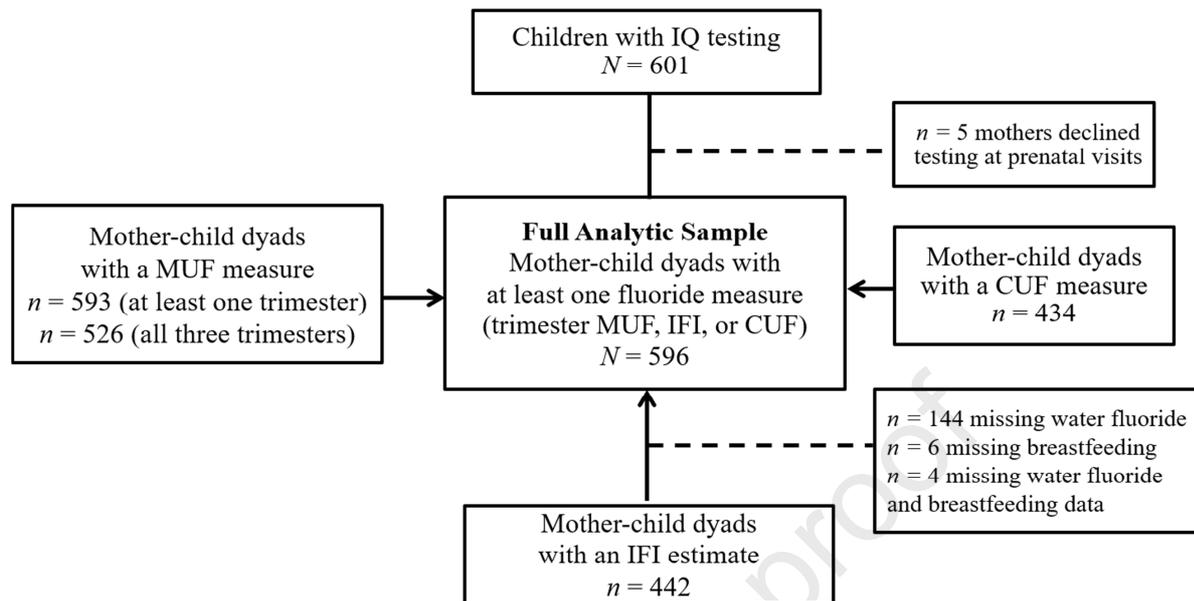
154 associated with postal codes matching each mother's residence during the third trimester of her
155 pregnancy. The number of months of exclusive breastfeeding was recoded so that mothers who
156 reported exclusive breastfeeding between 12 and 24 months were assigned a value of 12. Thus,
157 formula-fed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

165 *Child urinary fluoride (CUF)*. We measured CUF as an estimate of childhood exposure
166 using a spot urine sample taken when children were between 1.86 and 4.40 years old ($M = 3.25$,
167 $SD = 0.54$), also adjusted for specific gravity ($n = 437$) (Figure 1).

168

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

170



171

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

173

174 *Child Intellectual Abilities*

175 Trained research assistants assessed children's intellectual abilities at the age of 3 to 4
 176 years using the Wechsler Preschool and Primary Scale of Intelligence-III (WPPSI-III; Canadian
 177 norms; Wechsler, 2002). Outcomes included Performance IQ (PIQ), a measure of nonverbal
 178 reasoning, Verbal IQ (VIQ), a measure of verbal reasoning and comprehension, and Full-Scale
 179 IQ (FSIQ), a measure of overall intellectual ability. Examiners administered the WPPSI between
 180 2012 and 2015 prior to proposing our fluoride research; examiners are therefore considered
 181 blinded to exposure status.

182 *Covariates*

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

186 Caucasian), mother-reported exposure to second-hand smoke (yes/no) while pregnant, and a
187 continuous measure of quality of home environment using the Home Observation for
188 Measurement of the Environment (HOME) - Revised Edition (Caldwell & Bradley, 1984) at the
189 3-4 year-old home visit. We also included child age (in months) at urine sampling to control for
190 age-related differences in CUF. We did not include child age at IQ testing as the WPPSI-III is
191 age-normed in 2-month intervals. We also did not include city as a covariate in our GEE model
192 based on its redundancy with water fluoride that is used to calculate IFI. There was no
193 collinearity among the covariates or exposures included.

194

195 *Statistical Analysis*

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

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

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

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

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

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

227 Results

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

231 593 mother-child pairs with at least one MUF value, the mean child age at intellectual testing
 232 was 3.4 years (SD = 0.3); girls comprised 51.1% of the sample ($n = 303$). The average Full Scale
 233 IQ (FSIQ) score was 106.6 (SD = 13.7) for the study sample, which is consistent for a
 234 predominantly middle-to-upper class and educated group. Table 2 shows the descriptive
 235 statistics and correlations among fluoride exposure variables.

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

Characteristic	Samples				
	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 (13.7)
Verbal IQ	109.2 (13.7)	109.2 (13.6)	109.5 (13.3)	109.8 (13.6)	110.1 (13.7)

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

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

	N	Median	M (SD)	Range	Pearson correlations					
					MUF					
					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.

238

239 **1. Overall effects of exposure windows**

240 The association between MUF and IQ scores did not differ significantly across trimesters

241 [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).

242 Thus, for the remaining analyses, we used average MUF as a single prenatal exposure. We

243 compared average MUF, IFI, and CUF effects to examine the unique associations of prenatal,

244 infancy, and childhood exposures on IQ scores. Table 3 shows the associations between

245 *standardized* fluoride exposures and unstandardized IQ scores whereas Table 4 shows the

246 *unstandardized* coefficients per 0.5 mg/L MUF and CUF and per 0.1 mg IFI/day (to facilitate

247 comparison of average MUF, IFI, and CUF associations with IQ scores). The standardized

248 coefficient indicates the change in the dependent variable (i.e. age-normed IQ score) per one

249 standard deviation (SD) in the fluoride exposure variable; thus, a standardized coefficient of -1.9
250 means that the IQ score decreases by 1.9 points per one SD in the exposure variable. An
251 unstandardized coefficient represents the amount by which the dependent variable changes per
252 one unit change in the fluoride exposure (i.e. 1 mg/L) variable keeping other variables constant.
253 Combining across boys and girls, the two-way interaction between fluoride and time was
254 statistically significant for PIQ ($\chi^2(3) = 18.78, p < .001$) and VIQ ($\chi^2(3) = 8.28, p = .04$), but not
255 for FSIQ ($\chi^2(3) = 4.36, p = .23$). Controlling the FDR, there were significant negative effects of
256 standardized fluoride exposures for overall MUF and overall IFI on PIQ ($B = -2.36, 95\% \text{ CI: } -$
257 $3.63, -1.08; B = -2.11, 95\% \text{ CI: } -3.45, -0.76$, respectively). The associations between
258 standardized fluoride exposures and IQ scores are visualized in Figure 2.

259 ***2. Effects of exposure windows by sex***

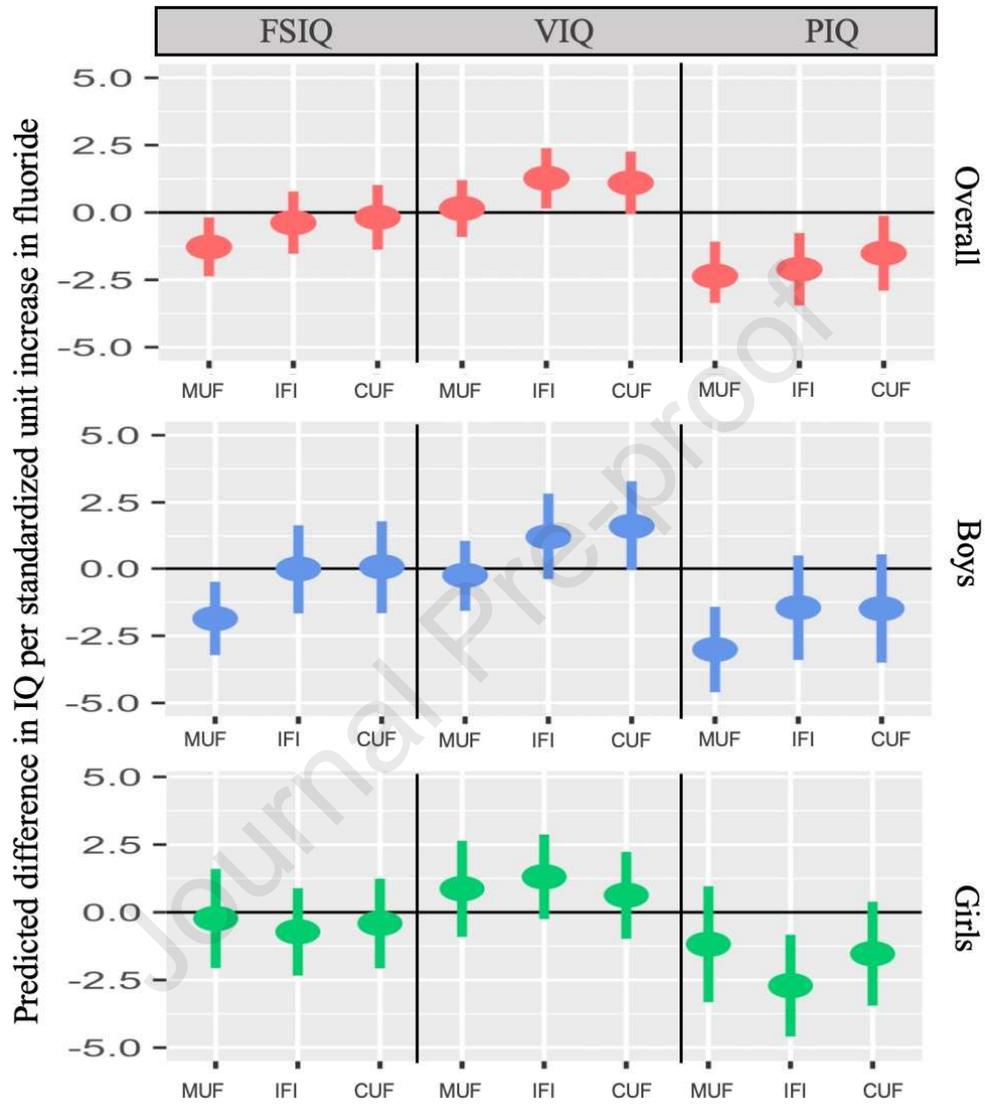
260 The three-way interaction between fluoride, child sex, and time was not statistically significant
261 for FSIQ ($\chi^2(2) = 2.74, p = .25$), PIQ ($\chi^2(2) = 2.72, p = .26$), or VIQ ($\chi^2(2) = 1.92, p = .38$).
262 However, among boys, the association between fluoride and PIQ significantly differed across
263 windows ($\chi^2(3) = 11.92, p = .01$), but not for FSIQ ($\chi^2(3) = 5.83, p = .12$) or VIQ ($\chi^2(3) = 5.80, p$
264 $= .12$) (Table 3). Similarly, among girls, the effect of fluoride exposure significantly differed
265 across windows for PIQ ($\chi^2(3) = 11.69, p = .01$), but not for FSIQ ($\chi^2(3) = 1.15, p = .77$) or VIQ
266 ($\chi^2(3) = 3.63, p = .30$) (Table 3). Probing the time (i.e. exposure windows) interaction within
267 boys and girls, significant effects of standardized fluoride exposures after controlling the FDR
268 were as follows: among boys, MUF had stronger negative associations with FSIQ ($B = -1.86,$
269 $95\% \text{ CI: } -3.22, -0.49$) and PIQ ($B = -3.01, 95\% \text{ CI: } -4.60, -1.42$) than IFI and CUF. Among girls,
270 IFI had a stronger association with PIQ ($B = -2.71, 95\% \text{ CI: } -4.59, -0.83$) than MUF and CUF
271 (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 (<i>n</i> = 291)	Females (<i>n</i> = 305)	Overall (<i>N</i> = 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)
<i>p_{int}</i>	.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)
<i>p_{int}</i>	.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)
<i>p_{int}</i>	.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).

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



275

276

277

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 (<i>n</i> = 291) ^{a,b}	Females (<i>n</i> = 305) ^{a,b}	Overall Participants (<i>N</i> = 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)
<i>p_{int}</i>	.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)
<i>p_{int}</i>	.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)
<i>p_{int}</i>	.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

278 3. Sensitivity analyses

279 Removal of six mother-child dyads that were influential (as per DFBETA) on the sex-
 280 specific estimates of fluoride exposures on FSIQ made the negative association between MUF
 281 and FSIQ among boys weaker and non-significant after adjustment for the FDR (*B* = -1.22, 95%
 282 CI: -2.41, -0.04). All other prenatal and postnatal sex-specific and overall effects remained
 283 significant (or non-significant) with removal of influential dyads for FSIQ, PIQ, or VIQ, adjusted
 284 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 (<i>n</i> = 288 ^a)	Females (<i>n</i> = 302 ^b)	Overall (<i>N</i> = 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)
<i>p</i> _{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)
<i>p</i> _{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)
<i>p</i> _{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).

^aFSIQ *n* = 287.

^bPIQ *n* = 301.

^cFSIQ and PIQ *n* = 589.

285

286

287 Discussion

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

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

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

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

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

334 Fluoride may target the hypothalamic-pituitary-thyroid axis (Malin, Riddell, McCague, & Till,
335 2018; Bai, Huang, Wang, & Guo, 2020; Du et al., 2020), though we are not aware of any
336 epidemiologic studies that have measured fluoride-induced changes in thyroid and sex steroid
337 hormone levels in pregnancy. In addition, the timing of neurologic development of specific brain
338 regions differs between the sexes (Perera & Herbstman, 2011; Lenroot et al., 2007), which might
339 increase susceptibility of fluoride exposure during a particular developmental window. In the
340 Mullenix et al. (1995) rat study, fluoride concentrations differed by sex in some brain structures
341 (e.g. hippocampus), which could also contribute to sexually dimorphic changes in behaviour. See
342 Green et al. (2020) for further discussion of mechanisms that may contribute to sex-based
343 differences of fluoride neurotoxicity.

344 The difference in magnitudes and divergence in the direction of some of the associations
345 between verbal and non-verbal intellectual abilities may have several progenitors that reflect
346 these distinct types of cognitive ability. While we would not expect higher fluoride intake in
347 infancy to be beneficial to VIQ, we would expect it to be detrimental to non-verbal (PIQ)
348 intelligence. Fluid (i.e. non-verbal) abilities are more biologically determined whereas
349 crystallized intelligence (i.e. VIQ) is more likely to be shaped by experience (Ashbury et al.,
350 2005; Luster & Dubow, 1992). Past studies have suggested that prenatal and early-life exposure
351 to some neurotoxicants, like lead, is more strongly associated with non-verbal intelligence than
352 verbal intelligence in young children (Bellinger et al. 1991; Dietrich et al. 1991, 1993; Factor-
353 Litvak et al. 1999; Jusko et al., 2008; Wasserman et al. 1997). Consistent with this pattern, our
354 findings showed a decrement of IFI to PIQ (statistically significant *decrease* of 1.6 points per 0.1
355 mg/day), but not VIQ (non-significant *increase* of 1.0 points per 0.1 mg/day).

356 Our current results are consistent with and extend our previous findings. The effect of
357 MUF on FSIQ was significant for boys (2.45-point decrement in FSIQ per 0.5 mg/L increase in
358 MUF; Table 2), reproducing our prior work (Green et al., 2019) in which we found a 2.2-point
359 decrement in FSIQ per 0.5 mg/L increase in MUF. We note that the current analysis did not
360 include city in the analysis because fluoride intake from formula (i.e. IFI) is a function of
361 residential water fluoride concentration and was therefore deemed redundant. Our finding of a
362 2.1-point decrement in PIQ per 0.5 mg/L increase in IFI ($B = -2.11$, 95% CI: -3.45, -0.76) was
363 also consistent with our prior finding that infancy is a critical period for non-verbal intelligence
364 in boys and girls (Till et al., 2020). Our current results extend our prior work by showing that
365 regardless of child sex or the exclusion of influential dyads, the associations of fluoride on PIQ
366 differs across exposure windows. However, exposures do not significantly associate with IQ
367 outcomes once city is controlled and FDR is applied.

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

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

379 effects, as reflected by narrow confidence intervals. We used the FDR method to guard against
380 false positive conclusions due to multiple comparisons, even though multiplicity control is rarely
381 imposed when evaluating multiple predictors in regression-based models (Cribbie, 2017). We
382 adjusted for numerous potential confounders and avoided problems with collinearity among
383 critical windows of fluoride toxicity by using GEE. Although several epidemiological studies
384 have applied GEE to test critical windows of environmental contaminants on neurobehavioral
385 outcomes (Jackson-Browne et al., 2018; Stacy et al., 2017; Vuong et al., 2017; Zhang et al.,
386 2017), this is the first study to use GEE to model critical windows of fluoride toxicity.

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

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

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

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

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

440

441

442

443 Supplemental Materials

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

	Males (<i>n</i> = 291)	Females (<i>n</i> = 305)	Overall (<i>N</i> = 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)
<i>p_{int}</i>	.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)
<i>p_{int}</i>	.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)
<i>p_{int}</i>	.49	.90	.53

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

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., Hidioglou, 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., Wateraux, 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](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-Miszczuk, 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 disproportionately 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](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., Lolocono, 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.

Journal Pre-proof

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:

Journal Pre-proof