URINARY FLUORIDE LEVELS AMONG CANADIANS AND THE ASSOCIATION BETWEEN FLUORIDE EXPOSURE AND ATTENTION DEFICIT HYPERACTIVITY DISORDER

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ABSTRACT

Fluoride has been identified as a developmental neurotoxin – a claim that is uncontested at high exposure levels, but remains debated at low exposure levels. In Canada, 39% of municipalities add fluoride to their drinking water at a recommended level of 0.7ppm. Exposure to fluoride has been linked with increased prevalence of attention deficit hyperactivity disorder (ADHD) in the United States and symptoms of inattention in Mexican children. This dissertation consists of one study examining the association between fluoride exposure and symptoms of ADHD in youth, and a second study examining the predictors of an individual's level of urinary fluoride. Study 1 used cross-sectional data on youth 6 to 17 years of age from the Canadian Health Measures Survey (CHMS, Cycles 2 and 3). Urinary fluoride concentrations were measured in spot samples and adjusted for specific gravity (UF_{SG}, n=1877) and water fluoride concentrations were measured in tap water samples (n=980). We used logistic regression to test the association between fluoride exposure and ADHD diagnosis and linear regression to examine the relationship between fluoride exposure and the hyperactive-inattention subscale score on the Strengths and Difficulties Questionnaire (SDQ). UF_{SG} did not predict ADHD diagnosis or SDQ score, whereas a 1 mg/L increase in tap water fluoride level was associated with 6.1 times higher odds of an ADHD diagnosis (95% CI = 1.60, 22.8) and a 1.5 increase in SDQ score for adolescents. Study 2 used data from Cycle 3 of the CHMS to examine factors that influence urinary fluoride levels in a sample of 1629 individuals aged 3 to 79 years. In the linear regression models, tap water fluoride level, community water fluoridation status, age, sex, BMI, smoking allowed in the home, and tea consumption were significant predictors of UF_{SG}. Community water fluoridation and tap water fluoride were associated with higher levels of fluoride excretion and with an increased risk of ADHD symptoms and diagnosis among Canadian youth. These findings highlight the need to identify subgroups who may have especially high levels of exposure and to further investigate the potential for fluoride-mediated developmental neurotoxicity in populations with community water fluoridation.

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Chapter One: Introduction

Environmental Exposures and Neurodevelopmental Disorders

The developing brain is particularly vulnerable to environmental toxins, including known neurotoxins such as mercury, polychlorinated biphenyls (PCBs), and lead (Eubig, Aguiar, & Schantz, 2010; Jacobson & Jacobson, 1996; Lanphear, 2015; Sagiv et al., 2010; Sagiv, Thurston, Bellinger, Amarasiriwardena, & Korrick, 2012). The effects of early exposure to environmental toxins appear to be longlasting. For example, lead exposure in early childhood has been linked to reduced school performance and behavioural difficulties in adolescence and adulthood (Fergusson, Boden, & Horwood, 2008; Wright et al., 2008; Zhang et al., 2013). A comprehensive report from the National Research Council (2006) raised concerns about potential adverse neurobehavioural effects of fluoride in drinking water and concluded that additional research is warranted, particularly for early life exposure. A meta-analysis found that children living in high-fluoride areas, mainly in China where fluoride exists as a natural contaminant, had substantially lower intellectual ability than those who lived in low-fluoride areas (Choi, Sun, Zhang, & Grandjean, 2012). In a review, Grandjean and Landrigan (2014) listed fluoride as a developmental neurotoxin – a claim that is uncontested at high exposure levels, but remains debated at low exposure levels. Most recently, a draft report by the National Toxicology Program (NTP; 2019) concluded that there is a moderate level of evidence that exposure to fluoride is associated with cognitive neurodevelopmental effects in children. This NTP report also stated that there are few available studies that evaluate whether fluoride exposure in humans is associated with other neurodevelopmental effects beyond IQ. The goal of the current dissertation was to better understand the levels of exposure in Canadian individuals and to examine the relationship between fluoride exposure and Attention Deficit Hyperactivity Disorder (ADHD).

Development of ADHD

ADHD is a neurodevelopmental disorder characterized by impairments in attention, organization, and hyperactivity-impulsivity. The newest edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5; American Psychiatric Association (2013) describes three subtypes of ADHD: predominantly inattentive, predominantly hyperactive/impulsive, and combined presentation. ADHD is one of the most common mental health conditions in children as it occurs in approximately 3% to 10% of the childhood population across countries (Davids & Gastpar, 2005; Biederman, 2005). The estimated prevalence of ADHD in adults ranges from 3 to 5% (Biederman, 2005). A recent meta-analysis of 175 studies found an overall point prevalence rate of 7.2% for ADHD across the lifespan (Thomas, Sanders, Doust, Beller, & Glasziou, 2015). Boys are three times more likely to develop ADHD compared to girls (Barkley, 2014). To meet diagnostic criteria for ADHD, symptoms must be present before 12 years of age and in two different settings (American Psychiatric Association, 2013; Friedman & Rapoport, 2015). ADHD is most often identified during elementary school years as difficulties with inattention become more prominent and impairing (American Psychiatric Association, 2013). The course of the disorder is persisent, with approximately 65% to 75% of individuals diagnosed in childhood continuing to meet criteria for the disorder through adolescence (Faraone, Biederman, & Mick, 2006; Davids & Gastpar, 2005) and over half continuing into adulthood (Davids & Gastpar, 2005). A family history of ADHD, psychiatric comorbidity, and psychosocial adversity all predict greater likelihood that the disorder will persist into adulthood (Biederman, 2005). Inattentive symptoms tend to persist from childhood through adolescence, whereas children with high levels of hyperactive symptoms tend to experience a reduction in these symptoms over time (Holbrook et al., 2016). In Canada, ADHD is more frequently

diagnosed by general practitioners (such as a family doctor) followed by either psychiatrists or pediatricians depending on the province (Vasiliadis et al., 2017).

Recent data suggest that the prevalence of ADHD has been increasing over time. A 5.4-fold increase in the prevalence of ADHD diagnosis in children was observed in the United States between 1979 and 1996 (Kelleher, McInerny, Gardner, Childs, & Wasserman, 2000). Similarly, the estimated prevalence of diagnosed ADHD in US children and adolescents increased from 6.1% in 1997-1998 to 10.2% in 2015-2016 (Xu, Strathearn, Liu, Yang, & Bao, 2018). A Canadian study using data from the National Longitudinal Survey on Children and Youth found that the prevalance of ADHD in school-age children doubled between 1994 and 2007 (Brault & Lacourse, 2012).

Although the cause of ADHD is currently unknown, numerous genetic and environmental factors have been linked to an increased risk of developing ADHD. The heritability of ADHD has been estimated to range from 30% to as high as 88% (Boomsma et al., 2010; Larsson et al., 2013; Larsson, Chang, D'Onofrio, & Lichtenstein, 2014; Larsson, Dilshad, Lichtenstein, & Barker, 2011; Lichtenstein, Carlström, Råstam, Gillberg, & Anckarsäter, 2010; Reiersen, Constantino, Grimmer, Martin, & Todd, 2008; van den Berg, Willemsen, de Geus, & Boomsma, 2006). Earlier heritability studies identified a two- to eight-fold increase in the risk for ADHD in children whose parents and siblings have ADHD (Biederman, 2005). In terms of environmental risk factors, a birth weight of less than 1,500 grams is associated with a two- to three-fold risk for ADHD (American Psychiatric Association, 2013). Smoking and alcohol use during pregnacy, diet, infections (e.g., encephalitis), and child abuse or neglect have all been linked to an increase risk of ADHD (American Psychiatric Association, 2013). A study using Rutter's indicators of adversity (i.e., severe marital discord, low social class, large family size, paternal criminality,

maternal mental disorder, foster care placement) found that the risk of an ADHD diagnosis increased significantly with each increase in the number of adversity indicators (Biederman et al., 1995).

Childhood exposure to neurotoxins has also been associated with an increased risk of ADHD, including Bisphenol A (BPA), lead, polybrominated diphenyl ethers (PBDEs), and fluoride. In a cross-sectional study, Tewar and colleagues (2016) reported that higher levels of urinary BPA were associated with a diagnosis of ADHD in children ages 8 to 15 from the United States. A meta-analysis on the association between lead exposure and ADHD revealed a small correlation between inattention symptoms and lead exposure (r = .16) and a similar correlation between hyperactivity/impulsivity symptoms and lead exposure (r = .13) across 33 studies and a total sample size 10,232 children and adolescents (Goodlad, Marcus, & Fulton, 2013). An updated systematic review on the association between lead and ADHD was completed recently by Donzelli and colleagues (2019). They found that 12 out of the 17 studies included showed a positive association between lead exposure and ADHD diagnosis, even at low levels of exposure (Donzelli et al., 2019).

Mixed findings have also been found between exposure to PBDEs and ADHD. Zhang et al. (2017) found that each 10-fold increase in serum PBDE concentration was associated with a 3.5-point increase in externalizing problems scores on the BASC-2 (Behavioral Assessment System for Children-2). Sagiv and colleagues (2015) reported associations of higher prenatal serum concentrations of PBDEs with decrements in attention on the Conners CPT II task (Conners et al., 2000), as well as with increased ADHD Index scores at both 9 and 12 years of age. Further, they found that higher prenatal PBDE exposure was associated with higher parent reported ADHD symptoms for 9 year-old children but not 12 year-old children. However, a

systematic review by Lam and colleagues (2017) commented that based on the heterogeneity of association estimates reported by a small number of studies and the fact that chance, bias, and confounding variables could not be ruled out, the current level of evidence to support an association between PBDEs and ADHD is considered to be low at this time.

Only two studies have examined the association between fluoride exposure and ADHD (Maliln & Till, 2015; Bashash et al., 2018). The first study examined the prevalence of ADHD among youth in the United States using data from the CDC (Malin & Till, 2015). Results showed that state-level prevalence of artificial water fluoridation in 1992 predicted state-level prevalence of ADHD in 2003, 2007, and 2011, controlling for socioeconomic status (Malin & Till, 2015). In particular, each 1% increase in artificial fluoridation prevalence in 1992 was associated with approximately 67,000 to 131,000 additional ADHD diagnoses from 2003 to 2011. The second study reported an association between fluoride exposure during pregnancy and more inattentive symptoms and total ADHD symptoms in Mexican children aged 6 to 12 years (Bashash et al., 2018). Participants in this study were exposed to fluoride added to salt at 250 ppm and to varying levels of naturally occurring fluoride in drinking water. More research is needed to understand the potential association between fluoride exposure and ADHD among Canadians exposed to fluoridated drinking water.

Artificial Water Fluoridation in Canada

While fluoride can be a natural contaminant in water and soil, fluoridation refers to the controlled practice of adding fluoridation chemicals (usually hexafluorosilicic acid) to drinking water for the purpose of preventing tooth decay (Jones, Riley, Couper & Dwyer, 1999). In 2009, approximately 45% of Canadians received artificially fluoridated water (Rabb-Waytowich, 2009); today, this prevalence has decreased to about 39% as some municipalities opt against

community water fluoridation (Public Health Agency of Canada, 2017). In the United States, 73.9% of the public water supplies are artificially fluoridated, compared with only 3% of European water supplies (Centers for Disease Control and Prevention, 2014). In Canada, each municipality decides whether to add fluoride to its water supplies, with the federal government setting the guidelines regarding the optimal concentration (0.7 mg/L) and maximum acceptable level (1.5 mg/L; Health Canada, 2010a). Within Canada, fluoridation varies considerably across the provinces. The lowest proportion of communities receiving fluoridated water are in the Yukon (0% of communities adding fluoride), followed by Newfoundland and Labrador (1.5%), British Columbia (1.2%), New Brunswick (1.2%) and Quebec (2.5%; Health Canada, 2017). Ontario has the highest proportion of communities receiving fluoridated water (71%), followed by Manitoba (69%) and the Northwest Territories (65%; Health Canada, 2017).

The optimal level of fluoride was determined based on studies conducted in the 1940s that examined the association between dental caries (cavities) and dental fluorosis (discolouration of tooth enamel) at different fluoride concentrations in drinking water (Health Canada, 2010a). However, an optimal concentration of fluoride has been disputed since it was first introduced into drinking water because of concerns related to differences in dose resulting from the indiscriminant delivery of fluoride through drinking water. The optimal concentration for fluoride does not consider individual variables that contribute to total intake, such as age, weight, and water consumption habits. These variables result in an uncontrolled dose of fluoride whereby young children, people with low BMI, kidney dysfunction, or those who drink more water (or have a higher exposure per kg of body weight) may be receiving a higher than recommended dose of fluoride per day.

Debate about Water Fluoridation

Fluoride is not an essential element in the human diet and there are no negative consquences from not ingesting enough fluoride; however, it is beneficial in the prevention of dental caries (Health Canada, 2010b). Numerous studies have shown water fluoridation to be effective for preventing dental decay (Horowitz, 1996; Ripa, 1993; Rugg-Gunn & Do, 2012; Truman et al., 2002). Based on studies conducted over the past 70 years, children living in communities with fluoridated water are estimated to have on average 1.8 fewer baby teeth and 1.2 fewer permanent teeth affected by dental decay (Iheozor-Ejiofor et al., 2015). In part due to the introduction of fluoridated toothpaste in the early 1970s, the benefits of community water fluoride (CWF) are recognized as being smaller today than in the past (Centers for Disease Control and Prevention, 2001). Despite widespread exposure to fluoride in drinking water and fluoride-containing dental products, dental decay remains a major public health problem, especially for socioeconomically disadvantaged populations for whom resources for oral hygiene and formal dental care are limited.

The Centers for Disease Control and Prevention (CDC) has named water fluoridation as one of the ten great public health achievements in the United States due to its role in reducing tooth decay and tooth loss (Centers for Disease Control and Prevention, 1999). Further, Health Canada has endorsed CWF as “the most cost effective and equitable method to deliver fluoride to the population” (Health Canada, 2017, p.3). Similarly, the Chief Public Health Officer and Chief Dental Officer of Canada released a position statement calling CWF a “safe, cost effective and equitable public health practice and an important tool in protecting and maintaining the health and well-being of Canadians” (Taylor & Taylor, 2016).

Recent studies have challenged the benefits of CWF because of the overall weak level of contemporary evidence supporting its effectiveness (Cheng, Chalmers, & Sheldon, 2007; Iheozor-Ejiofor et al., 2015; Wilson & Sheldon, 2006) and growing body of evidence suggesting potential negative health effects (discussed below). Some individuals object to the practice of water fluoridation in that it is a medical intervention which they perceive as imposed on them by the state and an infringement on their freedom of choice (Hileman, 1988; Cross & Carton 2003). A final concern relates to the source of the fluoride used in water fluoridation. Unlike the fluoride salts used in dental products (such as sodium fluoride and stannous fluoride), the main fluoride chemicals that are added to drinking water are not pharmaceutical grade or naturally occurring compounds. Silicofluorides, which are commonly used for water fluoridation, are a by-product from the manufacturing of phosphate fertilizers (National Research Council, 2006). Concerns have been raised about other contaminants that may arise from the use of these chemicals, such as arsenic. However, water safety standards monitor these additives to ensure they fall below permissible levels (Brown, Cornwell, & MacPhee, 2004; Centers for Disease Control and Prevention, 2014; NSF International, 2019).

Fluoride Exposure

Research from the United States has identified several sources of fluoride exposure, including beverages made with fluoridated water, tea, processed foods (e.g. mechanically deboned meat), fluoridated dental products (toothpastes, mouth rinses, fluoride gels, and fluoride varnishes), soil, pesticides, and air (National Research Council, 2006). Most toothpastes sold in North America contain fluoride, usually at 1,000-1,100 parts per million, which is equivalent to 1 to 1.1 mg fluoride ion per gram of toothpaste (National Research Council, 2006). For children, fluoridated toothpastes are a special consideration because many

children tend to use greater amounts of toothpaste than advised (Thornton-Evans et al., 2019) and their ability to expectorate toothpaste is not as well developed as that of adults. Moreover, many children under the care of a dentist undergo fluoride treatments (National Research Council, 2006) whereby unintended ingestion of fluoride may occur. Children ages 1 to 4 have the highest estimated fluoride intake from toothpaste (0.34 mg/day), followed by children ages 4 to 7 (0.22 mg/day); fluoridated toothpaste accounts for roughly 11 to 22% of total daily fluoride intake among young children (United States Environmental Protection Agency, 2010, p.98). The single largest contributing source of fluoride for all age groups is drinking water, which accounts for between 40% and 70% of total fluoride intake (United States Environmental Protection Agency, 2010, p.98).

The single largest contributing source of fluoride for all age groups is drinking water, which accounts for between 40% and 70% of total fluoride intake (United States Environmental Protection Agency, 2010, p.98). On average, young children ages 1 to 5 are exposed to twice as much fluoride (0.063 mg/kg/day) as adults (0.031 mg/kg/day) relative to their body weight (National Research Council, 2006). The exception to this age difference is a subgroup of adults who are exposed to high levels of fluoride, including those who work in coal-burning plants or in factories that manufacture phosphate fertilizer. This difference in dietary fluoride intake between children and adults occurs partly because children drink more water than adults relative to their body weight. Dietary fluoride intake is approximately 70 times higher among infants who are formula-fed using fluoridated water and powdered milk- or soy-based powdered formula than exclusively breastfed infants (Fomon, Ekstrand, & Ziegler, 2000; National Research Council, 2006; Zohoori, Omid, Sanderson, Valentine, & Maguire, 2019). In contrast, fluoride is found in extremely small amounts in breastmilk, regardless of

water fluoridation status (Brothwell & Limeback, 2003; Dabeka, Karpinski, McKenzie, & Bajdik, 1986; Ekstrand, Boreus, & De Chateau, 1981; Zohoori et al., 2019). A recent study found that 65% of fluoride intake in infants is retained in the body (deposited in calcifying tissues) (Zohoori et al., 2019). This is partly due to the fact that infants are limited in their ability to metabolize and excrete substances through the kidneys, which do not reach full capacity until age 2 (Zohoori et al., 2019). This level of fluoride exposure and retention increases the risk of developing dental fluorosis, which has been increasing over the past 40 years (Fomon et al., 2000). The critical period for development of fluorosis is from 4 months in utero until 11 months of age, and has been associated with higher water fluoride concentration, but not with the use of dentifrice or fluoride supplements (Warren, Levy, & Kanellis, 2001; Zohoori et al., 2019).

Pharmacokinetics of Fluoride

There are many ways in which individuals can be exposed to fluoride, although ingestion is the main method of absorption. Fluoride found in food or water enters the body through the gums, stomach, and intestinal lining, with most being absorbed by the small intestine (Martinez-Mier, 2012). Once absorbed, fluoride is rapidly distributed (i.e., within 10 minutes) throughout the body by the blood, with plasma fluoride levels reaching peak levels at 60 minutes following exposure and returning to baseline within 11 to 15 hours (Martinez-Mier, 2012; Whitford, 1996). About 50% of the fluoride ingested each day is excreted through the kidneys, and the remaining 50% accumulates in bones, the pineal gland, and other tissues (Chouhan & Flora, 2008). Fluoride readily penetrates cell membranes by simple diffusion and leads to a variety of effects on cell metabolism and function (Chouhan & Flora, 2008). Further, fluoride readily passes through the placenta to the developing fetus (Opydo-Szymaczek & Borysewicz-Lewickaa, 2007; Sastry,

Mohanty, & Rao, 2010). There is also some evidence that fluoride can cross the blood-brain barrier (Bhatnagar, Rao, Jain, & Bhatnagar, 2002; Gori et al., 2015; Hu & Wu, 1988; Jones & Iagaru, 2014; Luke, 2001; Pereira et al., 2011; Salgarello et al., 2016; Wu, Zhu, & Ji, 2013). Hu & Wu (1988) found that fluoride concentrations in adult cerebrospinal fluid are similar to those found in serum. Case reports of cancer patients have shown that radioactive fluoride used in treatment reaches the brain (Gori et al., 2015; Jones & Iagaru, 2014; Salgarello et al., 2016; Wu, Zhu, & Ji, 2013). Once in the brain, it is thought to accumulate within the hippocampus, which is responsible for memory and learning (Bhatnagar et al., 2002; Pereira et al., 2011). Finally, one study examining human cadavors found that fluoride accumulates in the pineal gland of adults (Luke, 2001).

Fluoride levels in plasma and urine have been shown to vary as a function of demographic variables including sex, age, Body Mass Index (BMI) and ethnicity (Aylward et al., 2015; Health Canada, 2015; Jain, 2017). In a report from Health Canada (2015) using data from the Canadian Health Measures Survey (CHMS) Cycle 3, females aged 3 to 79 had higher urinary fluoride (UF) adjusted for creatinine than males of the same age (0.53 mg/g for females and 0.40 mg/g for males). In addition to sex-based differences, the Health Canada (2015) report showed a number of differences by age; for example, children ages 3 to 5 had the highest UF concentration (0.76 mg/g) whereas adolescents ages 12 to 19 had the lowest UF concentration (0.29 mg/g). The values for adults also varied by age; mean UF concentration was 0.40 mg/g for those ages 20 to 39 and 0.52 mg/g for those ages 40 to 79. These age related differences may reflect differences in fluoride intake, as older adults consume more green, black and white tea which contains fluoride (Malinowska, Inkielewicz, Czarnowski, & Szefer, 2008), or differences in toxicokinetics. An individual's BMI has also been shown to be related

to an individual's level of fluoride exposure (Das & Mondal, 2016). Regarding ethnic differences, Jain and colleagues (2017) found that Hispanic adolescents aged 12–19 years from the United States had significant lower fluoride in plasma samples than non-Hispanic whites. Given that few studies examine fluoride levels across different demographic groups (e.g., sex, age, ethnicity), and even fewer studies present separate values for individuals who live in areas with and without CWF, more biomonitoring studies are needed to understand how fluoride levels (measured in urine) may vary based on demographic characteristics and CWF status.

Experimental Neurotoxicity

A systematic review on the effects of fluoride on learning and memory in animal studies was conducted with 32 studies that met the inclusion criteria (National Toxicology Program, 2016). Although the authors identified several methodological limitations with the studies, they concluded that there is a moderate level of evidence for negative impacts on learning and memory when animals are exposed to fluoride as adults, and a low level of evidence for developmental exposure. The exposure level for the included studies ranged from 0.45 to 272 ppm, and the authors noted limited evidence for lower levels of exposure that would be comparable to levels used for water fluoridation. It is important to note that rodents require approximately five times more fluoride in their water to achieve the same level of fluoride in their blood as humans. Thus, high levels of fluoride exposure in rats are necessary to ensure comparable exposure levels to those of humans (Dunipace et al., 1995; National Toxicology Program, 2016).

It is notable that the 2016 systematic review by the NTP noted a lack of animal studies examining sex effects. In an early study, Mullenix and colleagues (1995) injected pregnant rats with 0.13 mg/L of sodium fluoride two or three times daily, exposed juvenile rats to fluoridated

drinking water at concentrations of 0, 75, 100, and 125 mg/L, and exposed adult rats to drinking water with 100 mg/L of fluoride. Male, but not female, rats exposed prenatally to sodium fluoride demonstrated changes in behaviour such as symptoms of hyperactivity; in contrast, female rats demonstrated similar behavioural changes when fluoride exposure occurred in adulthood. In a more recent study, pregnant and lactating female mice received different doses of sodium fluoride (0, 25, 50, 100 mg/L NaF), and impairment in learning and memory was observed in male and female offspring (Wang et al., 2018). Bartos and colleagues (2018) found that exposure to low levels of fluoride during the gestational and lactation periods resulted in decreased memory in adult female rat offspring. Thus, further study examining sex effects associated with early-life exposure to fluoride is warranted.

As an attempt to address some of the methodological limitations noted by the 2016 NTP review, McPherson and colleagues (2018) conducted a behavioural study of male rats that were fed either a standard diet or a low fluoride diet, and were exposed to fluoride in drinking water at 0, 10, or 20 ppm. They did not observe any significant differences in motor, sensory, or learning and memory performance across the different levels of fluoride exposure. However, this study has been critiqued for beginning the exposure period at day 6 of gestation and for using Long Evans rats, a strain that has been previously shown to be more resistant to fluoride toxicity (Spencer & Limeback, 2018). Several other animal studies with rats have reported a negative impact of fluoride on attention, learning, and problem solving (Bartos et al., 2018; Chen et al., 2018; Liu, 2014; Sun, Zhang, Xue, Niu, & Wang, 2018; Wang et al., 2018; Zhao et al., 2019; Zhou et al., 2019).

Numerous mechanisms for impaired attention, learning, and memory due to fluoride have been proposed based on animal models. Chen, Shan, Wang, Nordberg, and Guan (2003) found

that rats with fluorosis have substantial decreases in neural nicotinic acetylcholine receptors (nAChR) and inhibited cholinesterase expression. Nicotinic acetylcholine receptors and cholinesterase expression both appear to play a role in attentional processes (Levin, Bushnell, & Rezvani, 2011). In particular, abnormalities at the α4 nAChR subunit have been implicated in all ADHD subtypes (Lee et al., 2008; Todd, Lobos, Sun, & Neuman, 2003). Several animal studies have found evidence of notable decreases in protein expression of α4 and α7 nAChR subunit genes in rats with fluorosis (Liu et al., 2010; Chen et al., 2003, Long et al., 2002). For example, a recent study found that low levels of fluoride exposure led to decreased mRNA expression of the α7 nicotinic receptor (nAChR) and decreased catalase activity in the hippocampus (Bartos et al., 2018).

Other neurological changes discovered through animal studies include alterations in neurotransmitters or their receptors (Chouhan et al., 2010; Liu et al., 2010; Reddy, 2014), reduced mRNA expression of glutamate receptor subunits (Sun et al., 2018), morphological changes in neurons (Bhatnagar, 2011; Zhao et al., 2019), increased catalase immunoreactivity (Güner, Uyar-Bozkurt, Haznedaroğlu, & Menteş, 2016), increased calcium inside the neuron (Yu, Shao, Zhang, Ouyang, & Zhang; 2019), excessive apoptosis (Jiang et al., 2019; Zhao et al., 2019; Zhou et al., 2019), abnormal mitochondrial dynamics (Lou, 2014; Lou, 2013), decreased neurogenesis and neuronal number (Jiang et al., 2019; Zhou et al., 2019), and the impairment of synaptogenesis (Chen et al., 2018). Finally, numerous studies have demonstrated greater oxidative stress in rats exposed to low levels of fluoride (Banala & Karnati, 2015; Bartos et al., 2018; Chouhan, Lomash, & Flora, 2010; Reddy et al., 2014; Zhang, 2015). For example, Chouhan and colleagues (2010) found that rats exposed to fluoridated water at a concentration of

1 mg/L for four months showed more oxidative stress and alterations in neurotransmitters compared to rats exposed to non-fluoridated water.

Potential Impacts of Fluoridation on Human Health

Evidence is accumulating that fluoride exposure may be neurotoxic and a cognitive developmental hazard to humans (National Toxicology Program, 2019). In epidemiological studies, exposure to fluoride in drinking water has been associated with lowered intellectual ability (Das & Mondal, 2016; Green, et al., 2019; Jiménez et al., 2017; Seraj, et al., 2012; Xiang et al., 2003; Zhao, Liang, Zhang, & Wu, 1996) as well as altered thyroid function (Malin, Riddell, McCague, & Till, 2018; Peckham, Lowery, & Spencer, 2015) and increased blood lead levels (Coplan, Patch, Masters, & Bachman, 2007), which may also influence intellectual ability. Studies examining high levels of fluoride in areas with endemic fluorosis have consistently found adverse effects on children's cognitive development. For example, Rocha-Amador, Navarro, Carrizales, Morales, and Calderón (2007) examined the concentration of fluoride in the drinking water of three rural communities in Mexico, which ranged from 0.8 to 9.4 mg/L. The authors tested the IQ of 132 children and found lower Verbal and Full Scale IQ scores in children exposed to higher levels of fluoride in their drinking water (as well as higher levels of urinary fluoride). Similarly, Seraj and colleagues (2012) classified villages in Iran as having low fluoride (0.8 ± 0.3 ppm), medium fluoride (3.1 ± 0.9 ppm), or high fluoride (5.2 ± 1.1 ppm) concentrations in the ground water. They found that children in the low fluoride group had higher IQ scores (mean IQ = 98) compared to children in the medium and high fluoride groups (mean IQ = 89 and 88, respectively). Recently, Jiménez and colleagues (2017) conducted a study with 65 mother-infant pairs in Mexico and found a relation between in utero fluoride exposure and impaired mental development. The authors found that each 1 mg/L

increase in maternal urinary fluoride corresponded to a decrease of 19.5 points on the Mental Development Index (MDI) of the Bayley Scales of Infant Development.

A meta-analysis of 27 studies indicated that children who lived in areas with high fluoride exposure had lower intelligence (equivalent to approximately seven IQ points) compared with those who lived in low-exposure areas (Choi, Sun, Zhang, & Grandjean, 2012). However, information on important covariates was not available for many of these studies. For example, the child's sex and parental education were not reported in over 80% of the studies and only 7% of the studies reported household income. As a result, Choi and colleagues (2012) were only able to account for two covariates: year of publication and mean age of the children in the study. Moreover, fluoride exposure from most studies included in the review was higher (> 2.0 mg/L) than recommended fluoride concentration levels in Canada (0.7 mg/L) making the results non-generalizable to North America. Other limitations include a lack of sufficient comparison groups and omission of important procedural details, such as whether the person administering the IQ test was blinded to participant condition.

Findings of research specifically focused on the health effects of community water fluoridation (CWF) have been mixed. Broadbent and colleagues (2015) studied the relationship between fluoride and intelligence by measuring the IQ of 992 children in New Zealand at ages 7, 9, 11 and 13 years, and again at age 38. They calculated fluoride exposure by examining residence in an area that received CWF as well as personal use of fluoride dentifrice and fluoride tablets prior to age 5 years. The authors did not find any statistically significant differences in intelligence linked to fluoride intake in this sample. However, Hirzy, Connett, Xiang, Spittle, and Kennedy (2016) criticized this study, stating that the difference in fluoride intake between the fluoridated and non-fluoridated communities (which was estimated to be 0.3 mg/day) was too

small to adequately detect a link between fluoride and intellectual ability. Other shortcomings included a failure to control for maternal tea consumption during pregnancy (an important source of fluoride in New Zealand), and lack of control for formula- versus breast-feeding.

A report from the National Research Council (NRC; 2006) in the United States warned that developmental toxicity and neurotoxicity may be associated with fluoride exposures at levels that are allowable in drinking water in the United States (the maximum allowable level is 4 mg/L in the United States versus 1.5 mg/L in Canada). This report highlighted the consistency of available evidence and recommended further research to address these potential concerns. In 2019, a number of public health organizations (e.g., the O'Brien Institute for Public Health, the National Toxicology Program of the U.S. Department of Health and Human Services, Canadian Agency for Drugs and Technologies in Health) reviewed scientific information given the large number of studies that have been conducted addressing the concerns raised in the 2006 NRC report. Although these independent scientific committees generally reviewed the same set of studies, including the results of four recent prospective cohort studies (Bashash et al., 2017; 2018; Green et al., 2019; Jimenez et al., 2017), the conclusions made in each report were quite different regarding risk of adverse effects on cognitive neurodevelopmental effects. In order to make evidence-based policy recommendations, further research is needed to elucidate potential risks of fluoride exposure and to address the limitations of the studies previously discussed. Few studies have been conducted at the individual level (i.e., those that collect individual biomarker data) and many previous studies did not include important covariates such as child age, sex, and socioeconomic status. Previous studies relied almost exclusively on global metrics such as general intellectual ability and did not examine specific domains of cognitive functioning such as learning,

memory, and attention. Finally, there are few fluoride studies examining behavioural outcomes related to fluoride exposure. The current study aims to address these gaps in the research and advance our understanding of the effects of fluoride exposure on Canadian children's behavioural functioning with a specific focus on ADHD.

Study Aims and Hypotheses

Fluoride is listed as an "emerging neurotoxic substance" that requires further in-depth study, especially for exposures that occur during sensitive periods for brain development such as prenatally and during childhood (Grandjean & Landrigan, 2014). Consistently, the draft report of the NTP released in October 2019 concluded that "fluoride is presumed to be a cognitive developmental hazard to humans" and that the level of evidence was judged to be moderate. Some past human studies have shown that higher levels of fluoride exposure are associated with lower intellectual ability, reduced attention and working memory, and increased risk of developing ADHD (Bashash et al., 2018; Choi et al., 2012; Malin & Till, 2015). Other studies have not found a relationship between fluoride and adverse outcomes such as learning disabilities (Barberio, Quiñonez, Hosein, & McLaren, 2017) or lower IQ (Broadbent et al., 2015). Given widespread exposure to fluoridated water among Canadians, rigorous research is needed to address the current controversy about the safety of water fluoridation, with a focus on vulnerable populations such as children and with sensitive outcomes. The specific aims of this dissertation are to (1) examine the association between fluoride exposure and attention outcomes in a representative Canadian sample of youth, and (2) characterize urinary fluoride (UF) levels by age, sex, and CWF status and determine how different factors impact UF. Of note, both studies were planned at the same time (as opposed to the results of the first study informing the design and hypotheses of the second study).

For study 1, it was hypothesized that UF, tap water concentration, and CWF status would predict a diagnosis of ADHD and symptoms of hyperactivity and inattention on the Strengths and Difficulties Questionnaire (SDQ score; Goodman, 1997). For study 2, it was hypothesized that UF would be higher among individuals with higher tap water fluoride levels and, accordingly, among those who live in cities with CWF compared to individuals who live in non-fluoridated cities. It was also hypothesized that UF will differ by both age and sex; specifically, adult women living in fluoridated regions will have the highest levels of urinary fluoride as found by a previous Canadian study (Health Canada, 2015). We also predicted that individuals who consume more tea (green, black, or white) will have higher levels of urinary fluoride. Exploratory analyses examined how the recency of dental product use impacts UF for individuals of different age groups.

Chapter Two: Association of water fluoride and urinary fluoride concentrations with Attention Deficit Hyperactivity Disorder in Canadian Youth

Rationale

Fluoride is listed as an "emerging neurotoxic substance" (Grandjean & Landrigan, 2014), and past human studies from Mexico and the United States have shown that higher levels of fluoride exposure are associated with increased risk of developing ADHD (Bashash et al., 2018; Malin & Till, 2015). Malin & Till (2015) used U.S. state-level measures of water fluoridation and ADHD prevalance. In the study by Bashash and colleagues (2018) with Mexican mother-child pairs, fluoride, which is added to salt, was measured in urine spot samples of pregnant mothers and compared with parent-rated symptoms of attention and hyperactivity using a questionnaire. In a Canadian study, using data from the Canadian Health Measures Survey (CHMS; Statistics Canada, 2013; 2015) Barberio and colleagues (2017) did not find an association between urinary fluoride and a diagnosis of a learning disability or a diagnosis of ADHD. Barberio and colleagues restricted their sample to youth ages 3 to 12, whereas we included youth between ages 6 and 17. This is because ADHD is not reliably diagnosed until age 6 (Kessler et al., 2005), and because changes produced by early and cumulative exposure to environmental toxins may manifest later in development. The upper limit of 17 years old was chosen because the SDQ was administered up until that age in the CHMS. Further, Barberio and colleagues focused their study on those with learning disabilities instead of selecting only those with a diagnosis of ADHD, as we did. Finally, the current study accounted for whether youth drink unfiltered municipal tap water, and controlled for many covariates that have been omitted from the studies described above (e.g., BMI, exposure to cigarette smoke and lead, etc.). Our study also built on previous work, such as the Malin & Till (2015) study, by using individual

biomonitoring data (e.g., urinary fluoride and the household level of tap water fluoride) to understand whether fluoride is associated with an increased risk of an ADHD diagosis in Canadian youth. Since 39% of Canadians currently receive CWF (Public Health Agency of Canada, 2017), it is essential to understand whether fluoride is associated with adverse outcomes at the level found in CWF.

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Author Contributions

As the first author of this manuscript, I selected the research questions, analyzed the data, and wrote the manuscript. The fifth author of this study, Dr. Christine Till, was my supervisor for this project. She provided input on the research questions and aims, helped select the dataset to use, and provided input on numerous drafts of this paper. The second author, Dr. Ashley Malin, provided input on the manuscript drafts. The third and fourth authors, Dr. Dave Flora and Dr. Hugh McCague, provided consultation on the statistical methods and provided input on the drafting of the manuscript.

Abstract

Background: Exposure to fluoride has been linked with increased prevalence of attention deficit hyperactivity disorder (ADHD) in the United States and symptoms of inattention in

Mexican children. We examined the association between fluoride exposure and attention outcomes among youth living in Canada.

Method: We used cross-sectional data collected from youth 6 to 17 years of age from the Canadian Health Measures Survey (Cycles 2 and 3). Urinary fluoride concentration adjusted for specific gravity (UFSG) was available for 1877 participants. Water fluoride concentration measured in tap water samples was available for 980 participants. Community water fluoridation (CWF) status was determined by viewing reports on each city's website or contacting the water treatment plant. We used logistic regression to test the association between the three measures of fluoride exposure and ADHD diagnosis. Linear regression was used to examine the relationship between the three measures of fluoride exposure and the hyperactivity/inattention score on the Strengths and Difficulties Questionnaire (SDQ).

Results: UF_{SG} did not significantly predict ADHD diagnosis or hyperactive/inattentive symptoms. A 1 mg/L increase in tap water fluoride level was associated with 6.1 times higher odds of an ADHD diagnosis (95% CI = 1.60, 22.8). A significant interaction between age and tap water fluoride level (p = .03) indicated a stronger association between tap water fluoride and hyperactivity/inattention symptoms among older youth. A 1 mg/L increase in water fluoride level was associated with a 1.5 SDQ score increase (95% CI: 0.23, 2.68, p = .02) for youth at the 75th percentile of age (14 years old). Similarly, there was a significant interaction between age and CWF. At the 75th percentile of age (14 years old), those living in a fluoridated region had a 0.7-point higher SDQ score (95% CI = 0.34, 1.06, p < .01) and the predicted odds of an ADHD diagnosis was 2.8 times greater compared with youth in a non-fluoridated region (aOR = 2.84, 95% CI: 1.40, 5.76, p < .01).

Discussion: Exposure to higher levels of fluoride in tap water is associated with an increased risk of ADHD symptoms and diagnosis of ADHD among Canadian youth, particularly among adolescents. Prospective studies are needed to confirm these results.

Introduction

Fluoride is beneficial in the prevention of dental caries (Health Canada, 2010b). It can naturally occur in water, but often at levels that are too low to prevent tooth decay. In the middle of the 20th century, the concept of adding fluoridation chemicals (usually hexafluorosilicic acid) to water supplies was introduced. Currently, approximately 39% of Canadians on public water supplies receive community water fluoridation (CWF; Public Health Agency of Canada, 2017) compared with 74% of Americans and only 3% of Europeans (Centers for Disease Control and Prevention, 2014). Consumption of optimally fluoridated water (i.e., 0.7 mg fluoride per liter of water) accounts for approximately 40 to 70% percent of daily fluoride ingestion (United States Environmental Protection Agency, 2010).

Fluoride has been classified as a developmental neurotoxin (Grandjean & Landrigan, 2014) – a claim that is uncontested at high exposure levels, but remains debated at the exposure levels associated with water fluoridation. Epidemiological studies conducted in endemic fluorosis areas (i.e., naturally occuring water fluoride concentrations >1.5 mg/L) have reported a negative association between fluoride concentrations in drinking water and intellectual ability in children (Das & Mondal, 2016; Rocha-Amador, Navarro, Carrizales, Morales, & Calderón, 2007; Seraj, et al., 2012; Xiang et al., 2003; Zhao, Liang, Zhang, & Wu, 1996). A meta-analysis of 27 studies concluded that children who lived in areas with high fluoride exposure had IQ scores that were approximately seven points lower than those who lived in low-exposure areas (Choi, Sun, Zhang, & Grandjean, 2012). Most of the studies included in this review were cross-

sectional and had deficient reporting of key information; however, the consistency of their findings supports the potential for fluoride-mediated developmental neurotoxicity at elevated levels of exposure. Recently, three longitudinal birth cohort studies addressed these limitations by examining the associations between maternal fluoride exposure indicators in pregnancy and offspring cognitive abilities. These prospective studies reported a negative association between prenatal fluoride exposure and cognitive development in infants (Jiménez et al., 2017), children living in Mexico (Bashash et al., 2017), and children living in Canada (Green et al., 2019).

Experimental and epidemiologic studies have also revealed an association between early-life fluoride exposure and adverse behavioural outcomes. One study demonstrated that prenatal fluoride exposure caused greater hyperactivity in male rat pups whereas females were more sensitive to postnatal exposures (Mullenix, Den Besten, Schunior, & Kernan, 1995). In an ecological study, higher prevalence of water fluoridation was associated with increased prevalence of Attention Deficit Hyperactivity Disorder (ADHD) diagnoses among youth in the United States (Malin & Till, 2015). In a cohort study, higher levels of fluoride exposure during pregnancy were associated with more inattentive symptoms and total ADHD symptoms in Mexican children aged 6 to 12 years (Bashash et al., 2018). In contrast, a study using data from the Canadian Health Measures Survey (CHMS; Statistics Canada, 2013; 2015) did not find an association between urinary fluoride corrected for dilution and a diagnosis of a learning disability in children aged 3 to 12 years (Barberio et al., 2017).

We examined the relationship between urinary and tap water fluoride concentrations and inattentive-hyperactive symptoms in a national sample of Canadian youth aged 6 to 17 years. We hypothesized that higher levels of urinary and water fluoride would be associated with increased odds of an ADHD diagnosis and more symptoms of hyperactivity and inattention.

Methods

Data Source and Participants

We used data from Cycle 2 (2009-2011) and Cycle 3 (2012-2013) of the CHMS collected by Statistics Canada. All aspects of the CHMS were reviewed and approved by Health Canada's Research Ethics Board (Day, Langlois, Tremblay, & Knoppers, 2006); the current study was approved by the York University Research Ethics Board.

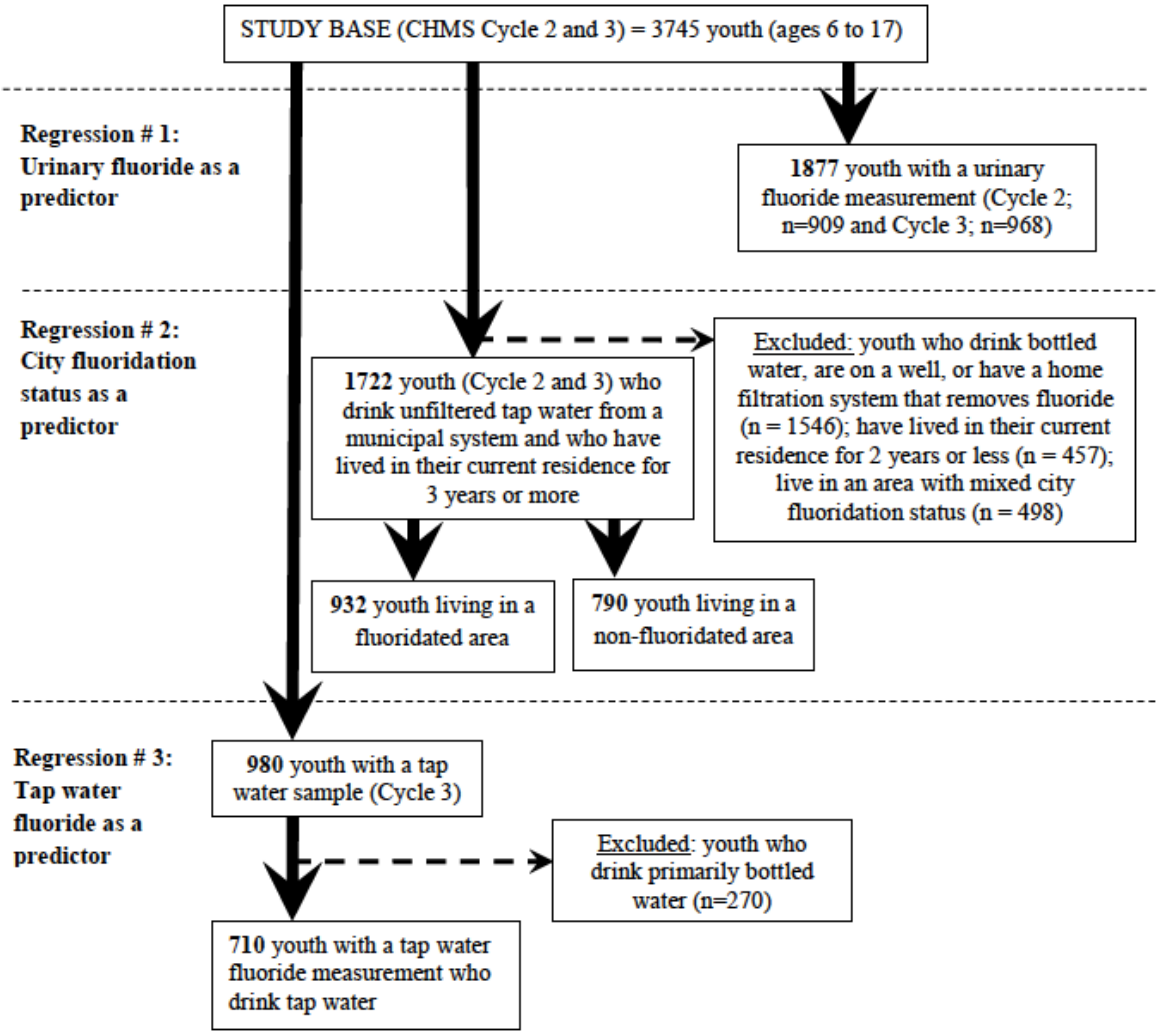
The CHMS randomly selected participants aged 3 to 79 years who lived in private households across Canada. A total of 6395 people participated in Cycle 2 of the CHMS, with 2520 people providing urine samples analyzed for fluoride concentration. Among those who provided urine samples, 909 (36%) were between 6 and 17 years of age. For Cycle 3, 5785 people were enrolled, with 2667 people providing urine samples analyzed for fluoride concentration; 968 (36%) were between 6 and 17 years of age. In Cycle 3 only, 980 youth ages 6 to 17 were selected to provide a tap water sample to be analyzed for fluoride content. Full details about the survey can be found at www.statcan.gc.ca.

Approximately half of the sites included in Cycles 2 and 3 received CWF, which was determined by viewing reports on each city's website or contacting the water treatment plant (see Supplemental Table 1). In total, 13 of 25 sites received CWF (eight from Cycle 2 and five from Cycle 3), corresponding to approximately 1,400 (51.9%) of 2,700 participants included in the study (rounded due to Statistics Canada data release requirements). Nine additional sites were considered to have mixed fluoridation status, corresponding to approximately 650 (24.0%) participants. A site was classified as mixed for one of five reasons: unclear site boundaries (n=150), having some municipalities within the site add fluoride while others did not (n=250), and stopping CWF during the period spanning CHMS data collection (n=50). We excluded all mixed sites from any analysis using city fluoridation status as a variable. We also

excluded sites which were labeled as fluoridated or non-fluoridated, but had an average water fluoride level that was either 2.5 times lower (n=100) or higher (n=100) than other fluoridated or non-fluoridated sites, respectively.

We utilized three participant samples for our analyses; see Figure 1.1 for a participant flow chart. The first sample, collapsed across both cycles, included 1877 youth ages 6 to 17 who had urinary fluoride measurements. The second sample categorized 1722 youth who were on a municipal water system (instead of a well) as either living in a fluoridated region (n=932; 54.1%) or a non-fluoridated region (n=790; 45.9%). This sample only included youth who primarily drank tap water (instead of bottled water), did not have a home filtration system that removes fluoride (i.e., reverse osmosis or distillation), and have lived in their current residence for three or more years. The third sample consisted of 710 youth from Cycle 3 who primarily drank tap water (instead of bottled water) and had fluoride levels measured in their tap water.

Figure 1.1 *Subject inclusion in each regression analysis*



Measurement of Urinary Fluoride Concentration

Urine spot samples were collected under normal (non-fasting) conditions and were not standardized with respect to collection time. Fluoride concentrations in spot urine samples were analyzed using an Orion PH meter with a fluoride ion selective electrode after being diluted with an ionic adjustment buffer (Statistics Canada, 2013). Urinary analyses were performed at the Human Toxicology Laboratory of the Institut National de Santé Publique du Québec (INSPQ; accredited under ISO 17025) under standardized operating procedures (Statistics Canada, 2013; 2015). The precision and accuracy of the fluoride analyses, including

quality control measures and quality assurance reviews, are described in previous publications (Health Canada, 2015). The limit of detection (LoD) for urinary fluoride was 20 μg/L for Cycle 2 and 10 μg/L for Cycle 3 (Health Canada, 2015). No urinary fluoride values in the Cycle 2 or Cycle 3 samples were below the LoD. Urinary fluoride concentrations were adjusted for specific gravity (UF_{SG}; mg/L); specific gravity shows no systematic variation within a day and is less dependent on body size, age, and sex than creatinine (Nermell et al., 2008; Moriguchi et al., 2005; Suwazono, Åkesson, Alfven, Järup, & Vahter, 2005).

Measurement of Water Fluoride Concentration

Tap water samples were collected at respondents' homes and were available for Cycle 3 only. Samples were analyzed for fluoride concentrations (mg/L) via a basic anion exchange chromatography procedure with a LoD of 0.006 mg/L. Concentrations at the LoD were assigned a missing value code by Statistics Canada, and these values were subsequently replaced with an imputed value of LoD/√2 (Hornung & Reed, 1990); 150 of 980 (16%) water samples had fluoride levels below the LoD.

Measurement of Outcome Variables

Primary outcomes included the hyperactivity/inattention subscale score from the Strengths and Difficulties Questionnaire (SDQ; Goodman, 2001) and a physician-made diagnosis of ADHD; these outcomes were measured in both Cycles 2 and 3. Data are presented for youth ages 6 to 17 because the SDQ was only administered to youth under age 18 and 90% of youth with ADHD are diagnosed after age 6 (Kessler et al., 2005). For children aged 6 to 11 years, information about ADHD diagnosis and SDQ ratings were provided by parents or guardians, whereas youth aged 12 to 17 years completed the questionnaire themselves, including the question about having a diagnosis of ADHD.

The SDQ consists of 25 items with a 3-point response scale (0 = *not true*, 1 = *somewhat true*, and 2 = *certainly true*). These items are divided among five subscales: emotional problems, conduct problems, hyperactivity-inattention, peer problems, and prosocial behaviour. Possible scores on each subscale range from 0 to 10. The five-item hyperactivity-inattention subscale was used in the current study to test the association between fluoride and ADHD-like symptoms.

The item pertaining to a physician-made diagnosis of ADHD differed between Cycles 2 and 3. In Cycle 2, respondents were asked *Do you have a learning disability?*, and if they responded *yes*, they were asked to specify the type of learning disability from among four options: (1) Attention Deficit Disorder, no hyperactivity [ADD]; (2) Attention Deficit Hyperactivity Disorder [ADHD]; (3) Dyslexia; and (4) Other. In Cycle 3, parents of children aged 6 to 11 were asked directly whether their child had ADHD, and if so, which subtype. Similarly, youth age 12 or older were asked whether they have received a physician-diagnosis of ADHD, and if so, which subtype. Across both cycles, all subtypes were combined into a single dichotomous variable of ADHD diagnosis (*no* = 0, *yes* = 1) as per current diagnostic classification schemes (American Psychiatric Association, 2013).

Covariates

Covariates related to ADHD diagnosis and to fluoride metabolism were selected based on literature review and consultation with an ADHD expert. The following covariates were included in each regression model: sex, age of child at household interview, ethnicity (white or ethnic minority), Body Mass Index (BMI), highest level of education obtained by either parent (less than a bachelor's degree vs. bachelor's degree or greater), total household income (Canadian dollars), exposure to cigarette smoke inside the home (yes or no), and $\log_{10}$-transformed value of concurrent level of blood lead (μg/dL).

Statistical analysis

Outliers that had high Cook's distance values were removed from all analyses with UF_{SG} as a predictor; these outliers (0.27% of values) represented individuals with urinary fluoride levels that were over 30 times the mean UF_{SG} level, likely representing an acute fluoride ingestion (e.g., swallowing toothpaste). Further, individuals with the highest incomes were identified as extreme observations; these values were replaced with the next highest income value (only 0.01% of income values were adjusted).

We used robust logistic regression to examine the associations between each fluoride exposure measure (UF_{SG}, CWF status, tap water fluoride) and ADHD diagnosis with the same set of covariates in each model. Box-Tidwell tests were used to check the linearity of the relationship between the log-odds of the dependent variable (ADHD diagnosis) and all continuous predictors; none of the non-linear terms were significant. Next, we used robust linear regression models with the same set of covariates to examine the associations between the three measures of fluoride exposure and the SDQ hyperactivity/inattention subscale score. Because the regression models produced heteroscedastic and non-normal residuals, all significance tests and confidence intervals were based on robust Huber-White standard errors. No issues with multicollinearity were detected from variance inflation factor (VIF) statistics. All regression analyses tested interactions between age and fluoride and between sex and fluoride, as hyperactivity is more common in younger boys and sex-dependent fluoride effects have been previously reported (Mullenix et al., 1995; Green et al., 2019). If an interaction with age was significant, we probed the interaction by calculating the predicted association between fluoride and the outcome at the 25^{th} and 75^{th} percentiles of age. When a tested interaction was non-significant, the model was re-estimated without the interaction term and the overall associations were interpreted. Finally, we conducted sensitivity analyses to test whether the

associations between the fluoride exposure variables and ADHD diagnosis differed between cycles given that the question about ADHD diagnosis was posed differently across the two cycles. A two-tailed $\alpha = .05$ was used as the threshold for statistical significance.

Results

Descriptive Statistics

Of all variables included in the CHMS, missing data were highest for household income (missing among 29% and 23% of CHMS respondents in Cycle 2 and 3, respectively); however, Statistics Canada provided imputed estimates for all participants missing the household income variable. Across Cycle 2 and 3 combined, most demographic variables (including sex, age, ADHD diagnosis, SDQ score, and length of residence) had less than 2% missing data. For highest household education, 2.6% of data were missing, and 7.4% of participants did not report their height or weight, which are needed to calculate BMI.

Table 1.1 presents descriptive statistics for the different samples used for the regression models. Among the 1,877 youth in Cycles 2 and 3 with a urinary fluoride measurement, 51.2% were male, 72.6% were white, and 47.0% had at least one parent with a university degree or higher. In this sample, 137 (7.3%) reported having received a diagnosis of ADHD; the mean for the SDQ Hyperactivity-Inattention subscale score was 2.7 ($SD = 2.7$; range: 0 to 10) with 201 (11.4%) youth having scores in the clinical range (i.e., above 90th percentile; Goodman, 2001). As expected, participants with an ADHD diagnosis had significantly higher scores on the SDQ Hyperactivity-Inattention subscale ($M = 6.5$, $SD = 2.5$) than those without a diagnosis ($M = 2.5$, $SD = 2.4$), $t = 24.8$, $p < .01$. Table 1.2 provides descriptive statistics for the 1,877 youth aged 6 to 17 years with and without an ADHD diagnosis.

Table 1.1

Demographic characteristics, fluoride exposure variables, and inattention-hyperactivity outcomes for youth included in the three different samples.

	Participants in Cycles 2 and 3 with:		Participants in Cycle 3 with:
Variable	Urinary fluoride Mean (SD) or %	CWF status† Mean (SD) or %	Water fluoride Mean (SD) or %
N	1877	1722	710
Child sex			
Male	51.2	50.8	52.7
Female	48.8	49.2	47.3
Child age at interview (years)	11.3 (3.4)	11.3 (3.3)	11.2 (3.5)
Ethnicity			
White	72.6	67.6	69.0
Non-white	27.3	32.5	31.0
Parental Education			
High School/College	53.0	43.4	44.9
University or higher	47.0	56.6	55.1
Smoking in the home			
Yes	11.5	7.7	8.3
No	88.5	92.4	91.7
Household income (per $1000 CDN)	91.7 (82.7)	97.3 (70.6)	104.0 (134.6)
Body Mass Index	19.8 (4.7)	19.7 (4.7)	19.6 (4.6)
Blood lead (μg/dL)	0.83 (0.41)	0.83 (0.41)	0.83 (0.41)
Lived in residence			
2.99 years or less	19.2	Excluded	21.1
3.00 years or more	80.8	100.0	78.9
Fluoride measures			
UF_{SG} (mg/L)	0.61 (0.39)	0.64 (0.45)	0.62 (0.48)
Water fluoride (mg/L)	0.23 (0.24)	0.26 (0.26)	0.23 (0.24)
Site adds fluoride			
Yes	50.9	53.3	53.7
No	48.1	46.7	46.3
Outcome Variables			
Diagnosis of ADHD	7.3	5.5	6.3
SDQ H/I Subscale Score	2.8 (2.7)	2.6 (2.6)	2.9 (2.6)

Abbreviations: ADHD = Attention Deficit Hyperactivity Disorder; BMI = Body Mass Index; SD = standard deviation; UF_{SG} = urinary fluoride adjusted for specific gravity; SDQ H/I= Strengths and Difficulties Questionnaire Hyperactivity Inattention.
† Youth who drink tap water and have a stable residence

Table 1.2

Comparison of youth with a urinary and water fluoride measurement with and without a diagnosis of ADHD

Variable	**ADHD Diagnosis** (n=137) *Mean (SD)/%*	**No ADHD Diagnosis** (n=1740) *Mean (SD)/%*
Sex		
Male	69.7	49.6
Female	30.3	50.4
Ethnicity		
White	85.1	72.4
Non-white	14.9	27.6
Parental Education		
High School/College	68.7	53.1
University or higher	31.3	46.9
Smoking in the home		
Yes	26.9	10.0
No	73.1	90.0
Age	12.0 (3.2)	11.2 (3.5)
Household income (per $1000 CND)	70.6 (54.1)	97.9 (95.4)
BMI	19.4 (4.1)	19.9 (4.8)
Blood lead (µg/dL)	0.83 (0.41)	0.83 (0.41)
Fluoride measures		
Tap water fluoride concentration†(mg/L)	0.29 (0.28)	0.22 (0.24)
UF_{SG} (mg/L)	0.57 (0.32)	0.62 (0.45)
Site adds fluoride – Yes	47.1	52.8
Site adds fluoride – No	52.9	47.2
Outcome		
SDQ H/I Subscale Score	6.74 (2.5)	2.51 (2.4)

† Cycle 3 only
ADHD = Attention Deficit Hyperactivity Disorder; BMI = Body Mass Index; SD = standard deviation; UF_{SG} = urinary fluoride adjusted for specific gravity concentration; SDQ H/I= Strengths and Difficulties Questionnaire Hyperactivity-Inattention

Fluoride Measurements

The mean UF_{SG} concentration was 0.61 mg/L (*Mdn* = 0.51; *SD* = 0.39; 10^{th} to 90^{th} percentile range = 0.27-1.06 mg/L) among the 1,877 youth from Cycles 2 and 3. The correlation between time of day and UF_{SG} was near zero (r = -.03). Mean UF_{SG} concentration was significantly higher among youth who lived in communities with fluoridated drinking water (*M* = 0.82 mg/L, *SD* = 0.54) than among youth who lived in communities without fluoridated drinking water (*M* = 0.46 mg/L, *SD* = 0.32), $t = 15.1$, $p < .01$. The mean water fluoride concentration was 0.23 mg/L (*Mdn* = 0.12; *SD* = 0.24; 10^{th}-90^{th} percentile = 0.01-0.65 mg/L) among the 710 youth for whom tap water measures were available, excluding those who reported drinking bottled water as their main source of water. As expected, water fluoride levels were significantly higher among those living in a fluoridated region (*M* = 0.49 mg/L, *SD* = 0.22) than non-fluoridated region (*M* = 0.04 mg/L, *SD* = 0.06), $t = 34.9$, $p < .01$. Table 1.3 presents the urinary fluoride and water fluoride levels across demographic characteristics, fluoride exposure variables, and attention outcomes.

Table 1.3

Urinary fluoride and tap water fluoride levels across binary demographic characteristics and fluoride exposure variables

Variable	**UF_{SG} (mg/L) Mean (SD)**	**Water fluoride (mg/L) Mean (SD)**
Child sex		
Male	0.61 (0.36)	0.23 (0.24)
Female	0.63 (0.51)	0.23 (0.24)
Ethnicity		
White	0.60 (0.42)	0.21 (0.23)
Non-white	0.68 (0.51)	0.28 (0.26)
Parental Education		
High School/College	0.62 (0.49)	0.21 (0.23)
University or higher	0.61 (0.38)	0.24 (0.25)

Smoking in the home		
Yes	0.57 (0.36)	0.17 (0.21)
No	0.63 (0.45)	0.24 (0.24)
Lived in residence		
2.99 years or less	0.64 (0.50)	0.23 (0.24)
3.00 years or more	0.61 (0.42)	0.23(0.24)
Type of water consumed		
Tap water	0.62 (0.44)	0.23 (0.24)
Bottled water	0.62 (0.45)	0.22 (0.23)
Source of water		
Municipal water system	0.63 (0.45)	0.25 (0.25)
Private well	0.54 (0.34)	0.13 (0.13)
Fluoride measures		
Site adds fluoride		
Yes	0.82 (0.54)	0.49 (0.22)
No	0.46 (0.32)	0.05 (0.06)

Abbreviations: ADHD = Attention Deficit Hyperactivity Disorder; SD = standard deviation; UF_{SG} = urinary fluoride adjusted for specific gravity

Association Between Fluoride Exposure and ADHD Diagnosis

No planned interactions were significant in the logistic regression of ADHD diagnosis on UF_{SG} concentration; thus, the final model did not include interaction terms. UF_{SG} did not significantly predict an ADHD diagnosis (adjusted Odds Ratio [aOR] = 0.96; 95% CI: 0.63, 1.46, $p = .84$) adjusting for covariates (Table 1.4). In the regression of ADHD diagnosis on CWF, there was a significant interaction between age and CWF ($B = 0.19$, $p = .02$), such that the association between CWF and the odds of an ADHD diagnosis was stronger among older youth. Specifically, at the 75th percentile of age (14 years old), the predicted odds of an ADHD diagnosis was 2.8 times greater among youth in a fluoridated region compared with youth in a non-fluoridated region (aOR = 2.84, 95% CI: 1.40, 5.76, $p < .01$), whereas among youth at the 25th percentile of age (9 years old), the predicted odds of an ADHD diagnosis was similar across CWF status (aOR = 0.91, 95% CI: 0.41, 1.99, $p = .81$; Table 1.4). Finally, tap water fluoride concentration was significantly associated with having an ADHD diagnosis, adjusting for covariates; this association did not significantly interact with sex or age. Specifically, a 1 mg/L

increase in tap water fluoride was associated with a 6.1 times higher odds of ADHD diagnosis (95% CI: 1.60, 22.8, $p < .01$; Table 1.4).

Table 1.4.

Adjusted Odds Ratio (aOR) and effect estimates for the association between fluoride exposure, ADHD diagnosis, and inattentive-hyperactive symptoms

	ADHD diagnosis		SDQ H/I subscale score	
	aOR[a]	95% CI	B[a]	95% CI
UF_{SG} (mg/L) [b]	0.96	0.63, 1.46	0.31	-0.04, 0.66
Fluoride in tap water (mg/L)[c]	6.10*	1.60, 22.8	0.31*	0.04, 0.58
75th percentile age	--[d]	--	1.52*	0.23, 2.80
25th percentile age	--	--	-0.33	-1.51, 0.84
CWF status[e]	1.21*	1.03, 1.42	0.11*	0.02, 0.20
75th percentile age	2.84*	1.40, 5.76	0.70*	0.34, 1.06
25th percentile age	0.91	0.41, 1.99	0.04	-0.38, 0.46

Abbreviations: ADHD = Attention Deficit/Hyperactivity Disorder; CWF = community water fluoridation; aOR = adjusted odds ratio; SDQ H/I= Strengths and Difficulties Questionnaire Hyperactivity-Inattention

* $p < .05$

[a]Adjusted for child's sex, age at interview, ethnicity (white or other), Body Mass Index, highest level of parental education, total household income, exposure to cigarette smoke inside the home (yes/no), concurrent blood lead level (log_{10}-transformed).

[b] Non-significant main effect of urinary fluoride level predicting ADHD diagnosis (*B*=-0.04, *p*=.84, N=119/1635 with ADHD/total sample) or SDQ subscale score (*B*=0.31, *p*=.08, N=1532).

[c] Interaction between age and water fluoride level predicting SDQ subscale score (*B*=0.31, *p*=.03, N=588).

[d] Since the interaction between age and water fluoride was not significant, only the main effects are presented for the logistic regression predicting ADHD diagnosis from fluoride in tap water (mg/L; N = 39/626 with ADHD/total sample).

[e] Significant interaction between age and CWF status predicting ADHD diagnosis (*B*=0.19, *p*=.03, N= 65/1173 with ADHD/total sample) and SDQ subscale score (*B*=0.11, *p*=.01, N = 1118).

Estimating the models with UF_{SG} and CWF for Cycle 2 and 3 separately showed a similar pattern (Table 1.5). For the regression with CWF, the interaction with age was not significant using Cycle 2 data only ($B = 0.12$, $p = .35$), but was significant using data from Cycle 3 only ($B = 0.30$, $p = .03$).

Table 1.5.

Adjusted[a] Odds Ratio (aOR) for association between fluoride exposure and ADHD for Cycle 2 and Cycle 3.

	ADHD diagnosis Cycle 2 only aOR (95% CI)	ADHD diagnosis Cycle 3 only aOR (95% CI)
UF_{SG}	0.75 (0.30, 1.85)	1.07 (0.61, 1.90)
CWF	1.40 (0.64, 3.09)	0.30 (0.03, 0.56)

Abbreviations: ADHD = Attention Deficit/Hyperactivity Disorder; CWF = community water fluoridation; aOR = adjusted odds ratio

[a]Adjusted for child's sex, age at interview, ethnicity (white or other), Body Mass Index, highest level of parental education, total household income, exposure to cigarette smoke inside the home (yes/no), concurrent blood lead level (log_{10}-transformed).

Association Between Fluoride and SDQ Hyperactive/Inattentive Scores

No planned interactions were significant in the regression of SDQ hyperactive/inattentive subscale scores on UF_{SG} concentration; thus, the final model did not include interaction terms. UF_{SG} did not significantly predict SDQ hyperactive/inattentive subscale scores ($B = 0.31$, 95% CI = -0.04, 0.66, $p = .08$; Table 4).

Next, there was a significant interaction between age and CWF status ($B = 0.11$, $p = .01$) such that the association between CWF and hyperactivity/inattention scores was stronger among older youth. Specifically, the regression model predicted that for youth at the 75th percentile of age (14 years old), living in a fluoridated region was associated with a 0.7-point higher SDQ hyperactivity/inattention score (95% CI = 0.34, 1.06, $p < .01$). In contrast, among youth at the

25th percentile of age (9 years old), CWF status was not significantly associated with SDQ hyperactivity/inattention scores (B = 0.04, 95% CI = -0.38, 0.46, p =.85).

Finally, the interaction between age and tap water fluoride level was also significant (B = 0.31, p = .03) such that the association between tap water fluoride and SDQ hyperactivity/inattention score was stronger among older youth. In particular, among youth at the 75th percentile of age (14 years old), an increase of 1 mg/L in water fluoride level was associated with a 1.52 increase in the SDQ hyperactivity/inattention subscale score (95% CI: 0.23, 2.68, p = .02). However, for youth at the 25th percentile of age (8 years old), the association between water fluoride level and SDQ hyperactivity/inattention subscale score was not significant (B = -0.33, 95% CI: -1.51, 0.84, p = .58).

Discussion

We found that Canadian youth exposed to higher tap water fluoride levels had a higher risk of receiving an ADHD diagnosis and reported more symptoms of hyperactivity and inattention. Specifically, an increase of 1.0 mg/L in water fluoride concentration was associated with 6.1 times higher odds of an ADHD diagnosis after accounting for potential confounding variables, such as exposure to second-hand smoke, household income, and blood lead level. Likewise, water fluoride concentration was positively associated with hyperactive/inattentive symptoms, especially among older youth. To contextualize these results, the difference in water fluoride concentration between cities with and without fluoridation is approximately 0.5 mg/L. Our finding of a 1.5-point increase in the SDQ hyperactive/inattentive symptom subscale for each increase of 1 mg/L in water fluoride level implies a 0.75-point increase per 0.5 mg/L water fluoride; this result is consistent with our finding of a 0.7-point increase on the SDQ's

hyperactivity/inattention subscale observed among older youth living in a fluoridated versus non-fluoridated region.

In contrast, urinary fluoride levels were not significantly associated with a diagnosis of ADHD or hyperactive/inattentive symptoms. Water fluoride concentration and CWF status may be more strongly associated with hyperactivity/inattention than urinary fluoride levels because fluoride concentrations in municipal water supplies vary within a limited range and therefore may serve as a proxy for early-life and chronic fluoride exposure. In contrast, urinary fluoride levels in spot samples are more likely to fluctuate due to the rapid elimination kinetics of fluoride. Additionally, urinary fluoride values may capture acute exposures due to behaviours that were not controlled in this study, such as professionally applied varnish, consumption of beverages with high fluoride content (e.g., tea), or swallowing toothpaste prior to urine sampling. Finally, the association between UF and hyperactivity/inattention could be obscured due to reduced fluoride excretion (i.e., increased fluoride absorption) during a high growth spurt stage (Jha, Mishra, Sharma, & Damodaran, 2011; World Health Organization, 1997). Despite these limitations, use of individualized biomarkers is considered an improvement over past ecologic studies (Malin & Till, 2015) examining the association between ADHD and fluoride exposure and it has the advantage of examining all sources of fluoride exposure, not just from drinking water.

Our findings are consistent with earlier studies showing a relationship between fluoride exposure and ADHD. In particular, Malin and Till (2015) found that a 1% increase in community water fluoridation prevalence in 1992 was associated with approximately 67,000 to 131,000 additional ADHD diagnoses from 2003 to 2011 among children and adolescents in the United States. Conversely, Barberio and colleagues (2017) did not find a significant relationship

between fluoride exposure and learning disabilities (including ADHD) using data from the CHMS Cycles 2 and 3. A direct comparison of our results to the results found by Barberio and colleagues is challenged by the differences in how the data were analyzed between the two studies. Our sample included youth between ages 6 and 17, whereas Barberio and colleagues restricted their sample to youth ages 3 to 12. Further, Barberio and colleagues included participants with learning disabilities instead of selecting only those with a diagnosis of ADHD. Finally, the current study accounted for whether youth in both Cycles 2 and 3 drank unfiltered municipal tap water. It may be that the effects of fluoride exposure are most pronounced in older youth, or that fluoride is specifically associated with ADHD-related behaviours as opposed to learning disabilities. Finally, inclusion of learning disabilities may also introduce selection bias due to differences in how learning disabilities and ADHD are diagnosed in Canada.

Our findings showed that age modified the association between fluoride exposure and the likelihood of ADHD diagnosis and symptoms of hyperactivity and inattention, such that the associations were stronger among older youth. This is surprising given that most studies of the association between fluoride exposure and lower IQ have focused on prenatal and early postnatal exposures, and it has been suggested that the prenatal period may be a key window of neurological vulnerability (Bashash et al., 2017; Grandjean, 2019; Green et al., 2019). The method used in the CHMS may not be as sensitive for young children who are at risk of an ADHD diagnosis but have not yet been diagnosed; given that 90% of youth with ADHD are diagnosed after age 6 (Kessler et al., 2005), we restricted our minimum age to 6 years. Cumulative exposure to fluoride over time may also impact neurobehavioural development such that youth show more symptoms as they age. Alternatively, because the developing brain is highly sensitive to environmental toxins (Grandjean & Landrigan, 2006; 2014) and because

gene expression later in life is impacted by epigenetic changes that occur earlier in development (Roth, 2012), changes produced by early exposure to environmental toxins may manifest later in development.

While ADHD is known to have a strong genetic component with an estimated heritability of 70% to 80% (Larsson, Chang, D'Onofrio, & Lichtenstein, 2014), environmental risk factors are also believed to contribute to the development of ADHD. Prenatal substance exposures, heavy metal and chemical exposures, and nutritional factors have been proposed to contribute to the rise in ADHD in the United States (Xu, Strathearn, Liu, Yang, & Bao, 2018; Sciberras, Mulraney, Silva, & Coghill, 2017) and an increase in behavioural difficulties as assessed by the SDQ (Philippat et al., 2017; Oulhote, Steuerwald, Debes, Weihe, & Grandjean, 2016; Luk et al., 2018). A recent systematic review (Donzelli et al., 2019) reported a significant association between lead exposure and risk of ADHD in 12 out of 17 studies; the adjusted odds ratios ranged from 1.09 to 7.25, which is within the range of the current study findings. Although the precise mechanism by which fluoride affects neurodevelopment is unclear, several possible mechanisms have been proposed. Animal studies have shown alterations in acetylcholine or cholinergic receptors due to fluoride exposure (Chouhan et al., 2010; Liu et al., 2010; Reddy, 2014). In particular, both nicotinic acetylcholine receptors and cholinesterase expression appear to play a role in attentional processes (Levin, Bushnell, & Rezvani, 2011). Other studies have shown morphological changes in neurons (Bhatnagar, 2011), mitochondria (Zhao et al., 2019), increased catalase immunoreactivity (Güner, Uyar-Bozkurt, Haznedaroğlu, & Menteş, 2016), more oxidative stress (Zhang, Lou, & Guan, 2015), and increases in apoptotic neurons and abnormal mitochondrial dynamics (Lou, Guan, Peic, & Guiyang, 2014; Lou et al., 2013). Further, some studies have suggested that fluoride may

suppress thyroid function (Trabelsi, Guermazi, & Zeghal, 2001); subclinical hypothyroidism during pregnancy has been linked with increased risk for attention disorders (Modesto et al., 2015; Päkkilä et al., 2014).

Our study has some limitations. First, tap water fluoride was measured in Cycle 3 only, which decreased the sample size for analyses using this predictor. However, we were able to determine CWF status for participants in both Cycle 2 and 3, which permitted examining the concordance between the effects associated with tap water fluoride level, CWF status, and urinary fluoride level. Second, use of exposure metrics obtained at the same time as the outcome of interest (cross-sectional data) is limited for making conclusions about the causal association between fluoride and ADHD. Exposure misclassification may have occurred for some participants due to changes in a city's water fluoridation status over the youth's lifetime. Tap water samples were collected between 2012 and 2013 when approximately 37.4% of Canadians had access to fluoridated water as compared with 42.6% in 2007 (Public Health Agency of Canada, 2017). Thus, water fluoride measures obtained at the time of CHMS data collection may not be consistent with water fluoride levels that were antecedent to the outcomes in our study. Because the CHMS only measured postnatal fluoride exposure, we were not able to distinguish the effects of fluoride exposure during different developmental periods (e.g., prenatal versus postnatal). Recent studies have identified pregnancy as a critical period during which fluoride exposure is linked to lowered IQ (Bashash et al., 2017; Green et al., 2019) and attention-related behaviours (Bashash et al., 2018) in offspring. Third, the method used by CHMS may not completely capture true ADHD prevalence. The CHMS relied on youth or parent report of a physician-made diagnosis of ADHD, whereas the gold standard for ADHD assessment would be a diagnostic interview with confirmation that symptoms began

before age 12 and occur in multiple settings. Further, because of the way that the CHMS items were phrased, the Cycle 2 sample may identify youth with a comorbid learning disability and ADHD, but not those who have ADHD and no learning disability. Nonetheless, the prevalence of ADHD in the current study (7.3%) is similar to the prevalence rate found in other studies. A meta-analysis including 175 studies from across the world obtained an overall ADHD prevalence rate of 7.2% (Thomas, Sanders, Doust, Beller, & Glasziou, 2015). In a 2012 sample of Canadian youth under age 24, the prevalence of ADHD was 5.4% (Hauck, Lau, Wing, Kurdyak, & Tu, 2017). Relatedly, the number of youth with ADHD in our study was relatively small, ranging from approximately 45 to 140 depending on the sample used for a given analysis, which limited statistical power and precision. Finally, the SDQ relies on youth or parent perceptions of symptoms. Future studies would benefit from prospective designs and more rigorous symptom assessment, particularly a structured diagnostic interview that assesses DSM-5 criteria for ADHD.

In conclusion, we found that higher tap water fluoride levels and fluoridation of municipal water supplies were associated with a higher risk of an ADHD diagnosis as well as increased symptoms of hyperactivity and inattention, especially among adolescents. These findings, which point to a potential cumulative effect of fluoride exposure, highlight the need for further investigation of the potential for fluoride-mediated developmental neurotoxicity in populations with water fluoridation.

Chapter Three: Urinary fluoride levels among Canadians with and without community water fluoridation

Rationale

In our first study, tap water fluoride and community water fluoridation (CWF) were associated with attention outcomes in youth, whereas urinary fluoride (UF) was not. To better understand this finding, we investigated the factors that influence UF. To understand how much UF is impacted by behaviours prior to urine sampling, we compared individuals who used a fluoridated product within 6 hours of urine sampling to those who used a fluoridated product more than 6 hours prior to urine sampling. Further, UF levels have been shown to vary as a function of demographic variables including sex, age, Body Mass Index (BMI) and ethnicity (Aylward et al., 2015; Barberio, Quiñonez, Hosein, & McLaren, 2017; Health Canada, 2015; Jain, 2017). However, prior studies of the differences in UF across demographic characteristics do not account for whether individuals live in areas with or without CWF. To address this gap, the current study presents UF concentration by age group, sex, and CWF status. Further, to build on previous literature on the contribution of water fluoride to UF (National Research Council, 2006; United States Environmental Protection Agency, 2010), we present the correlation between water fluoride and UF by age group. Finally, we assessed how numerous predictors identified from previous research impact UF levels. These predictors include water fluoride concentration, CWF, tea consumption, type of water consumed (tap or bottle), and recency of fluoride treatment at the dentist. Whether smoking is allowed in the home was also included, as cigarette smoke may modify fluoride metabolism and excretion (Jain, 2017). A strength of this study is that we controlled for numerous demographic characteristics including sex, age, ethnicity, income, and highest household education, as well as modifiers of exposure, including

BMI, in models examining the association between fluoride exposures and UF. Evidence is accumulating that fluoride exposure may be neurotoxic and a cognitive developmental hazard to humans (National Toxicology Program, 2019). Thus, it is essential to understand predictors of UF and identify subgroups who may have higher levels.

Publication Status

The work contained in this chapter has not yet been submitted for publication. When it is submitted, the authors will include: Julia Riddell, Ashley Malin, David Flora, Hugh McCague, and Christine Till.

Author Contributions

As the first author of this manuscript, I selected the research questions, analyzed the data, and wrote the manuscript. The fifth author of this study, Dr. Christine Till, was my supervisor for this project. She provided input on the research questions and aims, helped select the dataset to use, and provided input on numerous drafts of this paper. The second author, Dr. Ashley Malin, provided input on the manuscript drafts. The third and fourth authors, Dr. Dave Flora and Dr. Hugh McCague, provided consultation on the statistical methods and provided input on the drafting of the manuscript.

Abstract

Background: Urinary fluoride levels can vary as a function of demographic variables including sex, age, Body Mass Index (BMI) and ethnicity, as well as whether an individual lives in a fluoridated or non-fluoridated region. This study examines factors that may influence urinary fluoride levels in a Canadian population.

Method: We measured urinary fluoride and tap water fluoride levels in 1629 individuals aged 3 to 79 years participating in Cycle 3 (2012-2013) of the Canadian Health Measures Survey. We measured urinary fluoride adjusted for specific gravity (UF_{SG}) by age and sex, taking community water fluoridation (CWF) status into consideration. We also examined numerous predictors of UF_{SG}, including tap water fluoride levels, use of fluoridated dental products, smoking in the home, and tea consumption, while controlling for ethnicity, BMI, income, and highest household education.

Results: UF_{SG} concentration was significantly higher among participants who received fluoridated drinking water (M = 1.06 mg/L, SD = 0.83) than among those who did not (M = 0.58 mg/L, SD = 0.47), t = -13.7, $p < .01$. Females had significantly higher UF_{SG} concentration (M = 0.89 mg/L, SD = 0.76) than males (M = 0.77 mg/L, SD = 0.67), t = -3.4, $p < .01$. Females aged 60 to 79 living in fluoridated regions had the highest level of UF_{SG} at 1.56 mg/L, followed by females aged 40 to 59 at 1.51 mg/L. Participants who reported that they used a fluoridated product (such as toothpaste) less than six hours before the urine sample was collected had significantly higher levels of UF_{SG} (M = 0.94 mg/L, SD = 0.79) than those who did not (M = 0.74 mg/L, SD = 0.61), t = 4.0, $p <.001$. In the linear regression model estimated, we found a significant non-linear effect of age and UF_{SG} such that UF_{SG} remains relatively stable across childhood (ages 3 to 18), but increases over adulthood (ages 19 to 79). Further, CWF status predicted UF_{SG} concentration such that those who live in an area with CWF have a 0.39 mg/L higher level of UF_{SG} (95% CI = 0.24 to 0.53, $p < .01$) compared with those who live in an area without CWF. Further, tap water fluoride, sex, and tea consumption were significantly and positively associated with UF_{SG}, while BMI and smoking allowed in the home were significantly and negatively associated with UF_{SG}.

Conclusions: CWF and tap water fluoride are major sources of contemporary fluoride exposure for Canadian individuals across the lifespan. Lifestyle factors including tea consumption, as well as demographic variables such as age and sex, also predict an individual's level of UF, and may help identify groups at risk of fluoride overexposure.

Introduction

Since the 1940s, fluoride has been added to public drinking water supplies because of its ability to protect against dental caries. About three-fourths of the U.S. population receives fluoridated water as compared with one-third of Canadians (Public Health Agency of Canada, 2017) and only 3% of Europeans (Centers for Disease Control and Prevention, 2014). In the U.S. and Canada, 0.7 mg/L is the recommended concentration of fluoride in drinking water to prevent dental caries (U.S. Department of Health and Human Services, 2015; Health Canada, 2010a). In addition to water, tea is also an important source of fluoride (Li et al., 2009; Waugh, Potter, Limeback, & Godfrey, 2016; Yadav et al., 2007) via absorption of fluoride through soil (Fung, Zhang, Wong, Wong, 1999; Malinowska, Inkielewicz, Czarnowski, & Szefer, 2008). Other sources of fluoride include fluoridated dental products, supplements, and certain foods that contain naturally occurring fluoride (e.g., sardines) or are sprayed with fluoride-containing pesticides (e.g. grapes). Today, the Canadian population is exposed to more sources of fluoride compared to when fluoridation was first introduced in drinking water.

Water fluoride concentrations are moderately (Rango et al., 2014; Till et al., 2018) to strongly correlated (Kumar et al., 2016) with urinary fluoride levels and blood plasma levels (Jain, 2017). In adults (McLaren, 2016; Singh, Gaur, & Garg, 2007; Till et al., 2018) and children (Barberio, Quiñonez, Hosein, & McLaren, 2017; Kumar et al., 2016), urinary fluoride

levels typically approximate the concentration of fluoride found in water with average levels ranging from 0.46 mg/L to 2.48 mg/L. Fluoride levels in drinking water can vary from one geographic location to another depending on whether fluoride is added to public water supplies or because of variations in the concentration of naturally occurring fluoride (Ahmed et al., 2012; McLauren, 2016). Among individuals living in regions with water fluoridation, drinking water is the main source of fluoride intake, accounting for 25 to 70% of total fluoride intake (Jean, Wassef, Gagnon, & Valcke, 2018; United States Environmental Protection Agency, 2010). It is therefore important for surveillance studies to consider variability in water fluoride levels when assessing population fluoride levels.

Population-based biomonitoring studies examining fluoride exposure levels, such as the Canadian Health Measures Survey (CHMS; Saravanabhavan et al, 2017) and the National Health and Nutrition Examination Survey (NHANES; Jain, 2017), provide nationally representative reference values for fluoride, but do not present separate values for those who live in an area with community water fluoridation (CWF) and those who do not. This is important given that a bimodal distribution of fluoride exposure is expected. Likewise, the Human Biomonitoring Report (Health Canada, 2015) provides urinary fluoride levels by age and sex, but collapses across participants living in fluoridated and non-fluoridated regions. It is important to analyze exposure levels by CWF status in order to identify groups at risk of overexposure or fluoride-related health effects (e.g. dental fluorosis). Biomonitoring studies that do report urinary fluoride levels by CWF status show that levels of urinary fluoride are approximately 1.5 to 2 times higher in fluoridated regions compared to non-fluoridated regions (Green et al., 2019; McLaren, 2016; Till et al., 2018). Adverse health outcomes including dental fluorosis have been shown to be higher in areas with CWF (National Research Council, 2006). Because of multiple fluoride

exposure sources and growing concerns about the safety of CWF, it is critical for risk assessment studies to examine the relation between contemporary fluoride exposure levels as a function of water fluoride levels.

Plasma fluoride levels reach peak levels at 60 minutes following absorption and return to baseline within 11 to 15 hours, making the half-life between 5 and 8 hours (Martínez-Mier, 2012; Whitford, 1996). Fluoride is excreted in urine by the kidneys (Jain, 2017; Buzalaf & Whitford, 2011). Many factors can modify fluoride metabolism and excretion, including sex, age, skeletal growth, body mass index (BMI), ethnicity, and cigarette smoking (Aylward et al., 2015; Environmental Protection Agency, 2010; Jain, 2017; National Research Council, 2006). Medical conditions such as diabetes, kidney dysfunction, and iodine decificency can also modify fluoride absorption and metabolism (Buzalaf & Whitford, 2011; Malin, Riddell, McCague, & Till, 2018). Knowledge about how these factors are associated with exposure measurements is crucial for research aimed at understanding examining health outcomes associated with fluoride exposure.

The objective of this study was to describe how urinary fluoride levels differ by age, sex, and CWF, and to examine the association between water fluoride and urinary fluoride levels by age and CWF. We also examined sources of fluoride exposure, including tap water fluoride and tea consumption, and how they impact UF levels, while controlling for covariates such as age, sex, BMI, ethnicity, income, highest household education, and exposure to cigarette smoke.

Methods

Data Source and Participants

We used data from Cycle 3 (2012-2013) of the CHMS collected by Statistics Canada. All aspects of the CHMS were reviewed and approved by Health Canada's Research Ethics

Board (Day, Langlois, Tremblay, & Knoppers, 2006); the current study was approved by the York University Research Ethics Board.

The CHMS randomly selected participants aged 3 to 79 years who live in private households across Canada. Analyses were based on 2671 participants for whom water fluoride and urinary fluoride data were available (46% of the full environmental urine subsample). Tap water samples were collected in the home during the initial questionnaire and urine samples were collected at a mobile lab (Statistics Canada, 2017). Full details can be found at www.statcan.gc.ca.

Community water fluoridation (CWF) status was determined by viewing reports on each city's website or contacting the water treatment plant (see Supplemental Table 1). Of the 16 sites in total, 5 received CWF and 5 did not receive CWF between 2012-2013, corresponding to approximately 600 (22%) and 550 (21%) of 2617 participants included in the study, respectively (rounded due to Statistics Canada data release requirements). Six additional sites were considered to have mixed fluoridation status, corresponding to about 850 (31%) participants, and were excluded from all analyses. A site was classified as mixed for one of five reasons: unclear site boundaries (n=150), having some municipalities within the site add fluoride while others do not (n=150), and stopping water fluoridation during the period spanning CHMS data collection (n=150). We excluded all participants from sites that were labeled as fluoridated or non-fluoridated, but had an average water fluoride level that was either 2.5 times lower (n = 250) or higher (n = 150) than other fluoridated or non-fluoridated sites. We excluded all participants from mixed sites, leading to a final sample size of 1629.

Measurement of Water Fluoride Concentration

Tap water samples were collected at respondents' homes. Samples were analyzed for fluoride concentrations (mg/L) via a basic anion exchange chromatography procedure with a LoD of 0.006 mg/L. Concentrations at the LoD were assigned a missing value code by Statistics Canada, and these values were subsequently replaced with an imputed value of LoD/√2 (Hornung & Reed, 1990); 435 of 1629 (27%) water samples had fluoride levels below the LoD.

Measurement of Urinary Fluoride Concentration

Urine spot samples were collected under normal (non-fasting) conditions and were not standardized with respect to collection time. Fluoride concentrations in spot urine samples were analyzed using an Orion pH meter with a fluoride ion selective electrode after being diluted with an ionic adjustment buffer (Statistics Canada, 2013). Urinary analyses were performed at the Human Toxicology Laboratory of the Institut National de Santé Publique du Québec (INSPQ; accredited under ISO 17025) under standardized operating procedures (Statistics Canada, 2013; 2015). The precision and accuracy of the fluoride analyses, including quality control measures and quality assurance reviews, are described in previous publications (Health Canada, 2015). The limit of detection (LoD) for urinary fluoride was 10 μg/L for Cycle 3 (Health Canada, 2015). No urinary fluoride values were below the LoD. Urinary fluoride concentrations were adjusted for specific gravity (UF_{SG}; mg/L) because specific gravity shows no systematic variation within a day and is less dependent on body size, age, and sex than creatinine (Barr et al., 2005; Nermell et al., 2008; Moriguchi et al., 2005; Suwazono, Åkesson, Alfven, Järup, & Vahter, 2005).

Drinking Water Habits

Participants were asked the following questions: *When you drink water at home, what is your primary source of drinking water?* (tap water, bottled water, or other); and *What is the source of the tap water in this home?* (municipal, private well, or other). Of the total sample of 1629, 461 people did not answer these questions on drinking water habits, whereas 930 of 1168 (80%) participants reported drinking primarily tap water at home and 197 (17%) reported drinking primarily bottled water at home.

Modifiers of Exposure Levels

Variables that may modify fluoride metabolism and excretion were selected based on literature review (Aylward et al., 2015; Buzalaf, Leite, & Buzalaf, 2015; Buzalaf & Whitford, 2011; Jain, 2017). We examined the following covariates: sex, age at household interview, ethnicity (white or ethnic minority), BMI, highest household level of education (less than a bachelor's degree vs. bachelor's degree or greater), total household income (per $1000 Canadian), and whether smoking is allowed inside the home (yes/no).

Other Sources of Fluoride Exposure

Regarding tea consumption, 670 out of 1629 participants (41%) stated that they drink green, black, or white tea at least once per year. Those who responded that they drink tea at least once a year were asked follow-up questions, including the number of cups they typically drink (less than one cup, one to two cups, or more than two cups) and the last time they drank green, black, or white tea (within 24 hours of the urinary fluoride sample collection or more than 24 hours ago). Given that 959 participants (59%) reported that they do not drink tea or did not answer the question, tea consumption was coded as a binary variable (yes = 1, no = 0) for inclusion in the regression model.

Participants were asked about the last time they used a fluoride-containing product out of four options (less than 6 hours ago, 6 to less than 12 hours ago, 12 to less than 24 hours ago, and 24 hours ago or more). Due to the short half-life of fluoride, the response options were combined to create a binary variable (less than 6 hours ago = 1, 6 or more hours ago = 2). Participants were also asked about the last time they received fluoride treatments at the dentist out of five possible response options (less than 3 months ago, 3 to less than 6 months ago, 6 to less than 9 months ago, 9 to less than 12 months ago, and 12 or more months ago); these response options were combined to create a binary variable (less than 3 months ago vs. 3 months ago or more).

Statistical Analysis

One case was identified as an outlier based on a Cook's Distance greater than 4/N. This individual had the largest value of $UF_{SG,}$ which was about 7 times greater than the mean for their age group. The Cook's Distance value and UF_{SG} cannot be reported due to Statistics Canada policies regarding the release of individual data points. This outlying case was removed from all analyses. Individuals with the highest incomes were identified as extreme observations; these values were replaced with the next highest income value (only 0.01% of income values were adjusted). Consistent with previous publications (e.g., Health Canada, 2015; Jain, 2017), UF_{SG} values are presented for six age groups: 3 to 6, 7 to 11, 12 to 18, 19 to 39, 40 to 59, and 60 to 79 years. These age groups were also used when examining the correlation between UF_{SG} and tap water fluoride. For all other analyses, age was treated as a continuous variable. We used independent samples Welch t-tests to examine whether UF_{SG} differed by sex or CWF.

We used Spearman rank correlations to explore the relationship between UF_{SG} and tap water fluoride in the age groups described above. We then used linear regression to examine

the associations between UF_{SG} and predictors of fluoride exposure (CWF status, tap water fluoride, tea consumption, primary source of drinking water, time since last fluoride treatment at dentist, and time since use of a fluoride-containing dental product), controlling for covariates (age, sex, ethnicity, BMI, education, income, and exposure to smoking). Plots of the residuals by fitted values were examined, as well as plots of the residuals for specific variables such as age. Because age appeared to have a non-linear relation with UF_{SG}, the quadratic age effect was included in the regression model. No issues with multicollinearity were detected from variance inflation factor (VIF) statistics. We planned to test two interactions based on a priori hypotheses: age by sex and CWF by type of drinking water (tap or bottled). A two-sided $\alpha = .05$ was used as the threshold for statistical significance.

Results

Descriptive Statistics

For participants with non-missing tap water, UF measurements, and CWF status, most demographic variables (including sex, age, and highest household education) had less than 5% missing data. For ethnicity, 11% of data were missing and 15% of participants did not report their BMI.

In this sample, the proportion of males and females was approximately equal (49% male and 51% female). The mean age was 32 years old (range 3 to 79 years) and 73% were white. Regarding the highest level of household education, half of the sample (50%) had a high school, trade school, or college degree, while the other half (50%) had a university degree or higher. The mean household income was $87,700 (median = $73,000) and the mean BMI was 24. About half of participants (49%) reported that they drink green, black or white tea at least once per year, while the other half (51%) reported that they never drink any of these types of tea. Of those who

reported drinking tea at least once per year (n = 670), 210 (31%) reported drinking tea within 24 hours of the urine sample collection. Approximately half of the participants (53%) lived in a region that adds fluoride to the municipal tap water, whereas 47% do not. The mean UF_{SG} concentration was 0.87 mg/L (skewness = 4.0 and kurtosis = 34.6), and the mean water fluoride concentration was 0.28 mg/L.

Table 2.1
UF_{SG} *(mg/L) across sex and age groups in fluoridated versus non-fluoridated regions*

	FLUORIDATED					NON-FLUORIDATED				
Age group in years	*n*	*Mean*	*SD*	*Median*	*95% CI*	*n*	*Mean*	*SD*	*Median*	*95% CI*
Ages 3 to 6	**180**	**0.82**	**0.59**	**0.70**	**0.27, 1.68**	**185**	**0.57**	**0.41**	**0.46**	**0.18, 1.29**
Males	93	0.80	0.57	0.70	0.27, 1.58	86	0.52	0.32	0.46	0.16, 1.16
Females	87	0.83	0.61	0.72	0.27, 1.71	99	0.61	0.47	0.46	0.21, 1.43
Ages 7 to 11	**145**	**0.88**	**0.78**	**0.72**	**0.40, 1.52**	**123**	**0.46**	**0.28**	**0.38**	**0.18, 1.10**
Males	77	0.77	0.32	0.72	0.40, 1.27	57	0.43	0.27	0.38	0.18, 1.20
Females	68	1.00	1.07	0.75	0.40, 2.09	66	0.43	0.25	0.38	0.18, 1.01
Ages 12 to 18	**165**	**0.79**	**0.37**	**0.70**	**0.34, 1.52**	**140**	**0.47**	**0.34**	**0.40**	**0.21, 0.90**
Males	79	0.79	0.35	0.72	0.34, 1.50	67	0.43	0.27	0.38	0.18, 0.87
Females	86	0.78	0.38	0.67	0.36, 1.60	73	0.51	0.44	0.57	0.23, 0.93
Ages 19 to 39	**139**	**1.24**	**0.78**	**1.05**	**0.44, 2.47**	**119**	**0.59**	**0.38**	**0.51**	**0.23, 1.22**
Males	64	1.22	0.78	1.05	0.42, 2.47	58	0.50	0.28	0.44	0.23, 1.08
Females	75	1.25	0.77	1.05	0.49, 2.66	61	0.68	0.44	0.57	0.27, 1.22
Ages 40 to 59	**104**	**1.46**	**0.86**	**1.27**	**0.48, 3.61**	**102**	**0.73**	**0.66**	**0.50**	**0.21, 2.09**
Males	42	1.38	0.84	1.16	0.55, 3.42	55	0.65	0.62	0.49	0.19, 1.71
Females	62	1.51	0.88	1.34	0.42, 3.61	47	0.81	0.70	0.59	0.21, 2.28
Ages 60 to 79	**121**	**1.47**	**1.24**	**1.06**	**0.42, 3.80**	**106**	**0.72**	**0.64**	**0.54**	**0.23, 2.09**
Males	58	1.37	1.39	1.00	0.36, 4.37	49	0.51	0.33	0.44	0.21, 1.03
Females	63	1.56	1.09	1.14	0.46, 3.80	57	0.89	0.78	0.65	0.27, 2.66
Total	**854**	**1.06**	**0.83**	**0.84**	**0.36, 2.47**	**775**	**0.58**	**0.47**	**0.46**	**0.19, 1.43**
Males	413	1.00	0.78	0.80	0.34, 2.09	372	0.51	0.37	0.43	0.18, 1.08
Females	441	1.12	0.86	0.86	0.38, 2.66	403	0.64	0.53	0.49	0.21, 1.60

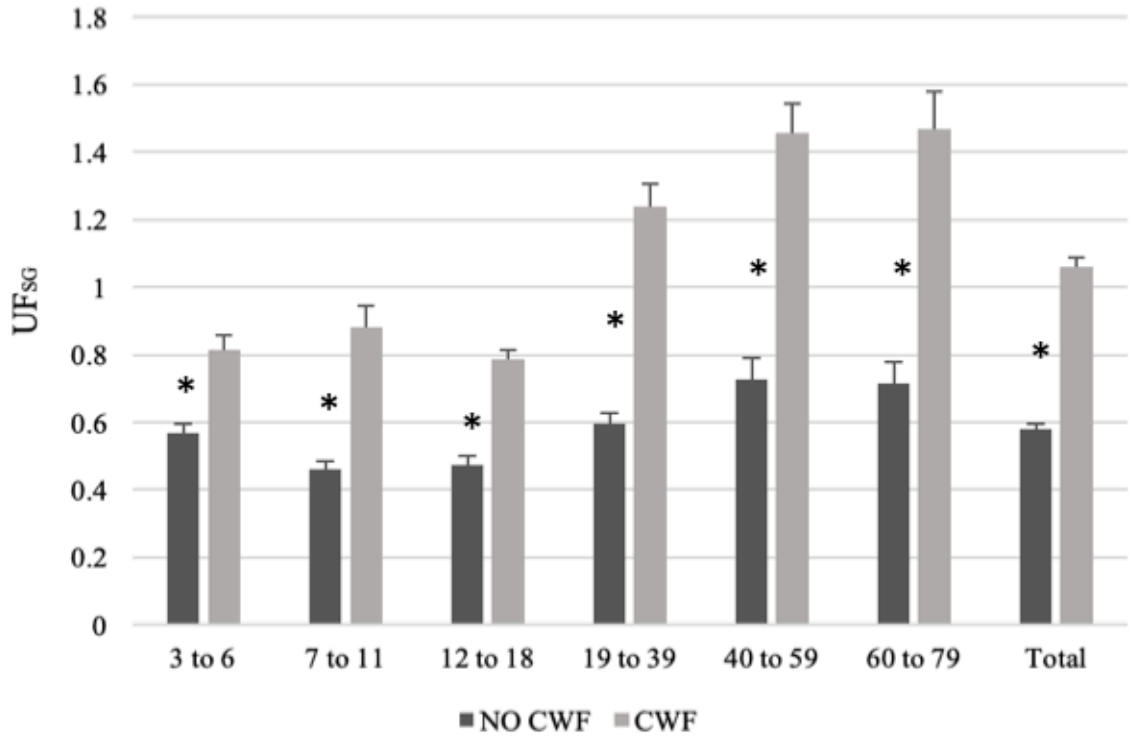


Figure 2.1

Comparison of UF_{SG} *(mg/L) by age group and CWF status*

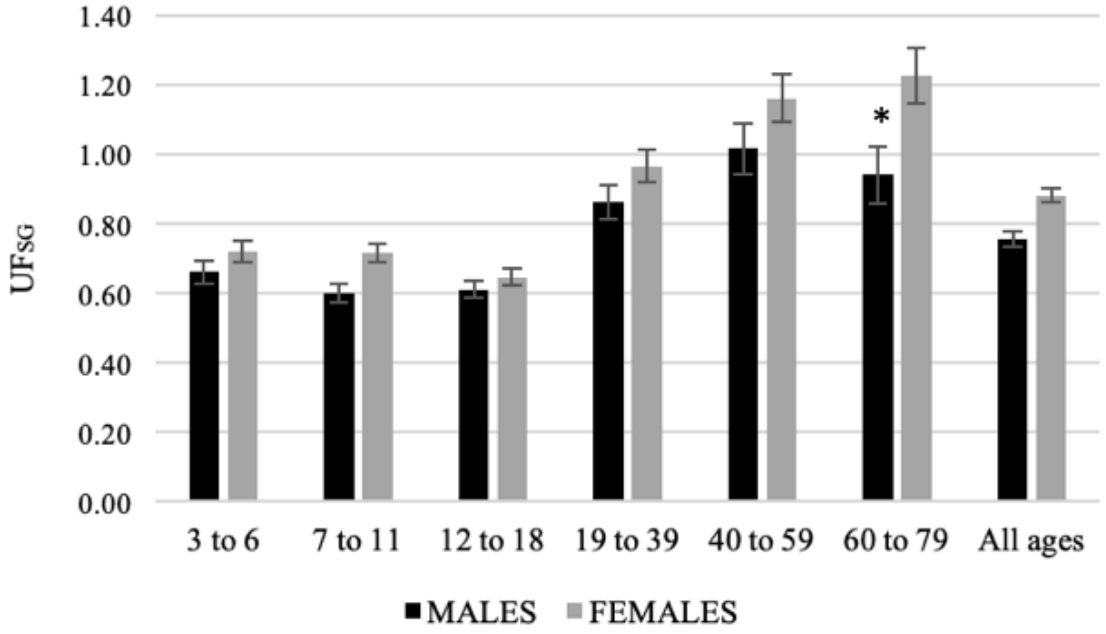


Figure 2.2

Comparison of UF_{SG} *(mg/L) by age group and sex*

Urinary Fluoride Measurements

The mean levels of UF_{SG} (mg/L) by sex, age, and CWF status are presented in Table 1. Figure 2.1 shows the mean levels of UF_{SG} by age group in fluoridated and non-fluoridated regions. For each of the six age groups, those living in a fluoridated region had significantly higher UF_{SG} than those living in a non-fluoridated region (t = -8.6 to -4.3, $p < .01$ after controlling for multiple comparisons using the Bonferroni correction). When collapsed across age groups, UF_{SG} concentration was significantly higher among participants who received fluoridated drinking water (M = 1.06 mg/L, SD = 0.83) than among those who did not (M = 0.58 mg/L, SD = 0.47), t = -13.7, $p < .01$. Figure 2.2 shows the mean levels of UF_{SG} by age group for males and females collapsed across CWF. When males and females were compared at each age group, collapsed across CWF, the only statistically significant difference was between females ages 60 to 79 (M = 1.16 mg/L, SD = 1.00) and males ages 60 to 79 (M = 0.94 mg/L, SD = 0.97), t = -2.0, p = .04. Overall, females had significantly higher UF_{SG} concentration (M = 0.89 mg/L, SD = 0.76) than males (M = 0.77 mg/L, SD = 0.67), t = -3.4, $p < .01$. Females aged 60 to 79 living in fluoridated regions had the highest level of UF_{SG} at 1.56 mg/L, followed by females aged 40 to 59 at 1.51 mg/L. Males age 7 to 18 living in non-fluoridated regions had the lowest levels of UF_{SG} at 0.43 mg/L.

Spearman rank correlations between UF_{SG} (mg/L) and tap water fluoride concentration (mg/L) by age group are shown in Table 2.2. All correlations were positive and statistically significant, with correlations between r =.18 and r =.44. The correlation between UF_{SG} (mg/L) and tap water fluoride concentration (mg/L) was strongest for adults ages 40 to 59 (r = .42), followed by adults ages 19 to 39 (r = .42). As expected, the correlation between UF_{SG} (mg/L) and tap water fluoride concentration (mg/L) was weakest for children ages 3 to 6 (r = .18).

Table 2.2

Correlations between tap water fluoride and urinary fluoride for each age group

Age group	r value (n = 1629)
Age 3 to 6	0.18*
Age 7 to 11	0.24*
Age 12 to 18	0.35*
Age 19 to 39	0.42*
Age 40 to 59	0.44*
Age 60 to 79	0.36*
All ages	0.31*

Differences in UF_{SG} by drinking water habits and dental product use

In fluoridated regions, UF_{SG} concentration was significantly higher among participants who report drinking primarily tap water (M = 1.09 mg/L, SD = 0.86) than among those who report drinking primarily bottled water (M = 0.95 mg/L, SD = 0.67), t = 2.06, p =.04. In non-fluoridated regions, UF_{SG} concentration was similar among participants who report drinking primarily tap water (M =0.58 mg/L, SD = 0.48) compared with those who report drinking primarily bottled water (M = 0.56 mg/L, SD = 0.39), t = 0.72, p =.47. In fluoridated regions, nearly all participants reported being on municipal tap water and very few reported being on a private well (t-tests not permitted due to Statistics Canada sample size requirements for data release). In non-fluoridated regions, UF_{SG} concentration was significantly higher among participants who receive their water from a private well (M = 0.73 mg/L, SD = 0.57) than among those who receive municipal tap water (M = 0.54 mg/L, SD = 0.43), t = -3.53, p <.01.
The UF_{SG} concentration among participants who report using fluoridated products at home (M = 0.88 mg/L, SD = 0.77) was similar among those who do not use fluoridated products at home (M = 0.86 mg/L, SD = 0.89), t = 0.23, p = .82. However, participants who reported that they used a fluoride-containing product (such as toothpaste) less than six hours before the urine sample was

collected had significantly higher levels of UF_{SG} (M = 0.94 mg/L, SD = 0.79) than those who reported using a fluoride-containing product six or more hours before the sample collection (M = 0.74 mg/L, SD = 0.61), t = 4.0, p <.001. Table 3 compares UF_{SG} across age groups based on the recency of their use of fluoridated products. Children age 3 to 6 years old who used fluoride-containing products within six hours of the sample collection had significantly higher levels of UF_{SG} than those who did not, t = 3.6, p <.001. Similarly, adults age 19 to 39 years old (t = 2.6, p =.01) and age 40 to 59 years old (t = 2.3, p =.02) who used fluoride-containing products within six hours of the sample collection had significantly higher levels of UF_{SG} than those who did not.

Table 2.3

Comparison of UF_{SG} *across age groups based on recency of fluoridated produce use*

Age group	Used a fluoridated product (e.g., fluoridated toothpaste or mouthwash) **less** than 6 hours before sample collection				Used a fluoridated product (e.g., fluoridated toothpaste or mouthwash) 6 or **more** hours before sample collection					
	n	*Mean (95% CI)*	*SD*	*Median*	*n*	*Mean (95% CI)*	*SD*	*Median*	*t*	*p*
Age 3 to 6	94	0.81 (0.72, 0.93)	0.62	0.72	144	0.62 (0.56, 0.67)	0.39	0.51	3.60	<.001
Age 7 to 11	88	0.64 (0.58, 0.69)	0.32	0.57	129	0.68 (0.55, 0.69)	0.67	0.53	0.28	.78
Age 12 to 18	136	0.65 (0.55, 0.64)	0.34	0.58	110	0.64 (0.54, 0.65)	0.37	0.55	0.04	.97
Age 19 to 39	139	1.01 (0.83, 0.99)	0.70	0.84	86	0.83 (0.66, 0.84)	0.58	0.67	2.65	.01
Age 40 to 59	93	1.27 (0.96, 1.18)	0.86	1.10	61	0.93 (0.73, 1.00)	0.85	0.67	2.27	.02
Age 60 to 79	103	1.29 (1.02, 1.40)	1.29	0.91	53	1.07 (0.81, 1.14)	0.85	0.84	1.88	.06
Total	653	0.94	0.79	0.72	583	0.74	0.61	0.59	4.00	<.001

Participants who reported that they drank green, black, or white tea within 24 hours of the urine sample collection had significantly higher levels of UF_{SG} (M = 1.31 mg/L, SD = 1.04) than those who did not (M = 0.87 mg/L, SD = 0.67), t = 5.6, p <.01. The amount of tea consumed was also significantly related to UF_{SG} concentration, such that participants who reported that they typically drink two or more cups of green, black, or white tea at a time had higher levels of UF_{SG} (n=110, M = 1.40 mg/L, SD = 1.15) than those who reported drinking one cup of tea (n=670, M = 0.93 mg/L, SD = 0.73), t = -4.1, p <.01.

Predictors of Urinary Fluoride Concentration

To determine how a number of predictors impact UF levels, we estimated a linear regression model with the following predictors: water fluoride in mg/L, CWF status, tea consumption, primary source of drinking water (tap or bottled), last fluoride treatment at dentist, and time since use of a fluoride-containing dental product, while controlling for age, sex, BMI, ethnicity, highest household education, income in $100,000 CND, and exposure to cigarette smoke. To account for the non-linear relationship between age and urinary fluoride, we included both age and age^2 in the model. We tested three two-way interactions: age by sex, age^2 by sex, and CWF by primary source of drinking water (tap or bottled). Because none of these interactions was significant, only main effects are presented.

Tap water fluoride level, CWF, age, sex, BMI, smoking allowed in the home, and tea consumption were significant predictors of UF_{SG} (see Table 2.4). Tap water fluoride predicted UF_{SG} concentration such that for every 1 mg/L increase in tap water fluoride, there is a predicted increase of 0.48 mg/L UF_{SG} (95% CI = 0.25 to 0.71), holding the other predictors constant. Compared with those who live in a non-fluoridated region, individuals who receive CWF have a 0.39 mg/L higher level of UF_{SG} (95% CI = 0.24 to 0.53). In the linear regression model estimated,

we found a significant non-linear effect of age and UF_{SG} such that UF_{SG} remains relatively stable across childhood (ages 3 to 18), but increases over adulthood (ages 19 to 79), as can be seen in Figure 2.3.

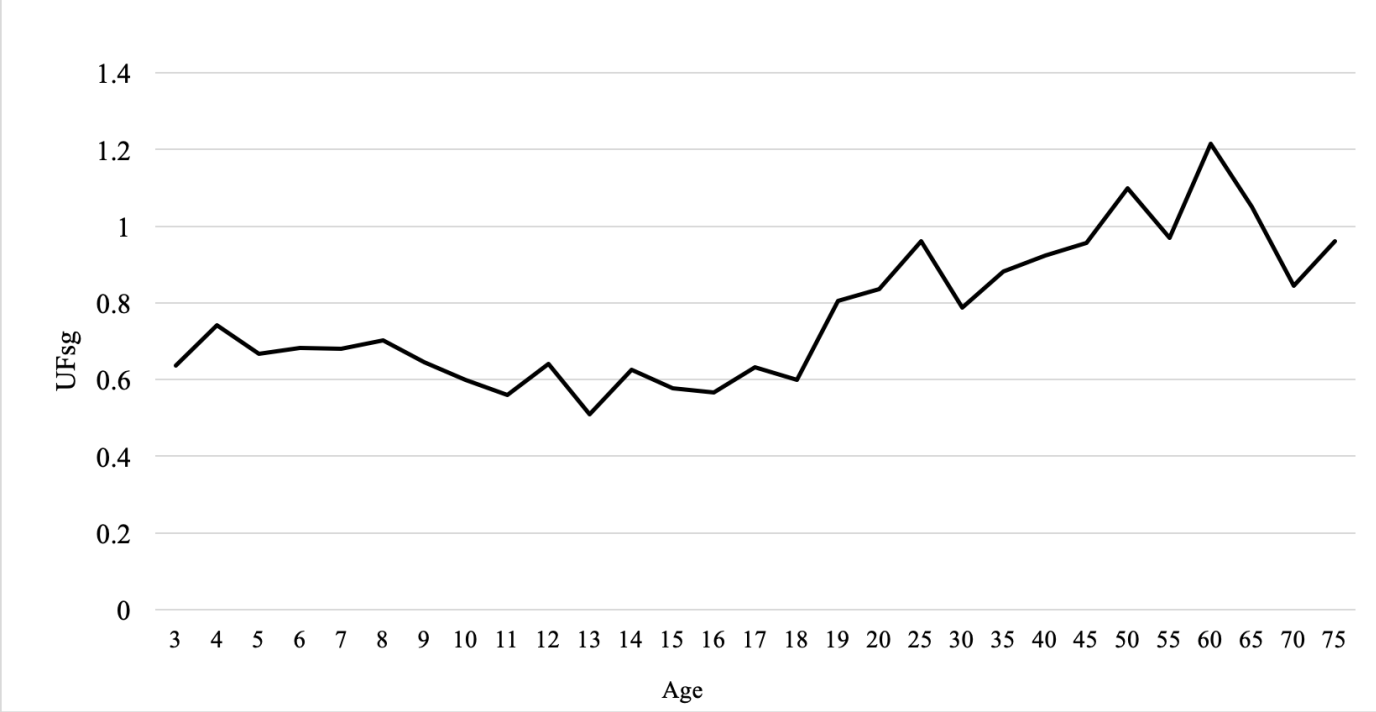


Figure 2.3

UF_{SG} *(mg/L) by age (in years)*

Compared to males, females had a 0.12 mg/L higher level of UF_{SG} (95% CI = 0.03 to 0.20), holding the other predictors constant. Further, BMI predicted UF_{SG} concentration such that for every kg/m2 increase in BMI, there is a predicted decrease of 0.02 mg/L UF_{SG} (95% CI = -0.02 to -0.01). Compared to those who allow smoking in their home, those who do not allow smoking in the home have a 0.25 mg/L lower level of UF_{SG} (95% CI = -0.41 to -0.09). Finally, compared to individuals who do not drink tea, people who report drinking green, black, or white tea had a 0.13 mg/L higher level of UF_{SG} (95% CI = 0.03 to 0.22). Overall, the predictors in this model accounted for 27% of the variance in UF_{SG}. Ethnicity, highest household education, income,

primary source of drinking water (tap or bottled water), and recency of receiving fluoride treatment at dentist were not associated with UF_{SG}

Table 2.4

Linear regression predicting UF_{SG} *(mg/L)*

	B	SE	*t*	*p*	95% CI
Water fluoride (mg/L)	0.48	0.12	4.10	<.01	0.25, 0.71
CWF status	0.39	0.07	5.23	<.01	0.24, 0.53
Age	0.03	0.01	4.65	<.01	0.01, 0.04
Age^2 (per 100 years)	-0.02	0.01	-3.02	<.01	-0.03, -0.01
Sex (ref: male)	0.12	0.04	2.60	.01	0.03, 0.20
BMI	-0.02	0.00	-3.26	<.01	-0.02, -0.01
Ethnicity (ref: white)	-0.07	0.05	-1.27	.20	-0.17, 0.04
Household education (ref: less than bachelor's degree)	0.05	0.05	0.93	.35	-0.05, 0.14
Income (per $100,000 CND)	-0.04	0.03	-1.36	.17	-0.10, 0.02
Smoking allowed in the home (ref: yes)	-0.25	0.08	-3.02	<.01	-0.41, -0.09
Tea consumption (ref: none)	0.13	0.05	2.67	.01	0.03, 0.22
Primary source of drinking water (ref: tap)	-0.01	0.09	-0.16	.87	-0.18, 0.16
Last fluoride treatment at dentist (ref: < 3 months ago)	-0.03	0.06	-0.55	.58	-0.15, 0.08
Time since use of a fluoride-containing dental product (ref: < 6 hours ago)	-0.09	0.05	-1.98	.05	-0.18, 0.00

Note. $N = 900$, $R^2 = .27$, $F(15, 883) = 22.16$, $p < .01$.

Discussion

In Canada, the current recommended level of fluoride in drinking water is 0.7 mg/L for community water fluoridation (CWF), though naturally-occurring levels of fluoride can exceed this standard in some regions. Given that drinking water is a main source of fluoride exposure for

most individuals (Jean et al., 2018; United States Environmental Protection Agency, 2010), this study sought to describe differences in urinary fluoride adjusted for specific gravity (UF_{SG}) as a function of CWF status, age, and sex. As expected, the mean UF_{SG} concentration was significantly higher among participants receiving fluoridated drinking water (M = 1.06 mg/L, SD = 0.83) than those receiving non-fluoridated water (M = 0.58 mg/L, SD = 0.47). This difference is consistent with other Canadian studies reporting that individuals living in fluoridated regions have between 1.5 and 2 times greater UF concentration than individuals living in non-fluoridated regions (Green et al., 2019; McLaren, 2016; Till et al., 2018). These findings underscore the importance of measuring exposure levels as a function of CWF status, especially in the context of risk assessment studies. For example, the Canadian Health Measures Survey 2007-2009 Oral Health Component reports a prevalence of less than 13% for mild to more severe forms of fluorosis (Health Canada, 2010b). However, given the strong influence of CWF on overall fluoride exposure, is it essential to understand what proportion of children with and without CWF have fluorosis.

Tap water fluoride predicted UF_{SG} concentration such that for every 1 mg/L increase in tap water fluoride, there is a predicted increase of 0.48 mg/L UF_{SG}, after controlling for covariates. However, the correlation between tap water fluoride and UF_{SG} was strongest for adults ages 19 to 39 (r = .42) and adults ages 40 to 59 (r = .44) and weakest for children ages 3 to 6 (r = .18). These results suggest that young children (i.e., those ages 3 to 6 years old) receive fluoride from sources other than CWF or are more likely to absorb fluoride into developing bone. These sources may include fluoride-containing products (e.g., Teflon), pharmaceuticals, pesticides, dental products (e.g., toothpaste, mouth rinses) or beverages such as soda and juice made with fluoridated water. For example, white grape juice has the highest level of fluoride

among juices (with a mean of 1.45 mg/L) due to the use of cryolite as a pesticide on grapes (United States Environmental Protection Agency, 2010). Another study found that a town receiving CWF at 1.08 mg/L had higher levels of fluoride in home-prepared juices, soups, tea, coffee, and other foods and beverages prepared with tap water than a Canadian town with low levels of naturally occurring fluoride at 0.23 mg/L (Clovis & Hargreaves, 1988 as cited in United States Environmental Protection Agency, 2010). A relative source contribution analysis by the United States Environmental Protection Agency (2010) determined that water and other beverages account for approximately 60% of dietary fluoride intake among adults, but only 40% of dietary fluoride intake for children 1 to 10 years old. Therefore, it is possible that tap water fluoride was only weakly correlated with UF_{SG} in young children because they receive most of their fluoride from other sources, such as commercially prepared juices and fluoridated dental products.

Demographic Predictors of Exposure

Demographic characteristics such as age and sex were related to important differences in urinary fluoride levels. For example, we found children and adolescents living in both fluoridated and non-fluoridated regions had lower levels of fluoride excretion compared with adults. Previous studies have found that young children (aged 1 to 4 years) have a higher daily intake of fluoride from various sources as compared with adults, regardless of CWF status (Health Canada, 2010a), suggesting that lower excretion of fluoride in children may be due to increased fluoride absorption during periods of bone growth (Jha, Mishra, Sharma, & Damodaran, 2011; World Health Organization, 1997). Further, women aged 60 to 79 living in fluoridated regions had the highest level of UF_{SG} at 1.56 mg/L, followed by women aged 40 to 59 at 1.51 mg/L. Men age 7 to 18 living in non-fluoridated regions had the lowest levels of UF_{SG}

at 0.43 mg/L. The overall finding that women have higher UF than men is consistent with the Canadian Health Measures Survey (CHMS) Cycle 3 Biomonitoring Report (Health Canada, 2015). One reason that older women have higher UF_{SG} than men is due to increased fluoride release from bone after menopause (Itai et al., 2010), as well as consuming more tea, which contains high levels of fluoride (Malinowska et al., 2008).

Urinary fluoride levels have also been shown to vary by ethnicity. In one study, Hispanic adolescents from the United States aged 12 to 19 years had significant lower fluoride in plasma samples than non-Hispanic whites (Jain, 2017). Our study did not find that ethnicity significantly predicted UF, although this could be due to the fact that our sample was 73% white.

Other Exposure Factors

Tea consumption over the past year predicted UF_{SG} concentration such that compared with individuals who did not drink tea, people who reported drinking green, black, or white tea had a 0.13 mg/L higher level of UF_{SG}, controlling for covariates. This finding is consistent with previous research which demonstrates the contribution of tea to urinary fluoride levels (Fung et al., 1999, Malinowska et al., 2008; Till et al., 2018).

Further, smoking cigarettes has been found to modify fluoride metabolism such that individuals who smoke cigarettes have been reported to have significantly higher levels of fluoride in plasma (Jain, 2017). Consistent with this finding, we found that individuals who live in homes in which smoking is not permitted have 0.25 mg/L lower UF_{SG} compared with those who live in homes in which smoking is permitted inside.

We found that participants who reported using a fluoridated product (such as toothpaste or mouthwash) less than six hours before the urine sample was collected had higher levels of UF_{SG} than those who did not. As expected, this effect was especially strong for children ages 3 to

6, who had a mean UF of 0.81 mg/L if they used a fluoridated product less than six hours before the sample compared with a mean UF of 0.62 mg/L if they had not used a fluoridated product within six hours of the sample. This result is consistent with a study from Nigeria which found that diet and toothpaste ingestion were the primary sources of fluoride intake in children ages 4 to 8 years old (Ibiyemi, Zohoori, Valentine, & Maguire, 2018). These findings show that urinary fluoride varies substantially depending on participant behaviour prior to sampling. To account for this variation, future studies that use urinary fluoride as a biomarker would benefit from the use of multiple spot samples whenever possible, especially if the sample includes young children.

Relatedly, because only a single spot sample was taken, it is also possible that the urinary fluoride levels reported were influenced by behaviours prior to sampling and may not be representative of long-term fluoride exposure; these behaviours include having varnish professionally applied or swallowing toothpaste prior to urine sampling. To further investigate this possibility, we examined urinary fluoride concentration by age and recency of toothpaste use. Young children (ages 3 to 6) who reportedly used a fluoridated product (such as toothpaste or mouthwash) less than six hours before the urine sample collection had significantly higher levels of UF_{SG} compared to those who reported using a fluoridated product more than six hours before the sample collection. This result is consistent with previous research on the impact of accidental toothpaste ingestion on urinary fluoride levels in young children whose spitting reflex is not fully developed (Ibiyemi et al., 2018; Thornton-Evans et al., 2019). Our findings also concur with previous studies showing increases in urinary fluoride in children aged 5–8 years after using fluoride containing-dental varnishes (García-Hoyos, Barbería, García-Camba, & Varela, 2012). These findings underscore the importance of inquiring about recent behaviours when measuring urinary fluoride levels.

Strengths and Limitations

A strength of this study was the use of biomarkers, corrected for dilution, and assessment of various sources of fluoride to understand how different factors contribute to fluoride levels across the lifespan. Other strengths include the large sample size and the fact that multiple sites were recruited. The relatively even sampling of sites that do and do not receive CWF facilitated our use of CWF status as a predictor.

One limitation of the current study is that the CHMS used only a single urinary fluoride spot sample. Because urinary fluoride levels in spot samples can fluctuate due to the rapid elimination kinetics of fluoride and also due to differences in lifestyle factors such as beverage consumption and use of fluoridated dental products, it would be ideal to obtain multiple urine samples over time to calculate more stable estimates of fluoride excretion. Further, we did not measure the amount of water each participant consumes per day to estimate total exposure (intake) from water consumption. Additional studies are needed to assess the relationship between fluoride intake, absorption, and excretion across demographic characteristics such as age and sex. We also cannot rule out the possibility that urinary fluoride values may capture acute exposures to very high fluoride exposures, such as professionally applied varnish or fluoridated anaesthetics. This study was also cross-sectional, examining contemporaneous fluoride excretion and predictors such as age. To better understand how urinary fluoride changes over the lifespan, it would be helpful to conduct longitudinal research with the same participants over time.

In conclusion, CWF was associated with UF_{SG}, demonstrating that fluoride in drinking water supplies is an important contributor to overall fluoride exposure for both children and adults. However, depending on age, there are other factors that impact UF concentration, such

as the recency of dental product use. There are multiple sources of fluoride exposure, including CWF, and a growing number of studies raise the possibility of adverse health effects from fluoride exposure in the developing fetus (Bashash et al., 2017; Green et al., 2019) young children (Choi et al., 2015; Riddell, Malin, Flora, McCague, & Till, 2019), and in other vulnerable populations, such as those who are iodine deficient (Malin, Riddell, McCague, & Till, 2018). The mean UF_{SG} levels in our sample (0.58 mg/L to 1.06 mg/L, depending on CWF status) are within the range reported by studies showing adverse effects. For example, Green and colleagues (2019) found that pregnant women living in areas with fluoridated tap water had a mean UF_{SG} concentration of 0.69 mg/L, and that each 1 mg/L higher maternal UF_{SG} was associated with a 4.49-point lower IQ score in boys. Similarly, Bashash and colleagues (2017) found that mothers in Mexico had mean UF levels of 0.9 mg/L, and each 0.5 mg/L higher maternal UF_{SG} was associated with 3.15-point lower IQ in their children.

More research is needed to understand fluoride intake and circulating fluoride levels in elderly populations, including those with disorders that may impact fluoride metabolism such as kidney dysfunction. Important questions remain regarding differences in UF across demographic groups (e.g. sex, age, ethnicity, etc.), and whether the levels of UF in the present study are associated with adverse outcomes for these groups. Further research is needed to quantify human fluoride exposure levels and identify subgroups who may have especially high levels of exposure, such as elderly women. This information is critical for informing public health recommendations and weighing the benefits of fluoride for the prevention dental caries with potential health risks associated with ingestion.

Chapter Four: General Discussion

This dissertation used Canadian biomonitoring data to examine urinary fluoride levels by fluoridation status and demographic variables and to assess the relationship between fluoride excretion and attention outcomes. In the first study, we found that Canadian youth exposed to higher tap water fluoride levels had a higher risk of receiving an ADHD diagnosis and reported more symptoms of hyperactivity and inattention. Specifically, an increase of 1.0 mg/L in water fluoride concentration was associated with 6.1 times higher odds of an ADHD diagnosis and a 1.5 increase in the SDQ hyperactivity/inattention subscale score while controlling for known predictors of ADHD such as child sex, lead exposure, and smoking in the home. Importantly, most of the participants had water fluoride levels lower than 1 mg/L, with the 95th percentile at 0.7 mg/L. To contextualize these results, the difference in water fluoride concentration between cities with and without community water fluoridation (CWF) is approximately 0.5 mg/L. An increase of 0.5 mg/L in water fluoride concentration would be associated with 2.5 times higher odds of an ADHD diagnosis. The prevalence of ADHD in Canada ranges from 4.8% to 6.1% depending on the study (Charach, Lin, & To, 2010), which is an average prevalence of 5.5%. In our study, the mean water fluoride level was 0.23 mg/L, which is much lower than the recommended level of 0.7 mg/L. Our findings suggest that if the average water fluoride level in Canada was raised from 0.23 mg/L to 0.73 mg/L, we could expect the prevalence rate of ADHD to rise from 5.5% to 14%.

Our second study examined how urinary fluoride levels differ by sex and age and as a function of living in fluoridated and non-fluoridated regions. Previous research has compared urinary fluoride concentration by sex, age, and ethnicity but has rarely separated by CWF status (Barberio, Quiñonez, Hosein, & McLaren, 2017; Jain, 2017). For those living in a fluoridated

region, women's average level of UF_{SG} was 1.12 mg/L, whereas men's average level of UF_{SG} was 1.00 mg/L. Similarly, women living in a non-fluoridated region had a higher level of UF_{SG} at 0.64 mg/L compared to men at 0.51 mg/L. Our finding that women have higher urinary fluoride than males is consistent with a recent Canadian study using urinary fluoride adjusted for creatinine (Health Canada, 2015).

Further, our study found that children ages 7 to 11 living in non-fluoridated regions had the lowest levels of UF_{SG} (0.46 mg/L), followed closely by children ages 12 to 18 with a mean UF_{SG} of 0.47 mg/L. Further, we found that adults age 60 to 79 living in a non-fluoridated region had 1.5 times the UF_{SG} level of children at 0.72 mg/L. To our knowledge, no other studies have examined differences in UF_{SG} by age and CWF. The human biomonitoring report produced by Health Canada (2015) also reported that children ages 12 to 19 have the lowest level of UF adjusted for creatinine at 0.29 mg/g. However, they reported that children ages 6 to 11 had a higher level of UF adjusted for creatinine (0.50 mg/g) compared to adults ages 20 to 39 (at 0.40 mg/g). Part of this discrepancy may be due to the use of creatinine instead of specific gravity to control for dilution, as creatinine has been shown to vary more than specific gravity by body size, age, and sex (Nermell et al., 2008; Moriguchi et al., 2005). This discrepancy may also be due to the lack of separation by CWF in the Health Canada (2015) report.

In our first study, we found that water fluoride concentration was more strongly associated with attention and hyperactivity than UF levels. This could be because UF levels in spot samples are more likely to fluctuate due to the rapid elimination kinetics of fluoride. It could also be the case that fluoride has an impact during particular critical periods of development, such as the prenatal period, and contemporaneous fluoride exposure may not represent the level of exposure during sensitive periods of development. In our second study, we found large

variability in urinary fluoride levels, particularly for adults (SD = 1.24 mg/L for adults ages 60 to 79 years old living in a fluoridated region compared to SD = 0.37 mg/L for children ages 12 to 18 years old living in a fluoridated region). This discrepancy is consistent with previous studies showing greater variability in the average daily fluoride excretion of adults compared with children (Villa et al., 2010).

Because UF only measures fluoride excretion, it is unclear whether sex and age differences in urinary fluoride levels are due to differences in fluoride intake, fluoride absorption, or both. In their review, the National Research Council (2006) estimated the total fluoride exposure level of different age groups if they were to receive fluoridated tap water at 1mg/L. They calculated the lowest fluoride exposures to occur in youth ages 13 to 19 years old (0.028 mg/kg/day) and nursing infants under age 1 (0.030 mg/kg/day). There are numerous factors that influence fluoride excretion, including age-related differences in excretion of fluoride by kidneys and increased fluoride absorption during periods of bone growth (Jha, Mishra, Sharma, & Damodaran, 2011; World Health Organization, 1997). Healthy adults (ages 18 to 75) excrete about 60% of the fluoride they ingest each day, whereas children under age 7 excrete only 45% of their daily ingested fluoride (Buzalaf, Leite, & Buzalaf, 2015; Villa et al., 2010). Converging evidence can be found when comparing age differences in absorption; healthy adults ages 18 to 75 absorb only 36% of daily fluoride intake whereas children under age 7 years absorb 55% (Buzalaf et al., 2015; Villa et al., 2010). Most fluoride absorption occurs in the small intestine and renal tubule, and can be influenced by nutritional status and diet composition (Buzalaf et al., 2015). For example, high dietary concentrations of calcium reduce fluoride absorption (Buzalaf et al., 2015; Whitford, 1994), resulting in higher excretion. Similarly, higher alkalinity in the body results in excretion of a higher proportion of ingested fluoride (World Health Organization,

2014). A vegetarian-based diet shifts one's pH towards alkaline, which reduces fluoride absorption in the small intestine and renal tubule (Buzalaf et al., 2015).

Further, smoking cigarettes has been found to modify fluoride metabolism such that individuals who smoke cigarettes have been reported to have significantly higher levels of fluoride in plasma (Jain, 2017). In our second study, we found that individuals ages 3 to 79 who live in homes in which smoking is not permitted have 0.25 mg/L lower UF_{SG} compared with those who live in homes in which smoking is permitted inside the home. Conversely, in our first study, we found that children ages 6 to 17 who live in homes in which smoking is not permitted have a higher level of UF_{SG} (0.63 mg/L) compared to those who live in homes in which smoking is permitted inside the home (0.57 mg/L). In this first study, the number of children who live in homes in which smoking is permitted was very small (n= 216 youth), and thus this finding may not be generalizable.

For adolescents (age 14) in our first study, living in a fluoridated region was associated with a 0.7-point higher SDQ hyperactivity/inattention subscale score and 2.8 times greater odds of being diagnosed with ADHD. The relationship between CWF status and attention outcomes, however, was not significant for younger children (age 8), even though UF levels were similar (or lower) among adolescents (0.79 mg/L) compared with younger children (0.88 mg/L) living in a region with CWF. This finding that the relationship between fluoride and attention outcomes is present only for adolescents is surprising, given that most studies have found strong relationships between prenatal/early postnatal fluoride exposure and lower IQ (Bashash et al., 2017; Grandjean, 2019; Green et al., 2019). It is possible that impairments in attention result from chronic fluoride exposure, meaning that effects are only evident in older children. Or, deficits in attentional processes may not emerge until early adolescence when attentional demands increase

and synaptic pruning occurs in the frontal lobes, which are responsible for executive functioning (Willcutt, Doyle, Nigg, Faraone, & Pennington, 2005). It is also possible that the association between urinary fluoride and attention/hyperactivity could be obscured due to reduced fluoride excretion (i.e., increased fluoride absorption) during a high growth spurt stage (Jha et al., 2011; World Health Organization, 1997). For example, Vatanparast, Bailey, Baxter-Jones, and Whiting (2010) found that boys ages 9 to 18 boys accrued an average of 199 grams bone mineral content per year, with the most bone growth occurring between of 13 and 14 years old, whereas girls accrued 138 grams of bone per year, with the most bone growth occurring between 12 and 13 years of age (Vatanparast et al., 2010).

Inflammation as a Potential Mechanism in the Association Between Fluoride Exposure and Attention Outcomes

It has been well documented in animal models that even low levels of fluoride result in greater oxidative stress (Banala & Karnati, 2015; Bartos et al., 2018; Chouhan, Lomash, & Flora, 2010; Reddy et al., 2014; Zhang, Lou, & Guan, 2015). For example, Chouhan and colleagues (2010) found that rats exposed to fluoridated water at a concentration of 1 mg/L for four months showed more oxidative stress and alterations in neurotransmitters compared to rats exposed to non-fluoridated water. Further, fluoride has been linked with increased catalase immunoreactivity in rats (Güner, Uyar-Bozkurt, Haznedaroğlu, & Menteş, 2016) as well as increases in reactive oxygen species formation and an inflammatory response (Akinrinade, Memudu, Ogundele, & Ajetunmobi, 2015). According to a recent meta-analysis, inflammation may play a role in the development of ADHD (Anand, Colpo, Zeni, Zeni, & Teixeira, 2017). Studies with rats illustrated that the administration of cytokines (e.g., interleukin-1β, interleukin-2 interleukin-6) can result in increased norepinephrine and reduced dopamine levels; these

changes in neurotransmitter levels are also observed in humans with ADHD (Anisman, Kokkinidis, & Merali, 1996; Zalcman et al., 1994). More recently, it has been suggested that several genes implicated in ADHD are also implicated in immune function (Dunn, Nigg, & Sullivan, 2019; Verlaet, Noriega, Hermans, & Savelkoul, 2014). The meta-analysis by Anand and colleagues (2017) showed elevated cytokine levels in blood or cerebrospinal fluid in individuals with ADHD compared with those without ADHD (Anand et al., 2017). A study of 40,000 adults from Taiwan found that individuals with ADHD had a significantly greater prevalence of autoimmune diseases including ankylosing spondylitis, ulcerative colitis, and autoimmune thyroid disease compared to controls without ADHD (Chen et al., 2017). Several maternal somatic diseases with immune components, including multiple sclerosis, rheumatoid arthritis, type 1 diabetes, and hypothyroidism, have been found to increase the risk of ADHD in offspring (Instanes et al., 2017). In particular, exaggerated central nervous system inflammatory response in the mother between 32 and 40 weeks gestation has been implicated as a potential factor in the development of ADHD in offspring (Strickland, 2014). Furthermore, chronic T-cell-mediated neuroinflammation and oxidative stress could lead to ADHD symptoms in those with genetic susceptibility (Verlaet et al., 2014). Other research has pointed to the roles of mitochondrial dysfunction, oxidative stress, inflammation, and decreased melatonin levels in the relationship between fluoride exposure and Autism Spectrum Disorder (Strunecka & Strunecky, 2019), which commonly co-occurs with ADHD (Antshel, Zhang-James, Wagner, Ledesma, & Faraone, 2016). More research is needed to explore the possibility of inflammation as a mechanism by which fluoride exposure leads to ADHD symptoms.

Limitations

Although UF is the most commonly used biomarker of fluoride exposure (Zohouri, Swinbank, Maguire, & Moynihan, 2006), there are several limitations regarding use of UF for this purpose, even with adjustment for dilution. Measurement error is more likely with one spot sample than 24-hour urine collection. Further, there is known to be temporal variation in UF concentrations due to rapid absorption and elimination of fluoride (Martinez-Mier, 2012; Whitford, 1996). This variation results in UF measuring excretion resulting from recent fluoride exposure (i.e., within the past 11 to 15 hours). Although the CHMS lacked standardization with respect to the time that the spot samples were collected, studies comparing first morning spot samples to 24-hour collections have found good correspondence (r = .83; Zohouri et al., 2006), and in our sample, the correlation between time of day and UF_{SG} was near zero (r = -.03). Further, the World Health Organization (2014) states that "plots of daily urinary fluoride excretion against total daily fluoride intake suggest that daily urinary fluoride excretion is suitable for predicting fluoride intake for groups of people, but not individuals." Nonetheless, uncontrolled lifestyle or environmental factors that were not measured in the current study, such as consuming low-fluoride bottled water prior to sampling, may have resulted in atypical UF values for that individual. Urinary fluoride is not a perfect measure of fluoride intake, but it is considered the optimal biomarker of concurrent fluoride exposure (Rugg-Gunn, Villa, & Buzalaf, 2011).

The CHMS measured fluoride in spot urine samples (i.e., excretion) and fluoride in tap water samples. Because fluoride from tap water is only one source of fluoride exposure, we were not able to examine the association between total fluoride exposure and ADHD. Although difficult, it would be advantageous to determine the extent of the relationship between overall

fluoride intake (i.e. from all sources) and the development of ADHD. It would be especially useful to conduct a longitudinal study to measure fluoride exposure during critical periods of brain development, such as prenatally, and subsequent attention outcomes. Future studies could use gold standard measures of ADHD diagnosis, such as a diagnostic interview and information from collateral sources to confirm that symptoms are present across settings (e.g., home and school). On one hand, the fact that the CHMS is cross-sectional limits our ability to make causal inferences concerning the development of ADHD due to fluoride exposure. However, in areas where fluoridation has existed for decades, one can assume that a cross-sectional study may serve as a proxy to prenatal exposure. Indeed, a recent report by the National Academy of Sciences used cross-sectional studies of dental fluorosis conducted in China as being indicative of early-life exposure to fluoride given that the presence of dental fluorosis is evidence that exposure to fluoride occurred in the first few years of life. Thus, they consider cross-sectional studies in areas that have had high levels of fluoride over a long period of time to be functionally prospective (National Toxicology Program, 2019).

Conclusions and Implications for Public Policy

Higher tap water fluoride levels and fluoridation of municipal water supplies were associated with a higher risk of an ADHD diagnosis as well as increased symptoms of hyperactivity and inattention, especially among adolescents. Additionally, adolescent boys had the lowest levels of fluoride excretion, suggesting that they may be absorbing higher levels of fluoride than adolescent girls or adults. Although this study is cross-sectional, it should be considered along with other studies showing the adverse neurodevelopmental impact of fluoride exposure. Higher levels of fetal fluoride exposure have been associated with lower IQ (Choi, Sun, Zhang, & Grandjean, 2012; Green et al., 2019; Bashash et al., 2017; Jiménez et al., 2017)

and increased risk of ADHD symptoms (Bashash et al., 2018). Furthermore, we have carefully considered numerous potential confounders which have been previously linked with ADHD diagnoses, including lead exposure and smoking in the home. We have also considered numerous variables which may modify fluoride excretion, such as age, sex, ethnicity, and BMI. Tap water fluoride and CWF status uniquely predicted ADHD diagnosis and SDQ score over and above the effects of these known risk factors for ADHD and covariates.

Due to their body weight, infants may be at particularly high risk of over-exposure to fluoride. To reduce the likelihood of fluorosis, breast-feeding or use of non-fluoridated water to reconstitute formula is recommended (Brothwell & Limeback, 2003; Fomon et al., 2000). However, national organizations across Canada and the United States continue to endorse use of fluoridated tap water to reconstitute infant formula (because fluorosis is seen as being very mild to mild and is thus not considered to be an adverse health effect). Health Canada (2013) states that infant formula can be prepared with water fluoridated at the "optimal" level. Similarly, the American Dental Association (ADA) recommends that parents use optimally fluoridated water to reconstitute infant formula, but also also recommends that formula should be reconsituted with non-fluoridated water if dental fluorisis is a concern (American Dental Association, 2010). This is where water fluoridation becomes a social justice issue as some families may not have the means to access non-fluoridated water for their infant. The potential impact of overexposure to fluoride in infancy is of concern given that about 75% of U.S. mothers who use powdered infant formula report that they reconstitute the formula using tap water (Centers for Disease Control and Prevention, 2015). More research and policy work is needed to ensure that infants, particularly those from families with low socioeconomic status, are not over-exposed to fluoride.

Controversy about the safety of water fluoridation has led scientific advisory groups to recommend additional research on fluoride toxicity. Because of the unique vulnerability of the developing brain, the potential for adverse neurodevelopmental outcomes as a consequence of early childhood fluoride exposure is of high public health relevance for Canadians and other populations. Some groups may be more at risk than others, such as individuals with genetic susceptibility, those consuming large amounts of fluoridated tap water, infants receiving formula reconstituted with fluoridated tap water (Till et al., 2020), young children ingesting toothpaste, postmenopausal women, individuals with renal disorders, and those with immune conditions. To make policy recommendations regarding CWF, it is essential to differentiate between water fluoride level and total fluoride exposure, as fluoride from CWF is just one source of exposure. Further, it is essential to understand how total fluoride exposure is linked with adverse outcomes such as increased risk of an ADHD diagnosis. Once these associations have been established, alternatives to this widespread public health intervention can be considered to minimize the risk of adverse outcomes, particularly among subgroups with higher exposure. When applied topically, fluoride is effective in reducing the incidence of dental caries, slowing the progression of existing lesions, and preventing new caries from forming (Centers for Disease Control and Prevention, 2001). When left untreated, dental caries can result in pain, tooth loss, and may lead to systemic infection, which has a negative impact on overall health. For example, research has found a link between oral infections and chronic cardiovascular disease (Slavkin & Baum, 2000). Thus, policy makers must find a way to promote the benefits of topically-applied fluoride for dental health while reducing the risks of ingesting fluoride on the developing brain.

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Appendix: CWF status by site in Cycles 2 and 3 of the CHMS

CWF status	Site	Region	Rationale for Classification
CYCLE 2			
No	Laval	Quebec	No evidence of fluoridation found in grey literature or online; classified as non-fluoridated by McLaren (2016).
	Coquitlam	British Columbia	Coquitlam's drinking water is provided by the Metro Vancouver watersheds, which are not fluoridated; classified as non-fluoridated by McLaren (2016).
	North shore Montreal	Quebec	The water treatment plant that services north shore Montreal (Atwater et Des Baillets) does not add fluoride; classified as non-fluoridated by McLaren (2016).
	St. John's	Newfoundland	Classified as non-fluoridated by McLaren (2016); confirmed by St. John's Water Services website: http://www.stjohns.ca/living-st-johns/city-services/water-services.
	Richmond	British Columbia	Richmond is served by the Metro Vancouver watersheds, which are not fluoridated: http://richmond.ca/__shared/assets/2015_Water_Quality_Report44511.pdf; classified as non-fluoridated by McLaren (2016).
	Kingston	Ontario	The municipality of Kingston does not add fluoride to drinking water: https://www.utilitieskingston.com/pdf_downloads/KingSt-WTP_Annual_Report_2010.pdf; classified as non-fluoridated by McLaren (2016)
	Gaspesie	Quebec	A representative from the Quebec government confirmed by email that there is no fluoride added to any municipalities in Gaspesie; classified as non-fluoridated by McLaren (2016).
CYCLE 3			
No	East Montreal	Quebec	Online sources including a map of water treatment plants in Montreal confirms that the water treatment plant that services East Montreal (Atwater et Des Baillets) does not add fluoride.
	South Central Laurentians	Quebec	This site is comprised of a number of regional municipalities likely including Les Laurentides, Argenteuil, Deux-Montagnes, La Rivière-du-Nord, Les Pays-d'en-Haut, Mirabel, Thérèse-De Blainville. Municipal government employees from Deux-Montagnes, Les Pays-d'en-Haut, and Mirabel confirmed via email that fluoride is not added to municipal water. Municipalities in Thérèse-De Blainville stopped fluoridation in 2002 according to: http://blogues.radio-canada.ca/rive-sud/2013/07/03/buvez-vous-de-leau-fluoree/
	Orillia	Ontario	A presentation by the regional health authority confirms that Orillia has never added fluoride: http://www.simcoemuskokahealth.org/docs/default-source/topic-oralhealth/cwffactsheetpublic
	Victoria-Saanich	British Columbia	The Greater Victoria Drinking Water Quality Annual Reports confirm that fluoridate is not added to the drinking water in Greater Victoria.
	Vancouver	British Columbia	The Metro Vancouver drinking water treatment processes website confirms that fluoride is not added to the water supply: http://www.metrovancouver.org/services/water/quality-facilities/facilities-processes/treatment-process/Pages/default.aspx

CYCLE 2			
Yes	Central and East Ottawa	Ontario	The website of the City of Ottawa's Water and Environment department confirms that fluoride is added to the drinking water: http://ottawa.ca/en/residents/water-and-environment/drinking-water-and-wells/fluoride
	Oakville	Ontario	Oakville is part of the region of Halton. According to the Halton region website, "the Region adds fluoride to the water supplies in Burlington, Halton Hills, Oakville, and the new developments in Milton to bring it up to the recommended levels: http://www.halton.ca/cms/one.aspx?pageId=15215.
	South of Brantford	Ontario	Annual drinking water reports confirm that Brantford continues to add fluoride to their water: http://www.brantford.ca/residents/health/water_quality/waterquality/Pages/MOEAnnualReport.aspx
	Southwest Toronto	Ontario	All areas of Toronto add fluoride to their drinking water: https://www.toronto.ca/311/knowledgebase/kb/docs/articles/toronto-water/water-treatment-and-supply/operations-efficiency/fluoridation-of-city-drinking-water-fluoride-levels.html
	East Toronto	Ontario	All areas of Toronto add fluoride to their drinking water: https://www.toronto.ca/311/knowledgebase/kb/docs/articles/toronto-water/water-treatment-and-supply/operations-efficiency/fluoridation-of-city-drinking-water-fluoride-levels.html
	Winnipeg	Manitoba	According to their city website, Winnipeg adds fluoride to their drinking water: http://www.winnipeg.ca/waterandwaste/water/fluorideFAQ.stm
	Edmonton	Alberta	Edmonton adds fluoride to their drinking water at a level of 0.7ppm: https://www.epcor.com/products-services/water/water-quality/Pages/facts-about-fluoride.aspx
	Calgary	Alberta	Calgary began adding fluoride to the drinking water supply in 1991, and stopped fluoridation in May 2011. Therefore, during Cycle 2 data collection, Calgary was fluoridated. For more information see: http://www.calgary.ca/UEP/Water/Pages/Drinking-water/Fluoride.aspx
CYCLE 3			
Yes	Oshawa-Whitby	Ontario	Annual water treatment reports confirm the addition of fluoride to the municipal drinking water: https://www.durham.ca/en/living-here/resources/Documents/WaterandSewer/OWAWaterQualityReport2016.pdf
	North Toronto	Ontario	All areas of Toronto add fluoride to their drinking water: https://www.toronto.ca/311/knowledgebase/kb/docs/articles/toronto-water/water-treatment-and-supply/operations-efficiency/fluoridation-of-city-drinking-water-fluoride-levels.html
	Brampton	Ontario	The city of Brampton is part of the region of Peel, which adds fluoride across the region: http://www.peelregion.ca/health/fluoride/
	Lethbridge	Alberta	Lethbridge has added fluoride to the drinking water since 1974: https://www.lethbridge.ca/living-here/waterwastewater/Documents/Consumer%20Confidence%20Report%202016.pdf
	Halifax	Nova Scotia	All water treatment plants in Halifax add fluoride to their drinking water supply: https://www.halifax.ca/home-property/halifax-water/water-services

EXCLUDED

MIXED SITES	South Monteregie	Quebec	The municipalities contained within this site were unclear and likely contained a mix of both fluoridated and non-fluoridated municipalities. Since postal code data were not available, sites could not be divided into separate areas and the whole site was therefore excluded from analyses using the CWF status variable.
	Southwest Monteregie	Quebec	The municipalities contained within this site were unclear and likely contained a mix of both fluoridated and non-fluoridated municipalities. Since postal code data were not available, sites could not be divided into separate areas and the whole site was therefore excluded from analyses using the CWF status variable.
	West Montreal	Quebec	Part of the region is supplied by a water treatment plant that adds fluoride, and another part of the region is supplied by a water treatment plant that does not add fluoride. Since postal code data were not available, sites could not be divided into separate areas and the whole site was therefore excluded from analyses using the CWF status variable.
	Windsor (stopped Jan 29, 2013)	Ontario	Windsor stopped fluoridation on January 29 2013, which was during the data collection for Cycle 3. About 78% of tap water samples from this site were collected after fluoridation was discontinued.
	Central and East Kootenay	British Columbia	This site contained some municipalities that added fluoride and some that did not. Since postal code data were not available, sites could not be divided into separate areas and the whole site was therefore excluded from analyses using the CWF status variable.
	Colchester and Pictou Counties	Nova Scotia	This site contained some municipalities that added fluoride and some that did not. Since postal code data were not available, sites could not be divided into separate areas and the whole site was therefore excluded from analyses using the CWF status variable.
	Southwest Calgary (stopped in 2011)	Alberta	Calgary began adding fluoride to the drinking water supply in 1991, and stopped fluoridation in May 2011. For more information see: http://www.calgary.ca/UEP/Water/Pages/Drinking-water/Fluoride.aspx. Since fluoridation stopped just before Cycle 3 data collection for this site, the tap water fluoride average for this site was almost 3 times higher than for other non-fluoridated sites. Therefore, this site was excluded from analyses using the CWF status variable.
	Brantford-Brant County	Ontario	The average tap water level for each site in Cycle 3 was calculated, and the average for all fluoridated and all non-fluoridated sites was also calculated. The average tap water level was 3 times lower in Brantford-Brant County than for the average of all non-fluoridated sites. This site was therefore excluded from analyses using the CWF status variable.
	Kent County	New Brunswick	The average tap water level for each site in Cycle 3 was calculated, and the average for all fluoridated and all non-fluoridated sites was also calculated. The average tap water level was 3 times lower in Kent County than for the average of all non-fluoridated sites. This site was therefore excluded from analyses using the CWF status variable.