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# Prenatal fluoride exposure and attention deficit hyperactivity disorder (ADHD) symptoms in children at 6–12 years of age in Mexico City

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ABSTRACT

*Background:* Epidemiologic and animal-based studies have raised concern over the potential impact of fluoride exposure on neurobehavioral development as manifested by lower IQ and deficits in attention. To date, no prospective epidemiologic studies have examined the effects of prenatal fluoride exposure on behavioral outcomes using fluoride biomarkers and sensitive measures of attention.

*Objective:* We aimed to examine the association between prenatal fluoride exposure and symptoms associated with attention-deficit/hyperactivity disorder (ADHD).

*Method:* 213 Mexican mother-children pairs of the Early Life Exposures to Environmental Toxicants (ELEMENT) birth cohort study had available maternal urinary samples during pregnancy and child assessments of ADHD-like behaviors at age 6–12. We measured urinary fluoride levels adjusted for creatinine ($MUF_{cr}$) in spot urine samples collected during pregnancy. The Conners' Rating Scales-Revised (CRS-R) was completed by mothers, and the Conners' Continuous Performance Test (CPT-II) was administered to the children.

*Results:* Mean $MUF_{cr}$ was 0.85 mg/L (SD = 0.33) and the Interquartile Range (IQR) was 0.46 mg/L. In multivariable adjusted models using gamma regression, a 0.5 mg/L higher $MUF_{cr}$ (approximately one IQR higher) corresponded with significantly higher scores on the CRS-R for DSM-IV Inattention (2.84 points, 95% CI: 0.84, 4.84) and DSM-IV ADHD Total Index (2.38 points, 95% CI: 0.42, 4.34), as well as the following symptom scales: Cognitive Problems and Inattention (2.54 points, 95% CI: 0.44, 4.63) and ADHD Index (2.47 points; 95% CI: 0.43, 4.50). The shape of the associations suggested a possible celling effect of the exposure. No significant associations were found with outcomes on the CPT-II or on symptom scales assessing hyperactivity.

*Conclusion:* Higher levels of fluoride exposure during pregnancy were associated with global measures of ADHD and more symptoms of inattention as measured by the CRS-R in the offspring.

*Abbreviations:* ADHD, attention-deficit hyperactivity disorder; $cm^3$, cubic centimeters; CNS, Central Nervous System; CPT-II, Conners' Continuous Performance Test – Second Edition; CRS-R, Conners' Rating Scale – Revised; $CUF_{sg}$, specific gravity adjusted child urinary fluoride; DSM-IV, Diagnostic and Statistical Manual of Mental Disorders – Fourth Edition; ELEMENT, Early Life Exposures in Mexico to Environmental Toxicants; EPA, U.S. Environmental Protection Agency; HOME, Home Observation for Measurement of the Environment; L, liter; mg, milligram; $MUF_{cr}$, creatinine adjusted maternal urinary fluoride; SD, Standard Deviation; SE, Standard Error

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## 1. Introduction

Fluoride, the ionized form of the halogen element fluorine, exists widely in the environment and is the most electronegative and reactive among all elements (ATSDR, 2010). Its well-known cariostatic effect led to the addition of fluoride to water, salt, and milk in some countries. Other sources of fluoride include dental products, such as toothpastes, mouth rinses, and varnishes, supplements, processed foods made with fluoridated water, fluoride-containing pesticides, teas, and fluorinated pharmaceuticals. Systemic ingestion of fluoride through water and water-based beverages is the main source of fluoride intake, accounting for approximately 75% of dietary fluoride intake among adults living in communities that fluoridate their water supply in the United States (U.S. Environmental Protection Agency, 2010; USDA (U.S. Department of Agriculture), 2005). However, in Mexico City, individuals are primarily exposed to fluoride through fluoridated salt (mean concentration of fluoride in salt is 250 ± 50 ppm), and to varying degrees of naturally-occurring fluoride in water, which have been reported to range from 0.15 to 1.38 mg/L (Juárez-López et al., 2007; Martinez-Mier et al., 2005). Public water supplies are not fluoridated in Mexico and the mean fluoride content of the water supply is not publicly available.

Long-term exposure to fluoride is regarded by the World Health Organization as being beneficial, including both prevention of dental caries and treating osteoporosis, though excess intake can also cause potential health hazards, including dental and skeletal fluorosis. Fluoride is also shown to readily cross the placenta (Shen and Taves, 1974) and accumulate in fetal brain tissues (Narayanaswamy and Piler, 2009), thereby inducing toxicity (Dong et al., 1993; Jiang et al., 2014). Several animal (Chen et al., 2003; Mcpherson et al., 2018; Mullenix et al., 1995) and human studies (Bashash et al., 2017; Choi et al., 2012) have explored associations between early-life exposure to fluoride and decrements in cognitive function and attention-related behaviors. An ecologic study reported an association between state level prevalence of community water fluoridation and prevalence of ADHD among youth living in the United States (Malin and Till, 2015). Given the increased vulnerability of the developing fetus to environmental exposures (Lanphear, 2015), as well as the widespread distribution of fluoride in society, the potential impact of prenatal exposure to fluoride warrants further study.

Attention-deficit hyperactivity disorder (ADHD) is the most common neurodevelopmental disorder in school-aged children and adolescents, with a worldwide prevalence estimated at about 5% (Polanczyk et al., 2007). Symptoms of ADHD include difficulties with attention, impulsivity, and/or hyperactivity at a level that is severe enough to be associated with impairments in academic and social functioning (American Psychiatric et al., 2013). Although genetics have been shown to play an important role in an individual's susceptibility to ADHD, with estimates of heritability from twin studies in the range of 60–70% (Posthuma and Polderman, 2013), several environmental factors have also been implicated. Environmental risk factors for ADHD include prenatal tobacco and alcohol exposure, heavy metal and chemical exposures, including lead (Huang et al., 2016), mercury, organochlorines, air pollution (Fuertes et al., 2016; Perera et al., 2018; Sentís et al., 2017) and nutritional factors (Polańska et al., 2012).

The purpose of the current study was to prospectively assess the relationship between prenatal exposure to fluoride and parent-reported behaviors associated with ADHD among 6–12 year old children born to mothers living in Mexico City. We tested whether fluoride exposure associated with inattentive and/or hyperactive behaviors.

## 2. Methods

### 2.1. Study population

Participants included a subset of mother-child dyads enrolled in various longitudinal birth cohort studies of the Early Life Exposure in Mexico to Environmental Toxicants (ELEMENT) project (Aafeiche et al., 2011; Bashash et al., 2017). We included mother-child pairs from two of the four ELEMENT cohorts (cohorts 2A and 3) for which maternal urinary samples during trimesters of pregnancy were available. Participants in cohort 2A (Bashash et al., 2017) were recruited between 1997 and 1999 whereas participants in cohort 3 were recruited between 2001 and 2003. We included participants if they had at least one archived urine sample from pregnancy, were ≤14 weeks of gestation at the time of recruitment, and their children underwent behavioral testing between the ages of 6 and 12, as described elsewhere (Bashash et al., 2017). Participants were excluded if they reported a history of psychiatric disorder(s), if there were medical complications (i.e. high-risk pregnancy, gestational diabetes, pre-eclampsia, renal disease, circulatory diseases, hypertension, continuous use of prescription drugs, or seizures during the index pregnancy), or if there was known maternal alcohol or illegal drug use during pregnancy. The study procedures were approved by the Institutional Review Boards of the National Institute of Public Health of Mexico, University of Michigan, Indiana University, University of Toronto, and Harvard School of Public Health, as well as participating clinics. Written informed consent was obtained from all participating families prior to study evaluation.

### 2.2. Fluoride measurements

Concentration of fluoride measured in maternal urinary samples was used as biomarker of prenatal fluoride exposure. Urine has been described as a suitable biomarker for fluoride since it serves as the main pathway through which fluoride is eliminated from the body and excretion is proportional to the total fluoride intake, but modified by factors like diet and various systemic conditions, such as recent fluoride exposure and urinary pH, as well as variation in creatinine excretion by muscle mass, age, sex, and other factors (Barr et al., 2005; Aylward et al., 2015). Ideally, overnight fasting or 24-hour urine samples are considered to be the optimal dosimeter for measuring chronic fluoride exposure in order to limit diurnal variations and the influence of diet associated with spot samples (Petersen et al., 2014). Because 24-hour urinary samples were not available in our sample, we used spot samples that were corrected for urinary dilution using urinary creatinine, as described elsewhere (Petersen et al., 2014). Each woman in the current sample provided at least one spot (second morning void) urine sample (Thomas et al., 2016) during pregnancy (range: 10 to 38 weeks). We then calculated the average of all available creatinine-adjusted maternal urinary fluoride (MUF) concentrations (Bashash et al., 2017). Further information regarding participant recruitment, data collection methods, as well as methods for fluoride sample shipping, storage, and analysis can be found elsewhere (Bashash et al., 2017).

### 2.3. Attention outcomes

Behaviors associated with ADHD were assessed using the Spanish version of the Conners' Rating Scales-Revised (CRS-R) (Conners, 1997), which has been validated for the evaluation of ADHD (Ortiz-Luna and Acle-Tomasini, 2006). The CRS-R contains three ADHD scales that correspond with the Diagnostic and Statistical Manual of Mental Disorders – 4th edition (DSM-IV) criteria for ADHD: 1) DSM-IV Inattention Index, 2) DSM-IV Hyperactive-Impulsive Index, and 3) DSM-IV Total Index (inattentive and hyperactive-impulsive behaviors combined). It also examines seven types of behavior problems that were derived through factor analysis, including: Oppositional, Anxious-Shy, Cognitive Problem/Inattention, Hyperactivity, Perfectionism, Psychosomatic, and Social Problems. In addition, the CRS-R contains four index scores that were derived based on theory and prior research: Conners' ADHD Index; Conners' Global Index (CGI): Restless-Impulsive; CGI: Emotional Lability, and CGI. For the purpose of the current study, we examined the three DSM-IV ADHD scales as our primary outcomes because these scales are intended to screen for ADHD, and are commonly used to