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Sex-Specific Neurotoxic Effects of Heavy Metal Pollutants: Epidemiological, Experimental Evidence and Candidate Mechanisms

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Abstract

The heavy metals lead (Pb), mercury (Hg), and cadmium (Cd) are ubiquitous environmental pollutants and are known to exert severe adverse impacts on the nervous system even at low concentrations. In contrast, the heavy metal manganese (Mn) is first and foremost an essential nutrient, but it becomes neurotoxic at high levels. Neurotoxic metals also include the less prevalent metalloid arsenic (As) which is found in excessive concentrations in drinking water and food sources in many regions of the world. Males and females often differ in how they respond to environmental exposures and adverse effects on their nervous systems are no exception. Here, we review the different types of sex-specific neurotoxic effects, such as cognitive and motor impairments, that have been attributed to Pb, Hg, Mn, Cd, and As exposure throughout the life course in epidemiological as well as in experimental toxicological studies. We also discuss differential vulnerability to these metals such as distinctions in behaviors and occupations across the sexes. Finally, we explore the different mechanisms hypothesized to account for sex-based differential susceptibility including hormonal, genetic, metabolic, anatomical, neurochemical, and epigenetic perturbations. An understanding of the sex-specific effects of environmental heavy metal neurotoxicity can aid in the development of more efficient systematic approaches in risk assessment and better exposure mitigation strategies with regard to sex-linked susceptibilities and vulnerabilities.

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Declaration of interests

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

Introduction:

Neurotoxicity is a term used to describe a variety of adverse effects on nervous system structure or function caused by exposure to biological, chemical, or physical agents. The neurophysiological changes induced by neurotoxic agents can include motor or cognitive symptoms and/or psychiatric and behavioral disturbances (Bjørklund et al., 2017; Mason et al., 2014; Vermeir et al., 2005). Common neurotoxic agents include drugs, bacterial and animal neurotoxins, synthetic pesticides, and heavy metals. Compared to other environmental contaminants to which humans are regularly exposed, metals have been known to be toxic since antiquity and their toxic effects have been extensively investigated and described (Florea and Büsselberg, 2006). However, as we will discuss later in this review, the precise mechanisms of heavy metal toxicity are still unclear. Due to their exceptional toxicity to the nervous system at low levels, their presence in the environment has been regulated to a certain extent (Jaishankar et al., 2014). Despite this, millions of people continue to suffer from chronic exposures to neurotoxic metals via food and water consumption or through other routes of exposure such as occupational inhalation, tobacco smoking, and more recently, electronic cigarette vaping (Aherrera et al., 2017; Olmedo et al., 2018; Zhao et al., 2019).

Heavy metals, classically defined as a group of elements with a specific density (d) greater than 5 g/cm^3, include lead (Pb; d = 11.34 g/cm^3), mercury (Hg; d = 13.53 g/cm^3), and cadmium (Cd; d = 8.65 g/cm^3), which are well known environmental pollutants (Tchounwou et al., 2012). They are found ubiquitously in the environment, often naturally (e.g., in earth's crust, products of volcanic activity), or as a result of man-made activities like mining, pesticide use, and food contamination. Due to their persistence in the environment, these metals have become an important part of the human exposome (Miller and Jones, 2014). Pb and Hg are highly toxic to the nervous system, kidneys, reproductive system, and immune system (Jan et al., 2015). The transition metal manganese (Mn; d = 7.47 g/cm^3) is also naturally abundant; it is the twelfth most abundant element and fifth most abundant metal. While less prevalent, the neurotoxic metalloid arsenic (As; d = 5.73 g/cm^3) is found in excessive concentrations in drinking water and food sources in many regions of the world. (Both Mn and As are found most commonly in drinking water and food sources). Mn and As can be classified as heavy metals based on their specific density. Like other heavy metals, they are also known to be toxic to multiple organs (including the brain and lungs for both, as well as kidneys for As) above certain levels (Cobbina et al., 2015; Riojas-Rodríguez et al., 2010; Tchounwou et al., 2012; Tyler and Allan, 2014).

Unlike Pb, Hg, Cd, and As, which have no known biological role in the human body and thus are only expected to cause adverse effects, Mn is an essential nutrient that needs to be absorbed by the human body in minimal quantities, otherwise, deficiency itself will result in toxicity that can culminate in death (Aschner and Aschner, 2005). However, when consumed in excess, Mn tends to accumulate, mainly in the liver, brain, and bone (O'Neal and Zheng, 2015). Although Mn is efficiently eliminated from the liver, its half-life is much longer in bone and also in the brain which is the target organ of Mn toxicity (O'Neal and Zheng, 2015; Peres et al., 2016). The human body absorbs Pb, Hg, Cd, Mn, and As at different efficiencies, and most of these metals can cause blood-brain barrier (BBB) disruption or

cross the BBB and accumulate preferentially in different brain regions where they have the potential to cause adverse neurotoxic effects even at low concentrations (Zheng et al., 2003). For instance, neurological deficits can be seen at exposure levels as low as 0.2 mg/L (ppm) of Mn in drinking water for children, and 0.07 to 0.97 mg/m^3 (roughly 0.03 to 0.43 ppm) Mn in occupationally-exposed adults (ATSDR, 2020). For Pb, accumulating evidence proposes that there is no safe level of exposure (Mason et al., 2014; Singh et al., 2018a).

While the precise mechanisms underlying each metal's neurotoxicity still remain unclear, oxidative stress, competition with essential metals such as zinc and iron, and dysregulation of gene expression have been supported by many studies as common fundamental processes implicated in metal toxicity (Jaishankar et al., 2014). Many of these processes are influenced by sex, and thus metal neurotoxicity can be expected to exhibit some significant sex-specific effects. *Sexual dimorphism*, defined as "*a condition where the two sexes of the same species exhibit different characteristics beyond the differences in their sexual organs*" is a well-documented attribute seen among many organisms (Ngun et al., 2011). Men and women are differentially exposed to and affected by environmental exposures and this may be attributed to sexual dimorphisms in anatomy, gray matter distribution, hormones, epigenetics, or metabolism (Mergler, 2012). While most epidemiological studies have classically used sex as a confounder to better estimate pollutant exposures' toxic effects, the patterns of sex-specific associations with outcomes haven't been given adequate attention in most cases (Buckley et al., 2017). Likewise, while numerous experimental studies have investigated the neurotoxic effects of heavy metal exposures, overall, relatively few have formally assessed sexual dimorphisms.

Nevertheless, more recently, the human and environmental health significance of understanding sex-related differences in metal neurotoxicity has been increasingly recognized, and several studies have started to tease apart the potential mechanisms. Various neurological diseases associated with heavy metal exposure including Alzheimer's disease, schizophrenia, and autism show an intriguing sexual dimorphism in their clinical expression (Weiss, 2011) that could be due to sex-specific effects in their underlying etiology. This review was thus conducted to survey the literature regarding the sex-specific effects of heavy metal exposure throughout the life course using Pb, Hg, Mn, Cd, and As as classical neurotoxic metals of interest in the context of environmental exposure. We report this connection from various perspectives ranging from epidemiological evidence to *in vivo* experimental studies to provide insight on how differences in the sexual makeup of an organism can affect his or her susceptibility to metal toxicity from a neurobiological perspective.

A note on gender and sex:

The Oxford dictionary of the English language defines "*gender*" as "*either of the two sexes (male and female), especially when considered with reference to social and cultural differences rather than biological ones*." The term is broadly used to denote a range of identities that do not correspond to the biologically established concept of male and female but rather to a social construct regarding culture-bound conventions, roles and behaviors, and relations among the two sexes (Timbs, 2012). "*Sex*," on the other hand, is defined as

"*either of the two main categories (male and female) into which humans and most other living things are divided on the basis of their [genetic makeup]*."

Both sex and gender influence health and disease, but because there is no current standardized method to measure gender, especially in laboratory animals, most scientists assume their behavior to be governed primarily by biological sex (Conger, 2017). In humans, however, sex and gender together form an amalgamation of biology and behavior that can be difficult to disentangle and evaluate. In the context of this paper, we have only considered the binary sex in terms of classical biological makeup (i.e., presence of XX chromosomes in females and XY in males), as the vast majority of data available on metal neurotoxicity pertain to binary sex and not gender differences, even if the term "gender" was sometimes used interchangeably with "sex" in the literature (e.g., in older publications and in non-human animal research).

Methods:

Literature search:

Exhaustive PubMed Central and Google Scholar searches sorted by best match were performed up to December 2020, using the terms:

> "Sex-Specific Susceptibility" *[Title/Abstract]* AND "Neurotoxic Metal Exposure" *[Title/Abstract]* OR "Heavy Metal Exposure" *[All Fields]* OR "Sex-Specific neurotoxicity" *[All Fields]* OR "sexual dimorphism, metal neurotoxicity" *[All Fields]* OR "heavy metals, gender, brain toxicity*" [All Fields]* OR "gender difference, metal neurotoxicity" *[All Fields]* OR "metal (lead/mercury/manganese/cadmium/arsenic), sex-specific, neurotoxicity" *[All Fields]* OR "metals, sex-specific, neurobehavior" *[All Fields]* OR "sex, neurons, metals" *[All Fields]* OR "sex, metabolism, metals" *[All Fields]* OR "sex, microbiome, metals" *[All Fields]* OR "gender/sex/girl/boy/male/female, toxicity, metals" *[All Fields]*.

The search strategy involved an initial screening of all titles and abstracts followed by a comprehensive assessment of existing literature reviews (including meta-analyses) and studies published on original data. Selected publications were further screened for relevant citations that were not directly identified via PubMed or Google Scholar searches.

Screening for inclusion:

This review systematically discusses both epidemiological and experimental studies. 65,217 records were identified by PubMed Central and Google Scholar using the above search terms (Figure 1). 405 full-text articles were assessed for eligibility following initial screenings of all titles and abstracts. Epidemiology studies reporting results of sex-specific analyses (both null and significant findings) were included if the following were reported: dose of exposure, biomarker(s) of interest, and the relevant neurological domain (physiological or behavioral) assessed. Some epidemiology studies involving cumulative life course exposure or assumed exposure were included despite the absence of a specific dose assessment when the certainty of exposure was supported otherwise (e.g., signs of classical symptoms in a population following extensive poisoning in a community).

Experimental studies reviewed in this paper were mainly restricted to *in vivo* rodent studies. Experimental studies were only included if the species analyzed, period of exposure, exposure dose, and domain of interest (physiological or behavioral) were clearly defined. After applying this inclusion criteria, 113 full-text articles written in English (and one abstract written in English) were included. Due to the lack of research specifically evaluating the mechanisms underlying sexual dimorphisms in metal neurotoxicity, we discuss some studies (both epidemiological and experimental) that assess sex differences in factors such as metabolism, methylation efficiency, metal transporter expression, and the gut microbiome that are suspected to have downstream effects on neurotoxicity.

Classification based on period of exposure:

For better structure and clarity, epidemiological and experimental studies for each metal were further organized by period of exposure. Epidemiological studies were broken down roughly into five windows of exposure: Prenatal, Postnatal, Childhood, Adolescence, and Adulthood based on human developmental stages. For *in vivo* experimental studies in rodents, period of exposure in mice and rats was correlated to the human equivalent following methods reported by Dutta and Sengupta (Dutta and Sengupta, 2016; Pallav Sengupta, 2013).

NHANES analyses:

Study population—We analyzed data from one cycle (2015–2016) of the National Health and Nutrition Examination Survey (NHANES), a cross-sectional, nationally-representative survey conducted by the Centers for Disease Control and Prevention (CDC) (National Center for Health Statistics, 2016). The survey is intended to assess the health and nutrition status of the general, non-institutionalized U.S. population. Data are collected by questionnaire surveys, household interviews, physical examinations, and laboratory tests and are publicly released in 2-year cycles. 2015–2016 were the study years with the most recent metal exposure data available at the time of analysis.

Blood Pb, Cd, Mn, and Hg (total and methyl) were measured in all examined participants aged 1–11 years old and a one-half random sample from participants aged 12 years and older. 4,987 participants had data available for Pb, Cd, and total Hg. 4,937 participants had methyl Hg data available. For descriptive analyses, age was categorized as follows: “child” (1–11 years of age), “adolescent” (12–21 years of age), “adult” (22–65 years of age), and “elderly” (65+ years of age). We restricted our analyses to metals measured in blood and thus did not examine total or speciated arsenic, which was measured in urine.

All NHANES protocols were approved by the National Center for Health Statistics (NCHS) Institutional Review Board (IRB), and all participants gave written informed consent. Our study was exempt from IRB approval because we used de-identified, publicly available data.

Blood sample—Whole venous blood specimens were collected by venipuncture from participants ages 1 year and older at a Mobile Examination Center. Specimens were then processed, stored, and shipped on dry ice to laboratories at the CDC (Atlanta, GA, USA) for analysis of blood metal concentrations.

Blood metals measurement—Whole blood Pb, Cd, Mn, and total Hg concentrations were determined using inductively coupled-plasma dynamic reaction cell-mass spectrometry (ICP-DRC-MS) after a simple dilution sample preparation step at the CDC's National Center for Environmental Health. The methodologic details of blood metal detection and measurements are described in the NHANES laboratory methods (Jeffery M. Jarrett, 2016). Inorganic, ethyl, and methyl mercury (MeHg) were quantified using a triple spike isotope dilution method employing gas chromatography to separate Hg species followed by introduction into ICP-DRC-MS for detection (Robert L. Jones, 2016).

Limits of detection (LOD) for blood metals, as well as the percentage of samples below the LOD, are presented in Supplementary Table 1. The number and percentage of samples below the LOD across sex and age group are shown in Supplementary Table 2. Values below the LOD were imputed by dividing the LOD by the square root of 2 (NHANES, 2018). Inorganic and ethyl mercury were not considered in this analysis as the levels for these Hg species were mostly undetectable in the NHANES population (Suppl. Table 1).

Statistical analysis—Blood metals were right-skewed and log-transformed for the analyses. We report the unadjusted geometric mean blood metal levels and 95% confidence intervals, stratified by sex and age group. Stratified analyses were conducted because it was anticipated that there might be sex differences in blood metal loads across age groups. We did not stratify by race/ethnicity because further stratification would have created small sample sizes per strata. Analyses to compare stratified groups were conducted using t-tests. Generalized linear models (Wald test) were used to explore the relationship between blood metal concentrations, sex, age group (categorical), and the 'sex × age' interaction. A two-sided alpha level of 0.05 was used to indicate statistical significance. NHANES incorporates sample population weights to account for the unequal selection probabilities caused by the cluster design, non-response, and planned over-sampling of certain subgroups. All analyses were performed in R (version 3.5.1) using the 'survey' package to account for NHANES complex sampling design and weights, producing estimates that are representative of the non-institutionalized civilian U.S. population (Lumley, 2004; R Core Team, 2015, R Studio Team, 2014).

Table 1 (Summary tables):

Interactive summary tables of 1) metal body burdens measured in biofluids, 2) epidemiological studies reporting sex-specific neurological outcomes, and 3) experimental evidence of sex-specific neurological outcomes for each metal were prepared using RShiny to allow the readers to filter the epidemiological and experimental studies discussed in this review by metal, sex, window of exposure, exposure dose, neurological domain assessed, and more. This table can be accessed at the following web page: https://meethila-gade.shinyapps.io/Sex_specific_neurotox/.

Sex-specific Neurotoxic Effects of Lead (Pb):

Pb is a heavy metal found naturally in earth's crust that has many different industrial, agricultural, and domestic applications. For example, Pb is currently used in the production of batteries, ammunition, metal products, and X-ray shields. Historically, Pb was used as an

additive to automobile gasoline and paint. Despite widescale control and elimination efforts, Pb exposure remains a public health challenge due to its persistence in the environment (Tong et al., 2012). Exposure to Pb occurs mainly via inhalation of Pb-contaminated dust particles and ingestion of Pb-contaminated food, water, and Pb-based paints found in older homes. *In utero* Pb exposure occurs due to a high degree of maternal-fetal transfer of Pb across the placenta (Chen et al., 2014). Individuals chronically exposed to Pb across various stages of the lifespan show diverse signs of toxicity including reduced birth weight, premature birth, anemia, hypertension, and nephropathy (Tarrago and Brown, 2009). However, Pb is especially toxic to the nervous system and thus causes extensive neurological deficiencies including deficits in intelligence, memory, processing speed, reading and comprehension, and visuospatial and motor skills. Infants in the prenatal/perinatal stages of development and children in adolescence are at the highest risk of Pb toxicity because: (1) developing children are more vulnerable to Pb exposure due to increased crawling and hand-to-mouth behavior; (2) children have a higher rate of gastrointestinal absorption of several metals including Pb; (3) a greater proportion of Pb absorbed in the systemic circulation enters the brains of children, who have an immature BBB; and (4) the immature brain is the most susceptible to Pb neurotoxicity (Lidsky and Schneider, 2003). Although it is believed that no amount of Pb exposure is safe (even at blood levels < 5 μg/dL [50 ppb]), large populations of children worldwide continue to show elevated blood Pb levels (> 10 μg/dL [100 ppb]) (“Childhood lead poisoning,” 2010).

Sex-specific body burdens of Pb measured in biofluids:

Large-scale epidemiological studies have consistently shown a pronounced sexual dimorphism in internal Pb exposure, reflected by levels of Pb measured in biofluids such as blood and cord blood (see summary of Pb levels in various biofluids across sex, accessible via this link: Table 1). For example, sex differences in blood Pb levels have been observed in NHANES (Annest et al., 1982; Brody et al., 1994). Specifically, in a sample of 16,563 people aged 6 months and above examined during NHANES II (1976–1980), researchers found a significant sex-specific difference in mean blood Pb levels at age 6–17 years which progressively increased with age. Males showed a mean blood Pb level of 13.68 μg/dL (136.8 ppb) as opposed to females who had a mean blood Pb level of 11.4 μg/dL at age 6–17 years (compared to the overall population mean blood Pb level of 16.1 μg/dL) (Annest et al., 1982). A similar trend persisted even as population Pb levels declined with the phasing out of leaded gasoline; in a sample of 13,201 U.S. persons aged 1 year and older examined during NHANES III (1988–1991), males showed consistently higher mean blood Pb levels compared to females (up to 1–2 μg/dL higher, compared to the overall geometric mean blood Pb level for the 1998–1991 U.S. population of 2.8 μg/dL). This sex difference persisted across all age groups except the youngest ages (1–2 years), and, consistent with the previous findings of Annest et al., the difference was not significant in children younger than 6 years of age. Also, interestingly these sex differences appeared to intensify at approximately 12 years of age, when sex differences amplify with the approach of sexual maturity (Brody et al., 1994).

This phenomenon has also been observed in a South Australian cohort, which found no difference in blood Pb concentration between sexes until children reached the age of 11–13

years; then, mean blood Pb concentration was higher in boys (8.4 μg/dL) than in girls (7.5 μg/dL) (Baghurst et al., 1999). A more recent NHANES study (1999–2014) examining mean blood Pb levels in U.S. children and adolescents (1–19 years old) reported overall a significant decrease over time in blood Pb across all age groups. This study also found a consistent difference in blood Pb levels among males compared to females (up to 0.2 μg/dL higher among males) that appears to exacerbate with age (Tsoi et al., 2016). To determine whether this pattern persists, we analyzed NHANES metal exposure data (2015–2016) and found that overall, males have significantly higher levels of blood Pb compared to females (Figure 2, actual values reported in Supplementary Table 3). In agreement with previous studies, this sex difference was apparent across all age groups (now also including participants as young as 1–2 years, Supplementary Figure 1). The difference was significant in adolescence and increased with age (Figure 3, Supplementary Table 4). Thus, these sex differences in Pb body burden may be more evident at lower Pb concentrations.

Supporting previous studies' observations that significant sex differences in blood Pb levels are not detected early in life, no significant differences in mean cord blood Pb levels were observed across sexes at birth in the Polish Mother and Child Cohort (REPRO_PL) study even though this cohort had much lower Pb exposures than the NHANES III (1988–1994) cohort. Though not significant, the mean cord blood Pb level still tended to be higher in boys (0.99 μg/dL) than girls (0.94 μg/dL) (Polanska et al., 2018). This trend may not have reached significance because cord blood may be more reflective of the mother's Pb exposure than the fetus. It is unclear the extent to which males are more prone to have higher exposures (e.g., behavioral and cultural factors such as playing outside more) or whether the underlying biology, such as sex differences in hematocrit levels, results in their higher blood Pb levels (Castro et al., 1987; Giorno et al., 1980; Pun et al., 1982; Rodger et al., 1987). Men have higher hematocrit levels and Pb in blood is bound to red blood cells (ATSDR, n.d.), so sex differences in Pb body burden could partly be explained by sex differences in hematocrit levels. However, Pb exposure itself also affects red blood cell count and hematocrit value, possibly in a sex-specific manner, and with the effect depending on the level of exposure (Bersényi et al., 2003; Iavicoli et al., 2003; Kim and Kang, 2017; Luo et al., 2020; Nikolić et al., 2013). These factors should be considered in studies comparing blood Pb levels across sexes.

In addition to blood Pb and hematocrit, a sexual dimorphism is also seen in serum brain-derived neurotrophic factor (BDNF) concentrations. BDNF plays a crucial role in neurodevelopment and is associated with learning and memory in childhood (Yeom et al., 2016). A study assessing the effect of metal (Pb, Hg, Mn, and aluminum) co-exposure on preschool children living in Taizhou City, China reported an inverse association of blood Pb level with serum BDNF concentrations in boys ($\beta = -7.32$, $p = 0.07$) after adjusting for confounders (Zhou et al., 2019). This underscores the need for further research to confirm the possible interaction between BDNF, Pb, and neurodevelopmental outcomes such as children's Intelligence Quotient (IQ).

Sex differences in the body burdens of neurotoxic metals such as Pb may contribute to observed sex differences in neurological diseases hypothesized to be associated with metal exposure. For example, males with multiple sclerosis (MS) exhibit a higher rate of disease

progression compared to females (Golden and Voskuhl, 2017). In fact, a study of 143 healthy adults and MS patients (mean age of females and males: 34.8 ± 10.7 and 31.5 ± 11.1 years, respectively) in Tehran, Iran found that overall, males had higher blood Pb levels compared to females, and this difference was significant among the MS patients (geometric mean: 5.71 ± 3.37 μg/dL vs. 3.67 ± 2.19 μg/dL, $p = 0.02$) but not in the controls (geometric mean, both sexes: 4.47 ± 2.9 μg/dL) despite a noticeable trend (Aliomrani et al., 2016). Exposure to heavy metals, including occupational Pb exposure (Dickerson et al., 2019), has also been suggested to play a role in the etiology of amyotrophic lateral sclerosis (ALS), which is more common among men with a male:female ratio that attenuates (from ~2.5 to 1.4) with age (Manjaly et al., 2010). While several case-control studies found an association between blood and/or cerebrospinal fluid (CSF) Pb levels and ALS risk, several others did not; for review see (Cicero et al., 2017; Vahter et al., 2007b). One of these studies specifically looked at potential sexual dimorphisms in metal exposure by assessing CSF concentrations of Pb, Cd, and magnesium (Mg) in 38 ALS patients (16 males, 22 females) and 38 hospital-admitted age- and sex-matched controls. While ALS patients were reported to have higher average concentrations of Pb (0.0155 μg/dL vs. 0.0132 μg/dL) in their CSF compared to controls, the difference was not significant and there were no differences in Pb CSF concentrations across sexes (Vinceti et al., 2017). Here, the lack of sex differences may be explained by the small sample size or by the fact that Pb circulating in CSF may reflect plasma Pb concentration rather than whole blood Pb levels that are usually measured (Cavalleri et al., 1984; Song et al., 2016). Alternatively, in ALS patients the typical sexual dimorphism in internal Pb levels may be altered by unknown pathogenic mechanisms. Thus, to evaluate possible sex differences in internal Pb exposure and to assess the contribution of Pb to associated neurological diseases, future studies should assess which biofluids or biosamples are the most relevant to predict actual patient central nervous system (CNS) exposure to Pb. This could be established by future studies investigating paired biofluids and post-mortem CNS samples collected from the same patients. In addition, large prospective cohort studies such as the *All of Us* research program may allow for the opportunity to investigate the contribution of Pb levels to the later development of neurological diseases (Sullivan et al., 2019).

Sex-specific neurological outcomes induced by Pb - Epidemiological evidence:

Prenatal exposure: There is a high degree of maternal-fetal transfer of Pb across the placental barrier (Chen et al., 2014). Prenatal exposure to Pb, while the CNS is still maturing, considerably influences neurological development. Males not only typically show higher body burdens of Pb, as discussed above, but they also appear to generally be more susceptible to the effects of prenatal Pb exposure, as previously reviewed by Singh and colleagues (Singh et al., 2018a). Here, we expand on this important discussion of the sex-specific adverse effects of Pb on the developing brain by including additional and more recent articles. In the following section, we specifically refer to this review for some aspects that we do not treat here with a similar level of detail.

The potential higher susceptibility of developing male brains to Pb is illustrated in the above-mentioned Polish REPRO_PL cohort, where cord blood Pb levels (geometric mean: 0.96 ± 0.16 μg/dL) showed a nearly significant interaction ($p = 0.1$) with sex;

boys, but not girls, were noted to have lower scores for cognitive function (psychomotor development assessed at 12 and 24 months of age using Bayley Scales of Infant and Toddler Development) with increasing cord blood Pb levels ($\beta = -2.07$; $p = 0.04$) (Polanska et al., 2018). An earlier Polish cohort study that was among the first to report the adverse effect of prenatal exposure to low levels of Pb (median cord blood Pb level: 1.23 μg/dL, range: 0.44–6.90 μg/dL) on mental development (assessing children at 12, 24, and 36 months of age using the Bayley Mental Development Index) noted a significantly higher mental function score in girls compared to boys at every age tested (Jedrychowski et al., 2009). This trend was also seen in a study by Bellinger and colleagues which reported boys to be more adversely affected than girls at high (≥ 10 μg/dL) prenatal Pb exposure. The study looked at children's cognitive development between 24 and 57 months (assessed at 24 months using Child's Mental Development Index [MDI] score from the Bayley Scales of Infant Development, and at 57 months using General Cognitive Index [GCI] score from the McCarthy Scales of Children's Abilities) and assessed blood Pb levels in cord blood and at age 6, 12, 18, 24, and 57 months. The change in developmental scores of girls and boys were almost identical among children with either low (< 3 μg/dL) or medium (6–7 μg/dL) exposures. However, among children with high prenatal exposures, girls had an appreciably higher mean change in developmental score between 24 and 57 months of age (difference between child's z-scored MDI at 24 months and z-scored GCI at 57 months) with a difference of 7.7 points ($p = 0.017$) (Bellinger, 2000). Collectively, these studies indicate that fetal Pb exposure, even at very low concentrations, may preferentially affect early cognitive development in boys (Jedrychowski et al., 2009; Polanska et al., 2018).

Postnatal and early childhood exposure: While the fetal period represents a critical period in brain development, the brain remains uniquely sensitive to environmental insults in the early postnatal period as well. The Cincinnati Lead Study suggests that neuro-mechanisms related to attention in adolescence are affected by both prenatal and postnatal Pb exposure (measured up to age 6.5 years), for males but not females, at Pb concentrations ranging from 5–27 μg/dL (prenatally) and 10–85 μg/dL (postnatally) (Ris et al., 2004). Specifically, this study reported a sexual dimorphism in the association between Pb exposure with attention span assessed at age 15–17 years using the Continuous Performance Test. The Pb x sex interaction was statistically significant for prenatal and average childhood (first 5 years) blood Pb and was near-significant ($p < 0.06$) for blood Pb measured at 6.5 years. (We also note that this study reported a significant association between prenatal Pb exposure and visuo-construction, measured by the block design subtest, in males only). These findings are consistent with the sex-based differences in prevalence of attention deficit hyperactivity disorder (ADHD), in which males are more affected (Pinares-Garcia et al., 2018). Thus, one could conjecture that elevated Pb exposure could increase the risk of ADHD, a hypothesis previously suggested (Kim et al., 2013) that warrants further investigation.

The Port Pirie cohort study, which enrolled children of a Pb smelter community in Australia with long-standing Pb pollution, was formed to examine the association between cumulative Pb exposure (experienced *in utero* and postnatally) and childhood growth and development. This was the first cohort to prospectively monitor the association between cumulative blood Pb exposure and prevalence of emotional and behavioral problems in children (Searle et

al., 2014). A study within this cohort reported that sex is a modifier of the association between Pb and child neuropsychological development for a given Pb burden at age 2 and 4 years. For a predicted increase in blood Pb concentration from 10 to 30 μg/dL, estimated covariate-adjusted decrements in GCI were 8.3 points for girls and 0.8 points for boys (Mcmichael et al., 1992). The findings of this study are similar to those reported by Baghurst and colleagues in the same cohort (Baghurst et al., 1992), reviewed by Singh and colleagues (Singh et al., 2018a). However, other studies reported male cognitive development to be more affected in relation to exposures assessed earlier in life, for instance at 3 and 6 months of age with prenatal and neonatal blood Pb levels < 30 μg/dL (Dietrich et al., 1987), at 4.75 years of age with cord blood Pb levels 10–25 μg/dL (Bellinger et al., 1990), and at 6 years of age with tooth Pb levels 2–34 μg/g (ppm) (Pocock et al., 1987).

The detrimental effects of Pb exposure early in life may persist into adulthood. A study examining prenatal, postnatal (average from 3 to 60 months), and 6-year-old blood Pb levels (median: 7.8, 12.3, and 6.8 μg/dL, respectively) suggested some sex-specific effects on criminal arrests in the U.S. in early adulthood, with increasing early-life Pb exposure significantly increasing the risk of total criminal arrests and arrests involving a violent crime (after adjusting for maternal IQ and socioeconomic status) (Wright et al., 2008). While the interaction term for sex was not statistically significant, the attributable risk for males (0.85 arrests/year) was considerably higher than for females (0.18 arrests/year).

Childhood and adolescent exposure: The brain remains vulnerable to environmental insults into adolescence as well, when myelination and synaptic pruning are actively shaping the still immature brain (Brenhouse and Andersen, 2011; Khundrakpam et al., 2016; Huttenlocher, 1979; Giedd et al., 1999; Paus et al., 1999). The Flemish Environmental and Health Study showed that an increasing body burden of Pb (mean blood Pb levels = 0.8–32 μg/dL) during adolescence (mean age 17.4 ± 0.8 years) was significantly associated with impaired symbol-digit substitution (an indicator of visuomotor performance) in males only (Vermeir et al., 2005). Sustained and selective attention as well as memory were not affected. The sex-specific effect on visuomotor performance could simply be explained by the higher mean exposure and range of blood Pb in boys (upper range > 10 μg/dL) compared to girls (upper range < 10 μg/dL) and not by a higher susceptibility of the male brain to Pb; this effect remains to be demonstrated in girls exposed to similar Pb levels. Another limitation in the interpretation of this study is that these adolescents were also exposed to other neurotoxic pollutants including Cd, compounds with ‘dioxin-like’ activity, and polychlorinated biphenyls (PCBs), opening the door for potential neurotoxic interaction. By analyzing the exposure individually, the authors reported that only Cd and Pb were significantly associated with visuomotor performance impairment in boys, and they did not find any correlation between the different exposures. However, with the recent development of various methods to study exposure mixtures in addition to potential interaction between congeners, it could be interesting to investigate the potential overall effects of these exposures as a mixture (Gibson et al., 2019).

Still, other studies have found that females may be more sensitive to Pb exposure later in life. For example, another study based on the Port Pirie cohort found that girls were more sensitive to the effects of Pb on IQ than boys (Baghurst et al., 1992). For an increase in

blood Pb from 10 μg/dL to 30 μg/dL, the expected covariate-adjusted decrease in full-scale IQ by age 7 was 7.8 points for girls and 2.6 points for boys. A more recent comprehensive review of the results of the Port Pirie cohort studies concluded that females appeared more susceptible to the effects of Pb exposure (Searle et al., 2014). They also noted a higher susceptibility of adolescent (11–13 years old) females to IQ deficits due to Pb exposure compared to adolescent males, a finding originally reported in a 2000 study (Tong et al., 2000). Thus, while it appears that males are generally more susceptible to the neurotoxic effects of Pb exposure, there are studies reporting that in some cases, females are more susceptible, and the sex difference may be related to the period of exposure (i.e., later in childhood for females).

Cumulative exposure: Unquestionably, time windows of exposure and the stage of brain development play a crucial role in determining toxicity and potentially sexual dimorphisms in response to metal exposure. A 1999 study conducted in the Port Pirie cohort noted that while the cumulative blood Pb exposure was similar for both sexes, the geometric mean was higher in boys (14.3 μg/dL) than girls (13.9 μg/dL), consistent with previous findings (Burns et al., 1999). In addition, this study observed a sexual dimorphism in neurobehavioral development (assessed using the standard Child Behavior Checklist) among adolescents 11–13 years old. For both sexes, the total mean behavior problem scores were significantly higher among adolescents with higher (> 15 μg/dL) lifetime average blood Pb than adolescents with lower (< 15 μg/dL) lifetime average blood Pb. Boys and girls with lifetime average blood Pb > 15 μg/dL had significantly higher externalizing behavior problem (e.g., attention and aggression) scores, but the difference across exposure groups for internalizing behavior problem (e.g., emotional reactivity) scores was significant only among girls. Researchers also calculated the expected increase in total behavior problem scores on the Child Behavior Checklist if lifetime blood Pb were to hypothetically increase. After adjusting for confounding, they predicted that if lifetime blood Pb were to rise from 10 μg/dL to 30 μg/dL, it would be associated with an increase in total behavior problem scores of 5.2 points for boys and 6.2 points for girls. The estimated increase in the internalizing score was 0.8 points for boys, but 2.1 points for girls. Together, these results and predictions suggest that females may show greater susceptibility to the effects of Pb exposure later in development, during adolescence. However, while the Child Behavior Checklist has been validated, it is a parent-report questionnaire and, in this study, mothers assessed the emotional and behavioral problems of their children. Thus, observed sex differences in certain scales of the Child Behavior Checklist (e.g., “anxious/depressed”, “withdrawn” behavior) could be due to mothers’ biased assessments of the behaviors of sons versus daughters. This is suggested by the estimated increase in externalizing scores, which was higher for boys than for girls.

The Mothers and Children’s Environmental Health study, a multicenter longitudinal study in South Korea, examined the possible sex differences in neurodevelopmental toxicity resulting from Pb exposure at various windows of development. This study examined the association between fairly low blood Pb levels and neurobehavioral development assessed at age five using the Korean version of Child Behavior Checklist which measures sleep problems as well as internalizing and externalizing behavior problems. Blood Pb levels were measured

from the prenatal period through age five (maternal blood Pb levels were assessed during early and late pregnancy, cord blood at birth; children's blood Pb levels at two, three, and five years). Consistent with previous reports, the blood Pb level was higher in males than in females at every timepoint measured, although the difference was only significant at age three. Also, consistent with the findings of the Port Pirie cohort studies that girls are more sensitive to Pb exposure later in development as compared to boys, the results of this South Korean study indicate that among males, the increased risk of total behavioral problems (internalizing and externalizing problems) was associated with the maternal blood Pb level during late pregnancy (a 3.00-point increase in the total behavioral problems score for every 1 μg/dL increase in maternal blood Pb during late pregnancy). On the other hand, among females, these behavioral problems were stronger and associated with the child's blood Pb at ages 2 and 5 years (a 3.82-point and 5.72-point increase in total behavioral problems score for every 1 μg/dL increase in child blood Pb). For all these results, the interaction effect of sex was significant or borderline significant (p-interaction: 0.13, 0.04 and 0.09, in association with Pb levels in prenatal maternal blood, and child blood at 2 years and 5 years, respectively). The effects observed among females were particularly strong for attention and sleep problems. This study again underscores the differential neurotoxic impact of Pb on the two sexes depending on the window of exposure, with males more susceptible to prenatal exposure and females more susceptible to postnatal exposure (Joo et al., 2018).

A summary of the epidemiological evidence of sex-specific neurological effects following Pb exposure is presented in Table 1. Taken altogether, epidemiological evidence indicate that males appear to exhibit higher body burdens of Pb, and sex differences in Pb levels are more discernible at lower levels of Pb exposure and become even more evident after puberty. In addition, males appear to be more susceptible to prenatal and neonatal Pb exposure while females are found to be more susceptible to effects associated with Pb exposure measured later in childhood. This either suggests that males and females have different time windows of susceptibility to Pb exposure, or that females are less resistant to chronic exposure sustained for several years into childhood and adolescence. While our present analysis agrees with the common view that, overall, males have a higher vulnerability, and possibly susceptibility, to Pb neurotoxicity, we are limited in our conclusion by a handful of studies that have independently analyzed outcomes from both sexes, particularly among adults.

Sex-specific neurological outcomes induced by Pb - Experimental evidence:

Compared to epidemiological studies, there have been many animal studies assessing Pb's sex-specific neurotoxicity. The results of these studies are summarized in Table 1. In most of these experimental studies, even if the level of internal exposure to Pb (or to the other metals we will discuss in the following sections) was rarely measured as is often the case (at least in blood) in human studies, the dose, timing, route of exposure, and the overall environment of the animals were tightly controlled, limiting the numerous confounding factors inherent to human studies. Alas, it is unfortunate that so many neurotoxicological studies missed the opportunity unique to animal studies to measure the levels of the metal of interest in the target organ (i.e., specific areas of the brain/spinal cord) at the conclusion of the experiment. In most instances, animals are experimentally exposed to Pb in the form of "Pb acetate"

via drinking water, a relevant route of exposure. The doses selected in experimental studies are often quite high as compared to the current action levels for Pb established by the U.S. Environmental Protection Agency (EPA), i.e. 15 ppb (equivalent to 1.5 μg/dL) in drinking water (ATSDR, 2017). However, EPA levels were established with safety factors and due to differences in metabolism, sources of exposure (multiple in humans), and duration of exposure (up to decades in the case of humans), rodents appear in general to require higher metal exposure levels to reach comparable internal levels/adverse outcomes as in humans. (Also note that the reported exposure dose is often not adjusted to the equivalent dose of the metal alone). Rigorous studies soundly justify the higher doses employed in the literature based on pilot dose-response studies and relevant internal biomarkers (e.g., dose-effects on relevant outcomes).

Developmental exposure: Numerous animal studies report effects of early Pb exposure on aggression, exploratory behavior, anti-depressant or anxiogenic behaviors, and hyperactivity (Ajarem and Abu-Taweel, 1992; de Souza Lisboa et al., 2005; Kasten-Jolly et al., 2012; Mansouri et al., 2012). Developmental exposure to Pb has been shown to differentially affect behavior in adult male and female BALB/cAnNTac mice. Mice developmentally exposed (from gestational day [GD] 8 to postnatal day [PND] 21) to 20 ppm (equivalent to 20 mg/L or 2,000 μg/dL) Pb acetate (a dose previously found to result in efficient maternal transfer and relevant blood Pb levels in embryos and pups) (Snyder et al., 2000) exhibited altered aggressiveness and exploratory behavior as adults (evaluated at 35 weeks of age) (Kasten-Jolly et al., 2012). The adult males developmentally exposed to Pb displayed aggressive behavior towards cage mates but did not react to a stranger in the intruder assay. The adult females developmentally exposed to Pb showed reduced exploratory activity in a variation of the open field test compared to exposed males and this difference was not due to impaired motor function (Kasten-Jolly et al., 2012). Thus, Pb differentially affected behavior in males versus females, with males showing increased aggression and females showing less exploration. Notably, these results agree with results of the epidemiological Port Pirie cohort studies that assessed the association between Pb exposure and affective behavior; compared to their unexposed counterparts and exposed females, exposed males show increased externalizing behavior (i.e., aggression), while exposed females show alterations in internalizing behaviors (i.e., withdrawal, anxiety) (Burns et al., 1999).

Consistent with this sexual dimorphism in externalizing and internalizing behaviors, Wistar rats prenatally exposed to Pb (10 ppm/day of Pb acetate through daily gavage over a period covering both pregnancy and lactation) showed sex-specific emotional disturbances, as assessed at PND 70 when their blood Pb residual levels were around 5 μg/dL (de Souza Lisboa et al., 2005). Notably, females exhibited depressive-like behavior during the forced swim test, while males showed a heightened emotional state (anxiety) in the open-field assay.

A study conducted among Long Evans rats evaluating the effect of developmental oral exposure to Pb acetate (0 vs. 250 ppm, exposed 10 days prior to breeding until PND 20) on spatial learning and reference memory (evaluated via the Morris water maze) showed a more pronounced and significant effect on impaired learning among females compared to

males (Jett et al., 1997). This effect was only observed when rats were tested at PND 21 (when the concentration of Pb in the hippocampus was highest; note that sex differences in hippocampal Pb concentration were not reported) and not at older ages (PND 56 and PND 91). However, due to the small sample size and absence of alternative behavior tests in this study, no definitive conclusion can be drawn about the relative sensitivity of females to the effect of developmental Pb exposure on spatial learning and memory. Also, there were no significant differences observed in overall performance of Pb-treated rats during 9 days of a working memory paradigm of the Morris water maze. However, male rats showed a noticeably longer escape tendency close to statistical significance on days 4 and 8.

A study in adult C57BL/6 mice brought some innovation to the field by investigating the seldom-explored enduring effects of perinatal Pb exposure. In this work, exposure via drinking water to the equivalent of 27, 55, and 109 ppm Pb from two weeks prior to mating through gestation to PND 10 resulted in late-onset obesity in one year old male offspring (Leasure et al., 2008). Other effects specific to males included decreased spontaneous motor activity, increased amphetamine-induced motor activity, and decreased rotarod performance across all Pb concentrations (negative nonmonotonic dose response curve: greater effect seen in low dose than high dose groups), while females remained unaffected. Further analysis showed altered levels of dopamine and its major metabolite and increased forebrain utilization with low gestational Pb exposure (27 ppm, leading to blood Pb ≤ 10 μg/dL, below the 2008 U.S. CDC's "level of concern" for blood Pb). The occurrence of sex-specific motor abnormalities and the incidence of late-onset obesity in a nonmonotonic dose-response pattern at levels comparable to low and supposedly safe human exposure reiterate the need to consider sex as an effect modifier instead of just a confounder and underscore the importance of seriously considering the potential long-term adverse consequences of even low levels of Pb exposure. This publication was among the critical studies that revolutionized Pb risk assessment by supporting the idea that there is no safe level of exposure to Pb.

In a study using trace fear conditioning to investigate the sex-specific effects of developmental exposure to Pb on associative learning and memory, Long Evans rats were exposed to Pb across three developmental periods: perinatal, early postnatal, and late postnatal periods, defined as 10 days before GD 0 through PND 21, PND 1–21, and PND 1–55, respectively. Testing started at PND 55 (Anderson et al., 2016). Female and male rats were fed chow containing three levels of Pb acetate (150, 375, and 750 ppm) previously shown to mimic real-life blood Pb levels (Schneider et al., 2013). Among unexposed control rats, there were no sex-specific alterations in the ability to acquire, consolidate, or recall associative memory until the last day of retention testing (tested at day 1, 2, or 10 post-conditioning). At this time, females showed a reduction in the freezing response compared to males. Only perinatal Pb exposure significantly impaired recall in males (150 and 750 ppm at day 2, only 750 ppm at day 10; note that there was a decrease in the freezing response in the 375 ppm group at day 2 that did not reach statistical significance). In contrast, this deficit in associative memory retention was observed in females only at the lowest dose (150 ppm) following both early postnatal (significant at days 2 and 10 post-conditioning) and late postnatal (only significant at day 10 post-conditioning) exposure to Pb. The absence of the retention effect tested at day 1 suggests that there is no impairment

in short term memory in either sex; however, a sex-specific effect based on developmental period of exposure is seen in consolidation and long-term recall of associative memory following developmental Pb exposure (Anderson et al., 2016). Interestingly, this sex-specific manifestation of cognitive effects is similar to the trend observed in humans, with males showing adverse effects following early prenatal/perinatal Pb exposure and females showing neurological deficits following exposure to Pb at later developmental periods (Burns et al., 1999; Joo et al., 2018).

Early postnatal exposure: Experimental studies have confirmed that early postnatal exposure to Pb is also detrimental to the brain independently of prenatal exposure. Subacute postnatal Pb exposure has been shown to impact olfactory memory of pre-adolescent mice in a sex-specific manner (Flores-Montoya et al., 2015). C57BL/6 mouse pups exposed to Pb acetate via dam's milk (dams drank water treated with either 0, 30, or 330 ppm Pb acetate from PND 0 to PND 28) showed a significant interaction between blood Pb levels of exposed mice (2–20 μg/dL) and sex, with males showing higher blood Pb levels. In the novel odor recognition test (tested at PND 28), among males, the amount of time spent exploring the novel odor compared with the familiar odor decreased linearly with increasing blood Pb levels (for every 1 unit increase in blood Pb, score decreased by 0.024). Females showed this response only at the lowest level of Pb exposure, 30 ppm, but the effect did not reach significance, suggesting that Pb affects olfactory recognition memory in both sexes differentially. Thus, in young males, blood Pb levels were linearly correlated with decreased olfactory recognition memory, whereas in females, a nonlinear and non-significant effect was observed, but this was likely due to the smaller range of blood Pb levels of the females compared to males. To better discriminate the dose-response curve for females, olfactory memory should be tested at additional Pb doses. This study was also limited by an unbalanced number of males and females; therefore, these results may not fully characterize sex differences in olfactory memory. However, a more recent study investigating the effect of orally administered Pb acetate (0, 30, or 330 ppm) on C57BL/6 mice (PND 0 to PND 28) showed sex-specific differences in long-term (at 9–12 months) hippocampal synaptic properties (Tena et al., 2019). The study found that low Pb exposure (30 ppm) in males but high Pb exposure (330 ppm) in females affected synaptic transmission and increased neuronal fiber excitability. This finding is in agreement with the novel odor recognition findings discussed above, where low Pb exposure affected the behavior of male mice without affecting the behavior of female mice, and suggests that early life Pb exposure can alter hippocampal neural circuits of adults in a sex-specific manner which may affect relevant behaviors such as recognition memory.

Post-weaning (young adulthood) exposure: The rodent brain remains vulnerable to Pb in adolescence and young adulthood as well. A subchronic 70-day exposure/30-day reversal study on weaned Swiss mice (PND 21) exposed to 0, 50, 100, or 500 ppm of Pb acetate orally via drinking water showed that exposure resulted in an anti-depressant-like effect in both sexes evaluated via the forced swim test (50 and 500 ppm) and tail suspension test (50 ppm) (Soeiro et al., 2007). However, this effect was not seen in other rodent species (Wistar rats) in a previously mentioned study that found earlier developmental exposure (prenatally and during lactation) to Pb (also via Pb acetate) yielded depression-like behavior

in females (tested at PND 70) only (de Souza Lisboa et al., 2005), indicating that the time window of exposure can result in opposite effects on the same neurological domain and also that there may be species differences in the response to Pb exposure. The discrepant findings could also be due to differences in exposure dose and duration. Also, the authors found an anxiogenic effect (assessed via the elevated plus maze) only in male mice exposed to 500 ppm Pb. Interruption of Pb exposure for 30 days showed a reversal of behavioral effects in both males and females exposed to 50 ppm Pb, but only in females exposed to 500 ppm Pb, suggesting a sustained susceptibility of males to prior elevated exposure.

Sexual dimorphisms in response to Pb exposure during adulthood have also been observed among other species. For example, young adult female Wistar rats showed significantly lower Pb concentration compared to male rats in blood (68 ppb vs. 88 ppb), plasma (0.6 ppb vs. 0.72 ppb), and brain (204 ppb vs. 239 ppb) after a month-long exposure to 50 ppm Pb acetate via drinking water (Mansouri et al., 2012). This confirms what is observed in humans and suggests that some evolutionarily conserved mechanisms may account for the higher burden of Pb consistently measured in males. This study also shows that blood Pb levels < 10 μg/dL (100 ppb) induce some significant behavioral alterations in tests related to hyperactivity in a novel environment (open field test) and impairment of spatial memory (Morris water maze) only in males, while object recognition (novel object recognition test) and motor coordination (rotarod) remained unaffected (Mansouri et al., 2012). Further analysis of females exposed to a higher dose (70 ppm Pb acetate) showed that despite the production of blood Pb and brain Pb levels (86 ppb and 250 ppb, respectively) similar to levels produced by 50 ppm Pb-exposed male rats, no behavioral alterations were seen. This suggests that the behavioral deficits selectively observed in male rats were not related to sex differences in internal doses of Pb, confirming that males show an increased susceptibility to Pb concentrations that do not cause behavioral impairment in females.

Transgenerational effects of adulthood exposure: More recently, a study by Sobolewski et al. investigating the transgenerational effect of Pb exposure and/or prenatal stress (PS) in C57BL/6 mice noted that exposures to Pb-PS may influence the brain and behavior across generations (Sobolewski et al., 2020). The study exposed 8-week-old female mice to 100 ppm Pb acetate two months prior to mating and bred them with unexposed males for up to three generations. Half the mice were also subjected to prenatal immobilization restraint stress for 30 minutes on GD 15–18, a critical window for glucocorticoid sensitivity. Assessment of behavioral outcomes (using schedule-controlled behavior [SCB], locomotor activity, elevated plus maze) showed consistent significant effects associated with Pb exposure in third filial (F3) female offspring suggesting a decrease in “anxiety-like” behavior, with an increase in overall response rates in SCB, increase in ambulatory locomotor activity, decrease in time spent in the closed arms of the elevated plus maze and increase in time spent in the center and open arms, and reductions in serum corticosterone levels. In contrast, fewer and less pronounced changes were seen across the Pb exposed F3 males for the above-mentioned outcomes. Thus, developmental exposures to Pb and Pb combined with PS can produce sex-specific transgenerational inheritance of behavioral phenotypes with effects persisting into the F3 generation and, when analyzed across lineages, consistent effects associated with Pb exposure were largely

observed among F3 females. The more pronounced effect in females compared to males could be attributed to heightened female sensitivity or sex-specific differences in gamete likewise the contribution of lineage transfer in relation to sex has received little attention.

Potential Mechanisms of Pb neurotoxicity:

Pb is known to interfere with neurotransmitter release, lowering serotonin levels and monoamine oxidase (MAO) activity, disrupting the function of dopaminergic and cholinergic systems and gamma-aminobutyric acid (GABA) signaling. Pb also inhibits N-methyl D-aspartate (NMDA) ion channels (Mason et al., 2014). Pb accumulation in the bone (a long-term site of deposition) has also been correlated with reduced total brain volume, lower gray matter volume in insula and cingulum, and diminished white matter volume in the parietal lobes in both sexes (Mason et al., 2014).

While most studies (Golden and Voskuhl, 2017) attribute sex differences in outcomes to the differential body burden experienced by males and females in terms of Pb accumulation, the study by Mansouri and colleagues performed in Wistar rats showed the higher susceptibility of the male sex to behavioral deficits even at levels of exposure lower than females' exposure (50 ppm in males vs. 70 ppm in females) (Mansouri et al., 2012). Prenatal exposure to Pb can produce morphological effects such as disrupted synapse formation through a reduction in neuronal sialic acid production and premature differentiation of glial cells (Mason et al., 2014). The perinatal susceptibility of males can also be explained by the differences in functional maturation of frontal cortex and ventral hippocampus expression of NMDA-R and/or $GABA_A$ receptors. Since this maturation occurs later in females, cognitive deficits related to Pb exposure among females are mostly seen following early postnatal and long-term postnatal Pb-exposure (Anderson et al., 2016). Apart from cognitive deficits, Pb is also known to affect a number of interrelated pathways associated with brain development and to promote inflammation-associated responses in the brain (Kasten-Jolly et al., 2012).

The persistence of adverse effects of developmental Pb exposure into adulthood indicates the possibility of long-term molecular reprograming. DNA methylation profiles in the brain are sexually dimorphic (Liu et al., 2010) and are often influenced by hormones and gene-environment interactions. The mechanisms underlying differential vulnerability (or resiliency) of males and females to adverse effects related to exposure to metals such a Pb, with an emphasis on the influence of genetics, epigenetics, hormones, and their interactions, are discussed in greater detail in the review by Singh and colleagues (Singh et al., 2018a). Therefore, here we will only discuss two studies from the same group published after their review. In the first study, they explored the effect of developmental Pb exposure on the methylome of the hippocampus, which is known to be particularly sensitive to Pb toxicity. Long Evans rats were exposed orally via chow diet to increasing Pb concentrations (0, 150, 375, and 750 ppm Pb acetate) either perinatally (10 days prior to breeding through PND 21) or postnatally with two postnatal exposure paradigms: early postnatal (PND 0–21) and long-term postnatal (PND 0–55) (Singh et al., 2018b). Methylation microarray profiling revealed insightful sex-specific effects, such as hypermethylation of genes involved in brain physiology (*Slc18a1* and *Pcdha1*) and hypomethylation of a gene known to be a suppressor

of learning and memory (*Ppp1cb*) in males. Females showed hypomethylation of genes involved in innate immunity (*Kif3b*, *Ceacam3*) and signal transduction (*Tect2*, *Rap2a*).

Then, in a more recent study (which we discussed previously in the context of transgenerational effects of Pb exposure), Sobolewski et al. found that Pb exposure and/or prenatal stress influence anxiety, locomotion, and stress markers in C57BL/6 mouse F3 female offspring possibly via changes in DNA methylation of genes of proteins involved in these different processes (Sobolewski et al., 2020). Specifically, they measured tyrosine hydroxylase (TH), glucocorticoid receptor (GR), and BDNF levels in frontal cortex and hippocampus, and DNA methylation of these genes. While fewer and less striking behavioral effects were seen across the Pb exposed F3 males, the authors observed a significant decrease in total percent DNA methylation of hippocampal *Bdnf* in the Pb group across lineages in addition to a lineage-specific significant increase in percent total hippocampal *Th* DNA methylation that was also observed among certain female F3 lineages. These results indicate that developmental Pb exposure can alter brain DNA methylation across generations in a lineage- and sex-specific manner. Therefore, these studies and the previous work from this group support the view that sex-specific effects in Pb toxicity during the developmental period might be attributed in part to dynamic changes in DNA methylation-associated modulation of transcriptional regulation in the hippocampus, a brain region associated with neuronal development, synaptic plasticity, and metal ion and chromatin modification (Schneider et al., 2013; Singh et al., 2018b; Sobolewski et al., 2018). These Pb-induced epigenetic effects may cause differences in the neurobiological and neuropsychological susceptibility among the two sexes (Qureshi and Mehler, 2010).

Human data on sex differences in Pb metabolism and accumulation hints that young or premenopausal women have better Pb retention or slower release than men leading to a somewhat protective effect of being female (Mansouri et al., 2012). Furthermore, differences in the distribution and density of receptors of estrogen, a hormone which plays a major role in neurodevelopment, may also contribute to neuroprotection among females (Gillies and McArthur, 2010; Varshney and Nalvarte, 2017) and in part explain the sexual dimorphisms observed in adverse cognitive effects of Pb exposure. In addition, Pb has a complex interaction with estrogen (Tchernitchin et al., 2011) and iron status (Lee and Kim, 2014) which may explain the differences sometimes observed in sex-specific effects. The effects of estrogen often dominate the association between iron deficiency and increased blood Pb concentrations, explaining why women have lower blood Pb concentrations than men despite having lower ferritin concentrations. (See Lee and Kim, 2014 for a more detailed discussion of the relationship between metal-iron interactions and sex-specific blood metal levels). Women during pregnancy and old age undergo bone demineralization and are more likely to release Pb into the bloodstream from bone, where it accumulates (Manocha et al., 2017), increasing the internal exposure to Pb. However, even at these older ages, males show higher blood Pb concentrations relative to females (Figure 3) (Brody et al., 1994). Thus, sex differences in Pb neurotoxicity and blood Pb concentrations are influenced by various factors in males and females rather than differences in exposure alone. Such factors, including hematocrit levels, rates of absorption and metabolism, estrogen effects, and iron status should be accounted for when analyzing sex-specific differences in blood levels of Pb and consequent neurotoxic outcomes.

From the experimental data on Pb, one can conclude that Pb neurotoxicity is detrimental to both sexes through a variety of molecular, hormonal, and epigenetic mechanisms affecting gene regulation, expression, function, and different domains of neurobehavioral development (Singh et al., 2018a). However, it appears that overall, males might be more susceptible to prenatal Pb exposure compared to females, who may be more susceptible later in development. There is a need to identify the precise factors that confer this differential susceptibility across sexes and developmental stages and integrate them into a mechanistic framework to discern how sex influences Pb neurotoxicity in order to revise recommendations for exposure and to inform interventions and therapeutics.

Sex-specific Neurotoxic Effects of Mercury (Hg):

Mercury (Hg), a naturally occurring, ubiquitous environmental toxicant, exists in its elemental form as well as many organic and inorganic forms (Farina and Aschner, 2019). Its most common organic and most toxic form, methyl mercury (MeHg), is produced as a result of methylation of inorganic Hg entering waterways by aquatic microorganisms (Aschner and Aschner, 1990). Hg is employed in the electrical industry (e.g., switches, thermostats, and batteries), dentistry (dental amalgams), and numerous industrial processes including mining. Despite Hg's now well-known toxicity, due to its property to amalgam with gold, it is still used in gold mining in certain parts of the world including the Amazon, west Africa, and the Philippines (Steckling et al., 2017). Some Hg compounds are also used as fungicides.

The major source of human exposure to MeHg is through fish consumption. MeHg is readily absorbed so it can accumulate in the food chain. MeHg has a high liposolubility facilitating its passage through cellular membranes and accounting for its enormous potential to bioaccumulate. Furthermore, MeHg easily complexes with cysteine in organisms, thereby mimicking essential amino acids like methionine, which it can then compete with for transport via specialized amino acid carriers that provide entry to normally protected complex and multilayer membranes such as the placenta and BBB. Through this pathway and its retention in the lipid-rich CNS, MeHg can reach high concentrations in the brain explaining its elevated neurotoxicity (ATSDR, n.d.). The Agency for Toxic Substances and Disease Registry (ATSDR) develops health-based values to inform populations and regulators about exposure risk for different pollutants. For instance, the minimal risk level is defined as "the estimate of daily human exposure to a substance that is likely to be without an appreciable risk of adverse effects (noncarcinogenic) over a specified duration of exposure" (ATSDR, n.d.). The minimal risk level for chronic duration (365 days or more) oral exposure to MeHg is 0.0003 mg Hg/kg/day (0.3 ppb/day). This derived value is based on adverse neurodevelopmental outcomes measured among children exposed to MeHg *in utero* from maternal fish ingestion (Davidson et al., 1998).

Sex-specific concentrations of blood Hg:

In contrast with Pb, there is only sparse evidence in the literature of a potential sexual dimorphism in internal Hg burden. A recent study of the Korean general population (Eom et al., 2018) and an older Japanese study (Sakamoto et al., 1991) reported higher levels of Hg in males as compared with females in blood and red blood cell Hg levels, respectively.

In the Korean study, the sex difference in blood Hg levels was observed only among adults aged 20 years and older. Our analysis of the 2015–2016 NHANES cycle (Figure 2) shows that among the general U.S. population there are no significant sex differences in total Hg or MeHg levels measured in whole blood (not overall, nor when stratified by age, Figure 3). However, the geometric mean total Hg concentration of subjects in the Eom et al. study (blood Hg 2.92 μg/L [2.92 ppb] for 0–83 years of age, 3.90 μg/L in adults > 19 years of age, 2010–2011) and Sakamoto et al. study (red blood cell Hg 20.4 μg/L for 40–89 years of age, 1980) were much higher compared to the general U.S. population (0.68 μg/L for 1–80 years of age in NHANES, 2015–2016, Supplementary Table 1), potentially indicating that sex differences in Hg metabolism for example are only apparent at high Hg concentrations. Blood Hg levels are often higher in Asian populations than in Western countries due to various factors including lifestyle, culture, customs, and diet. A diet favoring seafood is associated with the high level of blood Hg in Asian countries including Korea (N.-Y. Kim et al., 2012) and Japan (Yaginuma-Sakurai et al., 2009); thus, sex differences in fish and shellfish consumption could more likely explain the observed sex differences in Hg levels in these studies.

Sex-specific neurological outcomes induced by Hg - Epidemiological evidence:

Prenatal and early postnatal exposure: There is substantial evidence on the differential manifestation of neurotoxic effects induced by MeHg in males and females (Castoldi et al., 2003). The evidence presented in this section is summarized in Table 1. A study among four aboriginal communities (Cree Indians) in northern Quebec with high levels of MeHg exposure (Mistassini, Waswanipi, Great Whale, and Fort George) examined the association between prenatal MeHg exposure (measured via Hg in maternal hair, a particularly reliable biospecimen for exposure assessment of organic Hg such as MeHg) and differences in neurologic function among boys and girls between the ages of 12 and 30 months (McKewon-Eyssen and Ruedy, 1983). The average indices of prenatal exposure to MeHg were not clearly different for boys and girls (highly variable between 2.8–8.3 μg/g, equivalent to 2.8–8.3 ppm; with standard deviations as high as the means). In girls, no significant adverse effect of prenatal MeHg exposure was seen. In fact, in girls, there was a borderline significant negative association between MeHg exposure and incoordination (primarily attributed to delayed motor development). Boys, however, showed a significant positive association in abnormality of muscle tone and reflexes (Babinski reflex) with prenatal MeHg exposure. Comparing cases with abnormal muscle tone or reflexes with controls, the prevalence of abnormality of muscle tone or reflexes increased seven times with each increase of 10 μg/g of the prenatal exposure index.

Not only are boys more susceptible to motor deficits upon Hg exposure, but they also show heightened susceptibility to cognitive and neurobehavioral performance, as demonstrated by a cohort of singleton births in the Faroe Islands (Grandjean et al., 1998). Natives of the Faroe Islands, situated between Iceland and Europe, have high Hg exposure due to the cultural tradition of eating pilot whale, which contains large amounts of bioaccumulated Hg. Grandjean and colleagues assessed neurobehavioral effects of prenatal MeHg exposure comparing a group of children (~7 years old) with relatively high prenatal exposure (maternal hair Hg concentration of 10–20 μg/g) to matched children with low prenatal

exposure (maternal hair Hg concentration < 3 μg/g). They found that, among boys, high exposure was significantly associated with poor scores in neurobehavioral exams, while no significant effect was seen among girls. Specifically, boys showed increased errors in the Bender Gestalt Test, decreased average scores in a hand-eye coordination test, slowed reaction time in the Continuous Performance Test, and shorter delay retention in the California Verbal Learning Test.

In French Guiana, an area affected by MeHg pollution in the food web due to gold mining, a cross-sectional cohort of children from communities with low, medium, and high Hg exposure were administered neurological (9 months-12 years old) and neuropsychological assessments (5–12 years old only) (Cordier et al., 2002). Finger tapping, the Stanford-Binet copying test (including block and design scores, bead memory), the digit span test, and leg coordination were selected as outcomes for their good translation to non-occidental populations. Hg exposure levels were derived from both child and maternal hair (geometric mean concentration = 12.7 μg/g in the high exposure community). Among all sampled children, after adjustment for potential confounders, maternal Hg levels were significantly associated with increased tendon reflexes (in boys only) and decreased performance in Stanford-Binet copying scores (significant for both but much stronger association in boys) which assess visuospatial organization. Among the children from the community with higher exposure, maternal Hg levels were significantly associated with poorer leg coordination in boys and poorer block design scores in girls.

Children (< 5 years old) living in Bom Futuro (Rondonia, Brazil), another mining settlement in the Amazon with high levels of Hg exposure, showed significant sex differences in neurodevelopment with boys again showing a greater sensitivity (Marques et al., 2015). This study examined both pre- and postnatal organic Hg exposure, measured via hair and vaccination cards (to estimate total ethyl Hg exposure), and assessed neurodevelopment at 6 and 24 months of age using the Bayley Scale of Infant Development (BSID), Second Edition. Like the previous studies, this study's findings show the relative sensitivity of boys in the BSID at 6 months; compared to girls, boys had slightly (but statistically significant) lower Mental Developmental Index (MDI) and Psychomotor Developmental Index (PDI) scores, despite no significant difference in hair Hg across sexes. No significant differences between boys and girls were present at 24 months, but at this age only boys showed a significant association between BSID measures and hair Hg. In terms of milestone achievements (age at talking and walking), age of walking was positively and significantly associated with Hg levels in hair (at 6 months) in girls only (median hair Hg level = 0.98 μg/g).

The relationship between prenatal Hg exposure on neonatal development has also been examined among children of the Seychelles, an archipelagic island country off East Africa. Davidson and colleagues suggested some sex-specific effects in the Seychelles Child development study looking at MeHg (in maternal hair, median prenatal MeHg = 5.9 μg/g) and child development (cognitive ability [McCarthy Scales of Children's Abilities, MSCA], language development [Preschool Language Scale], drawing and copying [Bender Gestalt test], scholastic achievement [Woodcock-Johnson Test of Achievement] and behavior [Child Behavior Checklist]) at 66 ± 6 months of age (Davidson et al., 2004). Sex was found to

significantly influence drawing and copying scores. However, the regression coefficients for males and females were positive and negative, respectively, and neither of these estimates were significant. The authors noted that the study findings were not internally consistent, nor were they in line with previously reported results (Davidson et al., 1999, 1998; Huang et al., 2005) suggesting that these results might be due to chance. Yet a later study in Zhoushan City, China (Zhejiang Province) looking at Hg levels in maternal hair (geometric mean = 1.25 μg/g [ppm]) and cord blood (geometric mean = 5.58 μg/L [ppb]) and the impact of Hg exposure on neonatal neurobehavioral development observed a decrease in behavioral ability (assessed using the neonatal behavioral neurological assessment scale) for males but not for females with increasing Hg exposure (both maternal hair and cord blood) (Gao et al., 2007). In this study, the effects observed were significant even at Hg levels much lower than other fish-eating populations such as the Faroe Islands and Seychelles.

A study conducted within the Spanish INMA (INfancia y Medio Ambiente – Environment and Childhood) Project looking at the effect of cord blood total Hg (CB-Hg) levels on neuropsychological development (assessed using MSCA) in children ages 4–5 from populations with diets particularly rich in fish found sex-specific effects for motor scale (Llop et al., 2016). Contradicting previously mentioned studies, girls with higher Hg exposure scored significantly lower than boys on the motor scale at geometric mean CB-Hg 8.8 μg/L, suggesting that for some motor outcomes girls may be more susceptible than boys to early-life MeHg exposure. Indeed, the same group previously reported that similar levels of prenatal Hg exposure (geometric mean total CB-Hg 8.4 μg/L) may have an adverse effect on the psychomotor development of girls (assessed using BSID at 14 months of age); their multivariate analysis stratified by sex found a near-significant negative association between CB-Hg and BSID in female infants ($\beta = -1.09$, 95% confidence interval: −2.21, 0.03) (Llop et al., 2012). The lack of significant effect reported in this study compared to the more recent study could be explained by the use of a different assessment scale (the BSID compared to MSCA) which was also employed at a different time in development (14 months vs. 4–5 years), suggesting that the negative psychomotor effect among girls may be more apparent at a later age, and/or by differences in maternal fish intake during pregnancy. An important insight from the more recent 2016 study (not related to sexual dimorphisms but worth mentioning) is that higher maternal fish intake, while it led to higher levels of CB-Hg, were associated with beneficial effects across the outcomes tested by the MSCA. Hg neurotoxicity only revealed itself for children whose mothers had the lowest consumption of fish (i.e., less than 3 servings a week). Therefore, levels of the brain health-promoting nutrients provided by fish intake, such as certain types of polyunsaturated fatty acids, should always be taken into consideration when investigating the potential adverse outcomes of fish intake-related MeHg exposure.

In summary, epidemiological studies examining infants and young children have more often reported that boys are more susceptible to the neurotoxic effects of early life MeHg exposure than girls (González-Estecha et al., 2014; Grandjean et al., 1998; Marques et al., 2015; McKewon-Eyssen and Ruedy, 2017), particularly in neurobehavioral domains as the above evidence indicates. This suggests an important sexual dimorphism in MeHg neurotoxicity early in life. However, unlike Pb, most of these studies were conducted in populations

exposed to relatively high doses of Hg and it is unknown whether this trend persists at lower exposure levels and if the effects persist through childhood, adolescence, and adulthood.

Adulthood exposure: To observe whether sex modulates the effect of MeHg exposure on neurologic abnormalities in adults (18–82 years old) with fish-rich diets, a study among 302 Cree Indians of five villages in Quebec assessed hair and blood samples separately for organic and inorganic Hg (Auger et al., 2005). At a mean blood total Hg level of 37.7 μg/L [ppb] and a mean hair total Hg level of 6.4 μg/g [ppm], sex seemed to have only a minimal, non-significant impact on the association between Hg and neurologic outcomes (sensory/auditory/motor disturbances, incoordination, tremor, and reflexes). However, there were several limitations to this study including selection bias, potential effects of confounders like alcohol consumption and smoking which was likely under-reported, and the statistical assessment of multiple outcomes without correction for multiple comparisons which might have increased the chances of error. Also, note that while hair Hg is a biomarker of chronic MeHg exposure, it can only provide information on cumulative exposure over the last few months or years depending on hair length (hair grows on average 6 inches [or 15 cm] per year). Therefore, Hg in hair certainly does not reflect lifetime exposure, and especially exposure during early development. Thus, it is still unclear whether effects of Hg exposure during early development persist into adulthood.

Regarding current or recent exposure, it is likely that the mature adult brain may be less susceptible to Hg-induced toxicity mediated by the intake of MeHg-contaminated fish. Supporting this, an important insight from this study (again not related to sexual dimorphisms but worth mentioning) is that the authors found a significant interaction with age: increasing Hg was associated with increasing levels of tremor in younger subjects (< 40 years old), but not older ones. It could be the case that cumulative exposure is more imprecise in older adults and/or that in this population, environmental MeHg exposure was higher at the time of neurological development in the younger adults. Another analysis examined 361 individuals within the two Cree Indian communities with the highest MeHg exposure (Mistassini and Great Whale), comparing those with neurologic abnormalities to controls. After stratifying by sex and community, adjusted odds ratios revealed a positive association between neurologic abnormalities and MeHg which was significant in Mistassini but not in Great Whale (which had a smaller sample size), with a stronger effect observed in males than females in both communities (McKeown-Eyssen & Ruedy, 1983). Thus, sex-specific effects of MeHg exposure in adults may be evident only at higher exposure levels, further supporting the hypothesis that the mature adult brain is less susceptible to MeHg neurotoxicity.

In conclusion, the best documented evidence of sex-specific adverse neurotoxic effects of MeHg are the effects observed among exposed fetuses, infants, and young children. The evidence suggesting that MeHg can impact neurological function among adults in a sex-specific manner are inconclusive, though limited evidence suggests that males are more susceptible. More studies in the adult population or prospective studies following children into adulthood are needed.

Sex-specific neurological outcomes induced by Hg - Experimental evidence:

Prenatal and perinatal exposure: A summary of the experimental evidence reporting sex-specific neurotoxic effects following Hg exposure is provided in Table 1. Inherent sex differences in motor coordination have been widely studied and described in animals; therefore, sex is often taken into consideration when studying the effects of MeHg exposure on animal motor system control (in particular the dopaminergic system) (Farina et al., 2011). For instance, a study of prenatally exposed (MeHg at 0.5 ppm per day from GD 7-PND 7) Sprague Dawley rats tested as young adults at 6 months of age revealed a significant decrease in spontaneous motility and rearing only in male rats (Rossi et al., 1997). These motor alterations were reversed by administering a dose of D-amphetamine (a dopaminergic stimulant; effective at both 0.5 and 1.5 ppm), indicating that dopaminergic systems are likely involved in MeHg neurotoxic effects. However, these motor alterations are not mediated by dopaminergic cell loss, as immunohistochemical analysis showed no significant differences in tyrosine hydroxylase protein (TH, used as a marker for dopaminergic neurons) between control and exposed males. Rather, MeHg's neurotoxic effects could be mediated by alteration of the dopaminergic transmission, for instance at the receptor levels. The absence of altered locomotor activity in females cannot be explained by a differential burden of Hg in the CNS, since similar increases in total Hg levels in the cerebrum (10x) and cerebellum (20x) were measured in both sexes compared to controls. A noteworthy observation is that while the highest dose of D-amphetamine tested (1.5 ppm) not only reverses the effect of MeHg and significantly increases male rat locomotor activity, it tends to decrease female locomotor activity as well as the other motor outcomes tested (motility and rearing). This suggests that the relative balance between pre- and postsynaptic dopaminergic neurotransmission might be differentially affected by sex.

This hypothesis was supported by another prenatal MeHg exposure study in rats employing a similar exposure paradigm (MeHg at 0.5 ppm per day from GD 7-PND 7) but assessing motor effects earlier (on PND 21) (Giménez-Llort et al., 2001). They found that males, but not females, showed a significantly reduced response to postsynaptic dopamine D2 receptor stimulation (tested with U91356A, a D2 selective agonist; 0.1 ppm) which is involved in spontaneous motor activity both in the mesolimbic and nigrostriatal dopaminergic system. This effect, however, was not seen at PND 14 when both males and females displayed increased motor activity, highlighting the importance of the developmental period crucial for ontogeny of dopamine receptors in the brain.

Adulthood exposure: The dopaminergic system is also critically involved in non-motor function and has been suggested to be implicated in autism spectrum disorder (ASD) (Pavăl, 2017). Exposure to Hg was thus also studied in a neurobehavioral context and, to test the potential role of inorganic Hg in ASD, researchers conducted a study exposing sexually naïve adult prairie voles (≥ 59 day-old) to 60 ppm (mg/L) $HgCl_2$ via drinking water for 10 weeks (Curtis et al., 2011). Prairie voles are highly social animals and thus are a classical animal model for ASD to study the behavioral, neural, and physiological bases of social attachment. The results showed a significant male-specific increase in tumor necrosis factor alpha (TNFα; a marker of glial inflammation) protein expression in the cerebellum and hippocampus following subchronic Hg exposure compared to controls and exposed

females. However, no sex-specific effect or effect of treatment was observed for other mediators of glial activation such as the chemokines CCL2 and CXCL10. When monitored for social behavior in the choice test, Hg exposure significantly reduced social contact with an unfamiliar same-sex conspecific only among males, while no effects of exposure were seen in females. With regards to a dopamine-mediated non-social behavior, in agreement with previously reported effects of Hg on the dopaminergic system, exposed males showed an abnormal response to amphetamine treatment; Hg-exposed males did not display the expected increase in motor activity in response to D-amphetamine administration. However, differences in locomotion did not confound the choice test, as there were no effects of sex or Hg exposure on locomotor activity during this social behavior assay. This blunted amphetamine response only among Hg-treated males was not due to differences in ingestion or accumulation of Hg in brain tissue as confirmed by metal level assessment (Curtis et al., 2010). These findings demonstrate the role of non-motor dopaminergic pathways and neuroglial signaling that are altered in a sexually dimorphic manner by subchronic, low-dose Hg exposure *in vivo*.

To investigate whether some differences in MeHg whole body retention could explain sex differences reported in MeHg toxicity, male and female Long Evans rats were subcutaneously treated with an injection of 1 μmol radioactive ^{203}MeHg/kg at PND 7, 15, 20, 24, and 56 (Thomas et al., 1982). In rats dosed at PND 56, Hg whole body retention (observed up to 139 days) was significantly different across sexes (males showing higher retention) and this sex difference persisted when expressed as a function of body weight. However, no sex-specific differences were seen among younger age groups (7, 15, 20, 24 days). Knowing that Long Evans rats reach puberty at PND 50 on average (Pallav Sengupta, 2013), this suggests a role of hormonal influences on clearance in sexually mature subjects. These experimental data are in agreement with human data that showed a higher Hg burden in male blood as compared to female blood but only in adults above 20 years of age (Eom et al., 2018).

Another study investigating MeHg toxicity (40 ppm, diluted in drinking water) in 2-month-old Swiss albino mice reported that a 2-week exposure to MeHg significantly decreased locomotor activity in the open field test, with a stronger effect observed in males (−52%) than in females (−30%). This effect was prevented by administration of 17β-estradiol only in males (Malagutti et al., 2009). Also, a significant motor impairment (53% decrease in falling latency) on the rotarod was seen only in male mice. Again, these effects were reversed by the administration of 17β-estradiol demonstrating that the low susceptibility of females to MeHg neurotoxicity is associated with the neuroprotective effects of female sex steroids.

Noting that the higher susceptibility of the male sex is consistently observed across different species (humans, rats, mice, prairie voles), the sexually dimorphic neurotoxic effect of Hg exposure can be implicated with relative confidence. This high interspecies conservation suggests that the dopaminergic system and the neuroimmune axis are sexually divergent and confer higher susceptibility to males to metal exposures such as Hg.

Potential Mechanisms of Hg neurotoxicity:

MeHg is known to (1) affect cell membrane function and neuron delivery materials; (2) cause intracellular calcium overload, oxidative stress, lipid peroxidation, and mitochondria dysfunction; and (3) hinder synapse transmission, microtubule composition, amino acid transport, and cellular migration during developmental stages (Aschner and Aschner, 1990; Hong et al., 2012). MeHg was demonstrated to prevent astrocytic glutamate uptake and increase glutamate release which leads to elevated extracellular glutamate levels and overactivation of NMDA glutamate receptors, causing an increased influx of calcium cation (Ca^{2+}) into postsynaptic neurons and leading to activation of cell death pathways, as observed in cerebellar slices and cultured neuronal cells (Farina et al., 2011). These different molecular mechanisms linked to glutamatergic transmission particularly well-explain the clinical expression of MeHg neurotoxicity that includes paresthesia, constriction of visual fields, impairment of hearing and speech, cerebellar ataxia, seizures, loss of motor coordination, and cognitive disorders (Farina et al., 2011).

Upon finding that Hg exposure in sexually naïve adult prairie voles altered social behavior among males only, the same group looked further into the mechanism underlying these behavioral changes in a recent study (Soto et al., 2019). They hypothesized that metal-treated males find encounters with a same-sex conspecific to be more stressful, explaining the decrease in social interaction seen among $HgCl_2$-exposed males. To test this hypothesis, prairie voles (mean age 169 days) were treated with 60 ppm $HgCl_2$ via drinking water for 10 weeks and then made to interact with an unfamiliar vole of the same sex. Plasma corticosterone levels were used as an index of hypothalamic-pituitary-adrenal (HPA) activity. They found that exposure to $HgCl_2$ differentially affects the HPA axis in both sexes; $HgCl_2$-treated males actually had significantly lower circulating corticosterone compared to control males or $HgCl_2$-treated females. Since $HgCl_2$ treatment did not significantly affect female corticosterone levels in the present study (compared to controls) and reduced social contact was not seen among females in the previous study, one can say that the HPA axis response may underlie the sex-specific effect in social interactions observed following subchronic exposure to inorganic Hg, though not in the way that was expected.

As we previously discussed (Malagutti et al., 2009), female sex hormones have been found to play a role in alleviating MeHg toxicity, with administration of 17β-estradiol preventing motor effects observed in male mice. In this study, further biochemical analysis showed that exposure to MeHg caused a significant increase (+60%) in cerebellar lipid peroxidation and cerebral glutathione reductase activity (+25%), and a decrease (−43%) in cerebellar glutathione peroxidase activity in male mice, but not in females. Therefore, the neuroprotective effects of sex steroids appear to be mediated by the maintenance of antioxidant glutathione-related enzyme activity and point to an important role of oxidative damage in the sex-specific susceptibility observed in male mice. Maintenance of glutathione metabolism is itself linked to glutamate metabolism and thus is a primary target of MeHg neurotoxicity (Grady et al., 1978; Kimiko et al., 1987; Ruszkiewicz et al., 2016). The strong sexual dimorphism of the expression of antioxidant enzymes and their transcriptional regulation (TrxR, Trx, GPx, 2CPOX, COMT, TDO2, GRIN2A, GRIN2B, SLC6A4, KIBRA,

APOE, MT1M, MT2A, GSTT1, and SEPP1) has been suggested by several studies to account for the sex-specific effects of MeHg toxicity (Torres-Rojas and Jones, 2018).

Altogether, experimental evidence indicate the higher susceptibility of males to Hg neurotoxicity and suggest that this dimorphism is due to differences in neurotransmission as well as differences in neuroendocrine and antioxidant activity among the two sexes. As the differences in MeHg neurotoxicity among the two sexes (both humans and animals) are prominent during childhood and appear to be maintained into adulthood (at least based on animal evidence), it will be important to elucidate which neuroendocrine differences other than those related to sexual maturity (e.g., estradiol) may play a role in contributing to sex-specific effects. For instance, it is known that brain sexual dimorphisms start early during development under the influence of testosterone in males (Bakker, 2019). Besides, growing evidence supports the role of sex chromosomes in shaping brain sexual dimorphisms independently of sex hormones. The influences of these different factors remain to be investigated in MeHg neurotoxicity, as is the potential persistence of MeHg sex-specific neurotoxicity across the different stages of human life.

Sex-specific Neurotoxic Effects of Manganese (Mn):

Mn is a naturally occurring substance found in many types of rocks and soil and is used in a wide variety of other products such as nutritional supplements, fireworks, fertilizers, paints, and cosmetics (ATSDR, 2012). Unlike the other toxic metals discussed in this review which are foreign to human biology and thus expected only to potentially interfere with an organism's homeostasis when present above certain levels, Mn is an essential nutrient which plays an important role in a number of physiologic processes as a constituent and cofactor of multiple enzymes, including some critical enzymes of the nervous system (e.g., glutamine synthetase, arginase, Mn superoxide dismutase) (Peres et al., 2016). Thus, a minimal daily intake of Mn is required and Mn shows the typical nonmonotonic dose-response curve of essential nutrients, with too low levels (i.e., deficiency) causing toxicity and too high levels (i.e., excess) also causing toxicity. Being an essential micronutrient, Mn also plays a critical role in prenatal development and may be actively transported across the placenta to meet the nutritional demands of the growing fetus (Nandakumaran et al., 2016).

Occupational exposure is one of the primary sources of Mn intoxication and is commonly seen among workers involved in mining, welding, battery manufacturing, or application of fungicides containing the metal (Peres et al., 2016). The primary source of Mn exposure in the general population is through diet. Excessive Mn exposure through anthropogenic and dietary sources such as food, water, and air pollution or impaired excretion and transporter malfunction has been shown to lead to toxicity. Excess consumption of Mn-fortified food among children is also of concern in terms of toxicity (Aschner and Aschner, 2005). In the absence of a true minimal risk level, an interim guidance value of 0.16 mg Mn/kg/day (160 ppb Mn/day) is recommended for chronic oral exposure to Mn. This is based on reports of neurobehavioral effects in children associated with elevated concentrations of Mn in drinking water (ATSDR, 2012).

Sex-specific concentrations of blood Mn:

In contrast to Pb that consistently shows a higher burden in males, Mn blood levels were most of the time reported to be higher in women than men in adult population-based biomonitoring studies carried out in places as diverse as Southern Brazil, Northern France, and the U.S. (Da Silva et al., 2017; Nisse et al., 2017; Oulhote et al., 2014). This sexual dimorphism was suggested to be explained by increased Mn absorption in women of childbearing age due to their commonly observed deficiency in iron (Fe), which leads to a compensatory increase in Fe absorption pathways that can also be used by Mn (Lee and Kim, 2014). In keeping with the existence of puberty-triggered sex-related metabolic differences in the homeostatic mechanisms regulating blood Mn levels, in the U.S. NHANES study referenced above (Oulhote et al., 2014), females were observed to have higher levels of blood Mn (10.6 μg/L [ppb]) than males (9.2 μg/L) overall (ages 1–80 years), although the difference was greatly reduced during childhood as compared with adolescence and adulthood. In further agreement, sex-specific differences in Mn blood or hair levels were not observed in children in other studies (Broberg et al., 2019; Menezes-Filho et al., 2014). We also analyzed data from NHANES (2015–2016 cycle) and found that overall, blood Mn levels were significantly higher in females (geometric mean: 10.20 μg/L) compared to males (8.99 μg/L) (Figure 2, Supplementary Table 3). When examining sex differences in blood Mn concentrations stratified by life stage (i.e., child, adolescent, adult, and elderly) we found that blood Mn concentrations were higher in females across the life course, but this difference was significant only in adolescence (12–21 years of age) and adulthood (22–65 years of age) (Figure 3, Supplementary Table 4). Thus, our result that females have a higher Mn concentration and that this sex difference appears during puberty agrees with the existing literature and suggests that sex differences in Mn body burden may be due to puberty-triggered differences in sex hormones.

Sex-specific neurological outcomes induced by Mn - Epidemiological evidence:

Prenatal and early postnatal exposure: Despite the absence of a clear sexual dimorphism in Mn internal burden before puberty, several studies have reported sex-specific effects of Mn exposure on neurological outcomes assessed in children. These studies are summarized in Table 1. For instance, a study examining the impact of pre- and postnatal Mn exposure (measured in children's shed tooth dentin) on children's scores on the MDI and PDI found effect modification by sex for postnatal Mn levels and neurodevelopment at 6 months with stronger negative effects among girls on both indices (Gunier et al., 2015). Furthermore, girls born to mothers with low hemoglobin levels experienced larger decreases in the MDI and PDI, suggesting that maternal Fe deficiency may exacerbate the adverse effects of Mn exposure in their female offspring. The PHIME (Public Health Impact of Manganese Exposure in susceptible populations) cohort, which assessed the effects of prenatal and early postnatal Mn exposures (also via shed teeth) in older children (ages 10–14 years), observed a "U"-shaped association between prenatal Mn and virtual radial arm maze performance measures among girls only, suggesting that both low and high prenatal Mn may adversely affect visuospatial learning and working memory in girls. The fact that this association was not observed among boys, and furthermore wasn't observed in either sex for

postnatal exposure, demonstrates the importance of a critical window of exposure (prenatal period) as well as the differential effect of Mn based on sex (Bauer et al., 2017).

A study in the Californian CHAMACOS (Center for the Health Assessment of Mothers and Children of Salinas) cohort assessed the effects of pre- and postnatal Mn exposure, measured in teeth, on various behavior, cognitive, memory, and motor outcomes at ages 7, 9, and 10.5 years (Mora et al., 2015). Prenatal Mn levels in dentine of shed teeth were associated with increased behavior problems in boys at age 10.5, while early postnatal dentine Mn levels were associated with behavior problems at age 7 for both sexes. Surprisingly, higher pre- and postnatal dentine Mn levels were associated with *better* cognitive and motor outcomes and visuospatial and verbal memory in boys. Thus, overall, higher prenatal and early postnatal Mn measured in shed teeth was associated with behavior problems in boys and girls, but increasing Mn dentine levels were favorably associated with several measures of cognition, memory, and motor function in boys. These unexpected findings may be because dentine Mn levels in the CHAMACOS cohort could be within the range where Mn acts as a nutrient in a beneficial capacity.

In keeping with these surprising positive effects of Mn exposure, another study conducted in the aforementioned Italian PHIME cohort reported that Mn exposure had protective effects on neuromotor function (namely body stability, but perhaps also hand-related motor coordination) in boys and this sex difference was primarily attributed to prenatal exposure. Associations between Mn levels and resting hand tremor were dependent on sex as well as period of exposure (prenatal, early postnatal, childhood cumulative Mn). These findings suggest sex-specific critical windows of susceptibility in relation to neuromotor domains measured in male and female adolescents (Chiu et al., 2017).

The origin of these unexpected associations between increasing prenatal and early postnatal Mn levels and beneficial cognitive, memory, and motor outcomes remains unclear. On the other hand, studies have also found that higher postnatal tooth Mn was negatively associated with visual and motor scores among boys only (median age 8 years) (Claus Henn et al., 2017, 2010). A recent systematic review and meta-analysis investigating associations between Mn exposure and childhood neurodevelopment also emphasized the discrepant conclusions drawn from various studies and reiterated the inconsistencies in reported sex differences, although there was perhaps a trend indicating girls are more susceptible to Mn than boys (Liu et al., 2020). Inconsistencies in sex-specific exposure-response relationships may be due to differences in timing of exposures, differences in measured biomarkers of Mn exposure (e.g., hair, blood, and teeth; furthermore, the validity of dentine as a biomarker for total Mn exposure is still not fully characterized) (Liu et al., 2020), or to the methodological limitation inherent in cross-sectional study designs. The potential for selection bias, limited statistical power to evaluate sex interactions, and biological interactions with other metals may also in part explain the inconsistent findings. Thus, further studies (in particular, longitudinal analyses) should clarify this issue.

Childhood and adolescent exposure: Studies in Canada have found that Mn levels measured in drinking water, hair, and toenail are associated with intellectual function in children and adolescents up to 18 years of age, with different effects observed across sexes;

in girls, higher Mn levels were associated with lower Performance IQ, while the opposite effect was observed in boys (Bouchard et al., 2018; Dion et al., 2018). Building off these studies, to help regulators and decision-makers protect children from chronic Mn exposure, a recent Canadian study aimed to determine the maximum acceptable concentration of Mn in drinking water (Kullar et al., 2019). To reach this objective, they investigated which tap water Mn levels were associated with pre-defined levels of cognitive impairment (i.e., drop of 1%, 2%, and 5% in Performance IQ scores) in children aged 5.9–13.7 years (630 participants after pooling data from two previous studies). They confirmed that girls are much more vulnerable to the effects of Mn on cognition compared to boys; the Mn concentrations associated with a decrease of 1%, 2%, and 5% Performance IQ in girls were 78, 95, 192 μg/L (ppb) whereas for boys they were 185, 375 and 935 μg/L (ppb).

Sex-specific adverse effects of Mn exposure have also been reported in the context of neurodevelopment disorders and behavior problems. For instance, surface soil Mn levels (blood levels were not available for all subjects) were found to be associated with ADHD-related behavioral problems (evaluated by the Conners' Adolescent Self-Report Scale and revised versions of the Conners' Parents Rating Scales) particularly in girls among Italian children aged 11–14 years (Broberg et al., 2019). Specifically, girls showed "U"-shaped relationships, with higher (i.e., worse) self-reported Conners' scoring at elevated soil Mn levels. Positive linear relationships were found with increasing soil Mn and Conners' scores on several parent-reported scales. On the other hand, for boys, the only outcome showing a positive association with increasing soil Mn was parent-reported hyperactivity. Another smaller cross-sectional study in Brazil investigated behavioral traits (assessed by the Children's Behavior Check List) among airborne Mn-exposed children (exposure assessed by both child and mother hair Mn) living near a ferromanganese alloy plant (Menezes-Filho et al., 2014). Although similar hair Mn levels were measured in boys and girls (median 12.1 and 12.4 μg/g [ppm], respectively; about fifteen times higher than the general Brazilian population), this study reported a significant association between elevated child hair Mn levels and total Children's Behavior Check List scores (including both externalizing behavior problems and attention deficits) in girls only. However, the lack of a significant association among boys may be explained by the study's limited statistical power (N=70). Regardless, the higher susceptibility of girls to various levels of Mn exposure has been reported in multiple cohorts worldwide.

Adulthood exposure: In contrast with Mn's preferential neurotoxicity to girls during development and childhood, in adults, the only study that we found to report on sex-specific effects of Mn exposure suggests that excessive Mn more profoundly affects men, though women are also affected (Mergler et al., 1999). This community-based study reported that upon various sources of environmental exposure to Mn (median blood Mn = 7.3 μg/L; no sexual dimorphism on burden), both men and women exhibited signs of neurotoxicity, but men presented worse learning and recall scores, as well as poorer performance on different motor tests (e.g., a pointing task, frequency dispersion of hand-arm tremor, harmonic index, and pronation/supination arm movement velocity) in association with Mn levels. The differential neurotoxicity of Mn on adult men and women is a clear knowledge gap that future studies need to address. The imbalance of studies investigating Mn neurotoxicity in

women may be explained by the fact that most Mn neurotoxicity studies in adults focus on male-dominated occupations with higher risk of exposure to Mn such as welders, smelters, grinders, and miners (Furbee, 2011). However, knowing that in the general population women exhibit higher Mn levels than men (Da Silva et al., 2017; Nisse et al., 2017; Oulhote et al., 2014), it would be interesting to determine whether there are any neurological consequences of this differential internal burden.

Sex-specific neurological outcomes induced by Mn - Experimental evidence:

Adulthood inhalation exposure: As is the case for other metals, in contrast with human studies, there is an abundance of experimental animal studies investigating the sexual dimorphisms of Mn neurotoxicity (Table 1). However, strikingly, none of these studies investigated the sex-specific neurotoxic effects of early life exposure to Mn revealing a huge research gap in the field and a missed opportunity to learn more about the mechanisms underlying adverse effects of high Mn during development that were reported to differentially affect boys and girls in humans. Among all these experimental adulthood exposure studies, inhalation studies are well represented in the Mn neurotoxicity field, as this route of exposure is relevant to both occupational (e.g., welding, smelting) and environmental exposure (e.g., aerosol, dust, particulate matter). Regarding the pharmacokinetics of Mn, an inhalation study compared tissue accumulation in young (6-week-old) and senescent (16-month-old) female and male Sprague Dawley rats exposed to 0.01, 0.1, and 0.5 mg/m^3 Mn sulfate ($MnSO_4$), 0.1 mg/m^3 Mn phosphate (mineral hureaulite; less soluble), or HEPA-filtered air (control) 6 hours/day for 5 days/week over a 90-day period (Dorman et al., 2004). At the end of exposure, Mn levels were assessed in the olfactory bulb, cerebellum, striatum, lung, and blood of exposed rats (among other peripheral organs). The authors reported that young male rats exhibited the highest increases in olfactory bulb Mn concentrations as compared to age-matched females and old rats across the different exposures (the highest for 0.5 mg/m^3 $MnSO_4$ in agreement with dose and solubility). Young males also showed the highest Mn levels in lungs, in particular for hureaulite which may form deposits there due to its lowest solubility. Altogether, this suggests a sex- and age-driven dimorphism in Mn absorption at crucial portal entry sites (i.e., the lung alveoli and nasal olfactory epithelium). As discussed by the authors, this could be explained by the higher volume of inhaled air per minute of young males as compared to females and older animals. Also, olfactory nerve receptors are known to decrease with age due to a progressive regression of the olfactory neuroepithelium.

Adulthood intravenous and oral exposure: A study investigating the toxicokinetics of Mn in Sprague Dawley rats following various exposure paradigms, i.e. either a single intravenous or oral dose of $MnCl_2$ (equivalent to 6.0 ppm Mn) or single oral dose of methylcyclopentadienyl manganese tricarbonyl (MMT; a fuel additive in some gasolines; equivalent to 5.6 ppm Mn), observed a sex-dependent difference in toxicokinetic profiles of MMT-derived Mn (Zheng, 2000). In both sexes, rats administered MMT had significantly less clearance than rats dosed with $MnCl_2$ and accordingly, the area under the plasma-concentration time curve (AUC) of Mn in MMT-treated rats was nearly 37-fold greater than $MnCl_2$-treated rats, indicating that MMT-derived Mn appears likely to accumulate in the body following repeated exposure. Females showed a significantly greater AUC and

a longer half-life of plasma Mn than that of males due to slower elimination of MMT or MMT-derived Mn among females. Indeed, the volume of distribution was not different across sexes, but males had a significantly greater elimination constant than females; the clearance in females was about 1.7-fold less than that of males. This may be attributed to the differential metabolism of MMT and $MnCl_2$ in rats (Fitsanakis et al., 2010). MMT exposure in humans is mainly via inhalation yet unfortunately the studies that investigated this route of exposure in animals did not investigate sex-specific effects to confirm the potential preferential accumulation observed in female blood after oral exposure (ATSDR, 2012). One can speculate that an inhalation study may have provided counterevidence of females' greater susceptibility in this case due to the higher volume of inhaled air per minute in males as compared to females, as we discussed above in reference to another study (Dorman et al., 2004). Besides, as Mn levels measured in blood and brain are not well correlated (Marreilha Dos Santos et al., 2011), it remains difficult to interpret how sex differences in blood levels translate into neurotoxicity.

A very original recent study used a novel contaminated water sediment exposure model to recapitulate the aquatic microenvironment of heavy metals before exposure and assess whether metal-sediment interactions within the aquatic microenvironment alter metal toxicity. In this study, young adult (8–10 weeks old) male and female C57BL/6 mice were exposed via their drinking water to either water contaminated with Mn without sedimentation (0.5 g/L $MnCl_2$, roughly 500 ppm $MnCl_2$; corresponding to Mn levels in the water above the sediment) or to water incubated with Mn-contaminated sediment (1,000 mg $MnCl_2$/kg dry sediment, about 1,000 ppm $MnCl_2$) over six weeks (Freeman et al., 2020). The concentration of Mn used to spike sediment was selected based on actual Mn levels measured in sediments of the Baltimore harbor area. In this adult exposure paradigm, males in both water Mn and sediment Mn treatment groups were found to be more adversely affected by Mn than females based on a series of motor assessments classically used to detect Mn-induced Parkinsonism in experimental studies (beam traversal, cylinder, and accelerating rotarod tests).

Adulthood intraperitoneal exposure: In agreement with findings in humans, Mn exposure has been documented to lead to a variety of cognitive and motor neurotoxicological outcomes in animals, and some of these exhibited a sexual dimorphism. For instance, Wistar rats exposed to Mn in the form of $MnCl_2$ (equivalent to 1 or 5 ppm Mn) intraperitoneally for 30 days showed a sex difference in Mn-induced depressive behavior assessed by the forced swim and open field tests (Yamagata et al., 2017). In the forced swim test, treated rats showed an overall increase in immobility time compared to controls, especially among males exposed to 1 ppm Mn. In the open field test, male and female mice treated with 5 ppm Mn showed decreased crossings between quarters and fewer rearings. The accumulation of Mn was assessed in the cerebellum, brainstem, hippocampus, striatum, and cortex. Mn accumulation in the cerebellum and brainstem was higher overall in females and dose-dependent only in females; surprisingly, among males, the largest Mn accumulation occurred in the group exposed to 1 ppm Mn. In the other brain regions (hippocampus, striatum, cortex), accumulation was dose-dependent in both sexes and there was a sexual dimorphism only for the low dose of Mn in the hippocampus and cortex where

accumulation was again stronger in females. Overall, despite higher accumulation of Mn in females at some doses and in some brain areas, these results point towards a higher susceptibility of males to Mn-induced depressive behavior. While this seems to contradict the higher susceptibility of females generally reported by epidemiological studies, the age at which rats were exposed may partly explain the discrepancy. The paper's methods section did not report the age of the rats but they were likely young adults, as they weighed approximately 200 grams at the start of the study. As we discussed above in the context of adult exposure, a higher vulnerability of males was reported in humans at least in the only study interested in sex-specific effects (Mergler et al., 1999). Also, note that the males showed higher susceptibility at 1 ppm Mn but not 5 ppm Mn, indicating that these sex differences may be expressed only at low doses, and nonmonotonic effects are more difficult to unravel. This study was also limited by its relatively small sample size (n = 4 rats per sex and exposure group).

Adulthood subcutaneous exposure: Mn exposure also exerts sex-specific neurotoxic effects at the cellular level, with sex-dependent alterations in striatal medium spiny neuron (MSN) morphology observed following subcutaneous Mn exposure. Adult (12-week-old) FVB mice were injected subcutaneously in the hind leg with 50 ppm $MnCl_2$-$4H_2O$ (at exposure days 0, 3, and 6). Females exhibited a higher total spine density and males showed significantly longer total dendritic length and higher dendritic branching (Madison et al., 2011). These differences in MSN morphology were not due to sex differences in striatal Mn accumulation, and sex differences in neuron morphology persisted 3 weeks after the final Mn exposure. The authors proposed that the changes in total spine density they observed may lead to Parkinsonian-like symptoms by altering the input to the striatum which is critical to normal motor function. They also suggested that the sex-specific adverse effects of Mn exposure seen in males may play a role in the higher predisposition of males to develop Parkinson's disease in general, as well as to develop Parkinson's disease earlier than females.

Potential Mechanisms of Mn neurotoxicity:

One can anticipate that dysregulation in Mn homeostasis can have multiple and severe neurotoxic effects, given that Mn is an important metal used as a cofactor by numerous enzymes critical to metabolic and redox homeostasis in the CNS (e.g., glycosyltransferase, pyruvate decarboxylase, glutamine synthetase, and superoxide dismutase) (Liu et al., 2006). In agreement with its reported preferential accumulation in the basal ganglia including the globus pallidus and the substantia nigra (Guilarte et al., 2006a, 2006b), the neurotoxic effects of Mn include Parkinsonian-like features such as dystonia, bradykinesia, and rigidity. Mn has been shown to damage dopaminergic neurons, trigger gliosis, cause severe reduction in dopamine brain levels, alter Akt, ERK, p38, DARPP-32 and TH phosphorylation, and to lead to the upregulation of p53 (Liu et al., 2006; Peres et al., 2016).

Mn exposure can also influence antioxidant activity. In an inhalation study, young (6-week-old) and senescent (16-month-old) female and male Sprague Dawley rats were exposed to 0.01, 0.1, and 0.5 mg/m^3 Mn sulfate ($MnSO_4$), 0.1 mg/m^3 Mn phosphate (mineral hureaulite; less soluble), or HEPA-filtered air (control) 6 hours/day for 5 days/week over

a 90-day period. Mn was reported to sex- and age-specifically affect various markers of oxidative stress and antioxidant defenses (Erikson et al., 2004). For instance, young male rats (hureaulite-exposed) and aged male rats ($MnSO_4$-exposed) had decreased glutamine synthetase (GS; an enzyme particularly vulnerable to oxidative stress) protein levels in the hypothalamus but increased GS levels in the hippocampus. Also, glutathione was depleted in the olfactory bulb of Mn-exposed young male rats, accompanied by a decrease in both metallothionein (antioxidant enzyme) and GS mRNAs. Females exposed to $MnSO_4$ at the highest dose (0.5 mg/m^3) had increased GS protein in the olfactory bulb, perhaps due to a concomitant rise in total glutathione levels. Finally, only female rats were found to have reduced glutathione levels in the striatum (part of the basal ganglia and a target of Mn toxicity) following Mn inhalation. Puzzlingly, these changes were not directly correlated with Mn accumulation in these various brain areas. However, if we consider that the striatum is a central structure receiving various projections from the cortex, the substantia nigra, and the thalamus, among other brain regions, it certainly seems plausible that Mn accumulation in these projecting areas could mediate oxidative/antioxidant changes in the striatum. Thus, sex differences in brain Mn accumulation and differential effects of Mn on oxidative stress markers and antioxidant activity may underlie sex differences in Mn neurotoxicity.

Accumulation of Mn in the mitochondria could also contribute to Mn toxicity. Mn was shown to affect mitochondrial function in a sex-specific manner and reduce respiratory mitochondrial complex 2 and complex 4 activity in the adult rat brain (Zhang et al., 2003). In this study, daily intraperitoneal administration of $MnCl_2$ (7.5, 15.0, and 30.0 ppm) for 6 weeks resulted in (1) an overall decrease in body weight of male rats compared to females in the 15 and 30 ppm dose groups, and (2) decreased MAO activity in females of the 7.5 and 30 ppm dose groups and in males for the 7.5 ppm dose group only. Mn was also affected by mitochondrial Ca^{2+} levels; however, no sex-specific interaction was seen as compared with the effects on mitochondrial complex activity. Mn has been suggested to impair brain mitochondrial function by inhibiting energy transduction, inducing mutations, or via enhanced free radical generation (Zhang et al., 2003).

Mn might also differentially impact the gut microbiome of males and females which could then contribute to sex differences in Mn neurotoxicity. For instance, a study looking at the possibility of differential gut microbiome responses to Mn observed a strong sex-specific alteration of the gut microbiome in young adult (8-week-old) C57BL/6 mice exposed to 100 ppm (mg/L) Mn via drinking water over 13 weeks (Chi et al., 2017). Relative abundance of phyla Firmicutes (linked to obesity and metabolic disorders) and Bacteroidetes (linked to anti-inflammation) significantly increased in Mn-exposed male mice while females saw a decrease in Firmicutes. There was also a sex-specific effect on the expression of genes involved in some neurotransmitter biosynthesis pathways (serotonin and GABA). For instance, expression of genes encoding tryptophan synthase was reduced in females and increased in males while that of glutamate carboxylase was relatively lower in males. This indicates that one way metals may influence the gut-brain axis is through the alteration of neurotransmitter synthesis. Furthermore, two Mn ABC transporter genes (*Sitb* and *Sitd*) and the gene encoding a multicopper oxidase that oxidizes Mn^{2+} to Mn^{3+} were specifically enriched in females and largely reduced in males. This could have important sex-specific impacts on Mn oxidation, absorption, distribution, and utilization

in these animals. Thus, Mn may particularly impact bacterial genes of key metabolites of neurotransmitter synthesis, metal homeostasis, metal oxidoreduction, and pro-inflammatory mediators such as lipopolysaccharides that can stimulate immune cells to produce various pro-inflammatory cytokines and chemokines potentially affecting signaling of gut-brain interactions (Chi et al., 2017).

While the mechanisms behind the observed sexual dimorphism of Mn neurotoxicity are not fully elucidated, differences in uptake and regulation of the metal due to different nutritional demands between males and females could be a contributing factor (Llop et al., 2013). Evidence of sex-specific neurotoxic effects of Mn were also suggested to partly stem from the metal's differential uptake, absorption, and storage (Finley et al., 1994; Jenkitkasemwong et al., 2018). Due to the interdependency of Fe and Mn in homeostasis mechanisms, a dietary deficiency of Fe leading to abnormal Mn accumulation may also explain the sexual dimorphism in neurotoxicity (Fitsanakis et al., 2010; Lee and Kim, 2014). Studies in humans comparing premenopausal and postmenopausal women suggest that the Fe-Mn interaction is more influential in determining sex-specific differences in blood Mn levels than reproductive or menopausal factors influenced by hormones (Lee and Kim, 2014). Differential uptake and body burdens alone, however, do not explain observed sexual dimorphisms in alterations to striatal neuron morphology following Mn exposure in mice (Madison et al., 2011), pointing to the role of other mechanisms such as interactions with hormones and neurotransmitters and differential regulation of oxidative stress (Llop et al., 2013). In agreement, Mn-treated female mice exhibit higher neostriatal dopamine and norepinephrine levels (Vorhees et al., 2014). Also, primary cortical neurons and astrocytes *in vitro* and young male mice *in vivo* are protected from Mn-induced toxicity by pretreatment with estrogen or tamoxifen (Lee et al., 2009). Together, this suggests a protective role of estrogen in Mn neurotoxicity. It is speculated that progesterone may also be involved in modulating Mn neurotoxicity (e.g., glutathione peroxidase activity is increased in brain tissue when progesterone levels are high) (Erikson et al., 2004). Female susceptibility to the neurotoxic effects of Mn at low doses is observed due to a reduced Mn elimination rate. Remarkably, brain Mn does not seem to decline in response to blood Mn status indicating that intracellular binding and sequestration of Mn in the brain may prevent its movement to the extracellular space, slowing its release into the plasma and explaining the numerous contrasting findings reported in the epidemiological literature (Zheng, 2000).

Lastly, polymorphisms in the Mn transporter genes *SLC30A10* and *SLC39A8* could also influence Mn homeostasis mechanisms in early development, providing a potential explanation for the differences in sensitivity between males and females to environmental Mn exposure (Broberg et al., 2019; Wahlberg et al., 2018). Results from these studies suggest that girls that are genetically less efficient at regulating Mn may be particularly susceptible to the adverse effects of Mn. Therefore, interactions with polymorphisms in Mn transporter genes essential to homeostasis should be considered more often when studying the sexual dimorphism of Mn neurotoxicity and future studies in adults should confirm whether these polymorphisms could also be critical in determining sex-specific neurotoxicity later in life.

Sex-specific Neurotoxic Effects of Cadmium (Cd):

Cd is a heavy metal naturally found in the earth's crust (ATSDR, 2012b). It is used in manufacturing in the production of other metals such as zinc, lead, or copper, and is used in consumer products such as batteries. Cd enters soil, water, and air primarily through mining and refining, fossil fuel combustion, and waste incineration and disposal. Cd from polluted soil and water can accumulate in crops and organisms, contaminating the food supply. A major source of Cd exposure is through cigarette smoking, which can double the Cd body burden, as 5–50% of inhaled Cd is absorbed through the lungs. Among non-smokers, the primary source of Cd exposure is dietary intake. About 1–10% of Cd can be absorbed in the digestive tract by ingestion of contaminated food and water (ATSDR, 2012b). While Cd is also capable of crossing the placenta and accumulating in this organ during gestation, it appears Cd is less efficient at trans-placental passage than other heavy metals and that the placenta is an efficient or partially efficient barrier against Cd (Chen et al., 2014; Esteban-Vasallo et al., 2012; Sakamoto et al., 2010).

The minimal risk level for chronic duration oral Cd exposure is 0.1 μg/kg/day (0.1 ppb/day) (ATSDR, 2012b). Due to its long half-life and poor elimination, Cd accumulates with age in organs including the kidneys, lungs, and testes and also reaches the CNS. Cd-dependent neurotoxicity has been related to neurodegenerative diseases including Parkinson's disease, Alzheimer's disease, Huntington's disease, and MS (Aliomrani et al., 2016). Cd has also been associated with lower attention, olfactory impairment, and memory deficits in humans living around Superfund hazardous waste sites where Cd is often found (Leal et al., 2012). However, the underlying mechanisms behind these associations remain unknown.

Sex-specific body burdens Cd measured in biofluids:

Blood Cd tends to reflect recent exposures while urinary Cd can reflect the cumulative Cd body burden. In the general population, like for Mn, women have been reported to have higher blood Cd levels than men (ATSDR, 2008). In our analysis of the NHANES 2015–2016 data, we also found that females had significantly higher blood Cd compared to men (Figure 2, Supplementary Table 3). Females displayed higher blood Cd levels across the life span, although the difference was most pronounced among adults aged 22–65 years (Figure 3). A similar trend was reported in a Korean population with higher Cd exposure (Eom et al., 2018). It is interesting that adult females would exhibit higher blood Cd levels, considering that adult men are more likely to smoke than women (although the gender gap is decreasing) and working adults could include the population subjected to occupational exposures which present a high risk of exposure. On the other hand, females generally absorb greater amounts of Cd in the gastrointestinal tract, which could be due to differences in biology or the fact that females have higher tendencies to eat Cd-contaminated foods such as leafy greens and soybeans (ATSDR, 2012). Furthermore, similar to Mn, women with lower lifetime average Fe stores are thought to be at risk for greater Cd absorption after oral exposure (Olsson et al., 2002), supporting a common homeostatic mechanism for Mn and Cd. A study of men and women aged 20–59 in Northern France found no sex difference in blood Cd levels, but found that women had significantly higher creatinine-adjusted urinary Cd (Nisse et al., 2017). Women in NHANES (2003–2010) and in a Hispanic New Mexico population aged

40–85 years also displayed higher levels of urinary Cd (Adams et al., 2016) in agreement with numerous previous reports (Vahter et al., 2007a). The higher body burden of Cd in women can translate to susceptibility to disease. Interestingly, the severe Cd-induced Itai-Itai disease, the name given to the historical mass Cd poisoning in Japan, mainly affects women (Vahter et al., 2007a, 2002b).

In contrast, in the context of occupational exposure, significantly higher levels of Cd (and Pb) were found in blood of men compared to women electronic waste workers in Thailand despite similar ages and hours worked across sexes (Neitzel et al., 2020). However, this can likely be attributed to smoking status, as smoking was significantly associated with higher blood Cd and Pb levels among males, but this could not be assessed for females because none of the 58 women included in the study were smokers. Lastly, a study in Iran comparing the association between blood Cd and blood Pb concentrations in MS patients and healthy controls found not only that MS patients had significantly higher blood Cd than controls (mean 1.82 ± 0.13 μg/L [ppb] vs. 1.47 ± 0.11 μg/L), but also that across all subjects, males had higher concentrations of blood Pb, As, and Cd compared to females, although the difference was not significant for Cd (Aliomrani et al., 2016). Further investigation of glutathione S-transferase (GSTM1) polymorphisms in this population revealed that while there was no difference in the frequency of the GSTM1 null genotype between MS patients and healthy controls, there was a statistically higher frequency of the GSTM1 null genotype in female patients compared to males which may have enhanced the risk of MS in this population. Also, Cd levels were significantly higher in MS patients with the GSTM1 null genotype than healthy controls (with no differences between groups for Pb and As concentrations) and among the MS patients, those with the null genotype had an earlier age of disease onset, pointing to susceptibility to Cd toxicity among these individuals with poorer ability to metabolize metals and detoxify reactive oxygen species (Aliomrani et al., 2017).

While this trend (higher blood Cd levels in males) has been reported in other research studies (Farzin et al., 2008) in the Tehran population, it might not have reached significance in this study due to an imbalanced study design (66% female participants) and reporting bias regarding smoking habits. Also, this study only examined smoking habits across patients and controls (both sexes included) rather than stratified by sex and disease status. Overall, evidence from the literature suggests that women have higher Cd levels than men, but in order to accurately ascertain potential sex differences in Cd body burdens to determine how that might affect susceptibility to disease (e.g., MS), future studies should assess urinary Cd in addition to blood Cd and also examine smoking status stratified by sex as smoking is a significant source of Cd exposure.

Sex-specific effects induced by Cd - Epidemiological evidence:

Prenatal exposure: Recent data indicate that Cd has estrogenic effects that disproportionately affect female offspring (Johnson et al., 2003). Furthermore, among pregnant women with moderate prenatal Cd exposure (mean = 0.56 μg/L [ppb]) in the U.K.-based ALSPAC (Avon Longitudinal Study of Parents and Children) observational birth cohort study, birth weight, head circumference, and crown-heel length were significantly

adversely associated with maternal whole blood Cd levels collected at 11 weeks of gestation in girls only (Taylor et al., 2016). Another study from the same group examining the association of prenatal blood Cd concentration (again assessed via whole blood samples of women 11 weeks pregnant, mean = 0.45 μg/L) and motor skills at 7 years of age (assessed by Movement Assessment Battery for Children) found no association (both sex-specific or in general) between the exposure and the outcome, which might be due to the long gap in timing of assessment, non-persistent effects, or unidentified confounding factors (Taylor et al., 2018). Of note, when the Cd levels in maternal blood at 11 weeks of pregnancy in the ALSPAC cohort were analyzed based on the sex of the fetus, no statistically significant differences were observed, suggesting that prenatal exposure should be identical across sex and that any sex-specific effect observed cannot be accounted for by differential exposure (Taylor et al., 2014).

Cd was also found to impact weight at birth, possibly in a sex-specific manner (significant only when considering all children possibly due to limited power) in a longitudinal study in Bangladesh that showed that Cd impacts fetal size, measured by ultrasound at week 14 and 30 of pregnancy, for an exposure to Cd (median = 47 μg/kg [ppb] via rice) that was evaluated using maternal urinary Cd at gestational week 8 (median = 0.63 μg/L [ppb]) (Kippler et al., 2012b). Stratifying the analysis based on sex showed a significantly stronger association among females compared to males in an inverted “U”-shaped manner (turning point = 1.5 μg/L). This might be due to the increased intestinal uptake of Cd due to undernutrition, especially low Fe stores, and differences in epigenetic mechanisms. Another study in Bangladesh looking at the association between maternal Cd exposure (median = 0.63 μg/L) and neurobehavioral development (assessed using Wechsler Preschool and Primary Scale of Intelligence and Strengths and Difficulties questionnaire) of children at 5 years of age found a pronounced and significant inverse association between IQ and maternal exposure. Moreover, stratification by sex and socioeconomic status revealed somewhat stronger associations in girls than in boys and in higher-income than in lower-income families. The highest maternal and child exposure (≥ 2 and ≥ 0.6 μg/L urinary Cd) were associated with a 1.7-point reduction in full-scale IQ points (Kippler et al., 2012a).

Childhood exposure: A recent Chinese population-based birth cohort study of 296 school-age children examined sex-specific associations of child exposure to potentially neurotoxic trace metals (Mn, Cd, Pb, Cr, Co, Cu, As and Se) with cognitive abilities. Cd levels were measured at birth in cord blood (geometric mean = 0.36 μg/L) and in childhood in spot urine (geometric mean = 0.18 μg/L), and cognitive abilities were assessed using the Wechsler Intelligence Scale for Children-Chinese Revised (WISC-CR). In agreement with the Bangladesh cohort studies, this study found that girls may be more adversely affected by childhood Cd exposure (Zhou et al., 2020). Specifically, childhood urinary Cd was negatively associated with full-scale IQ and Performance IQ in girls, while neonatal cord blood Cd displayed inverse associations with verbal IQ only in boys. The epidemiological evidence of sex-specific neurotoxic effects following early-life Cd exposure is summarized in Table 1.

Adulthood exposure: While the number of human studies that have investigated sex-specific neurotoxic effects of Cd exposure remains limited, overall, the existing literature suggests a potential higher vulnerability and susceptibility of girls, especially when exposure occurs during development or early in life. Studies in adults are even more rare; we found only one study reporting sex differences in Cd neurotoxicity among adults, but the article was written in Chinese. Thus, the comments here are based solely on information presented in the article's abstract, which was written in English. This adult exposure study compared the mental health of villagers living in a mining area in China's Hubei province to a control group of residents and found that Cd, Pb, and As levels were significantly higher among those living near the mining area and furthermore, that this was associated with poorer mental health (Dang et al., 2008). Anxiety levels were particularly affected and differentially affected women of the exposed group. However, this study did not discriminate the effects of the three metals in the mixture. Further research is thus needed to explore whether exposure to Cd later in life exhibits similar or opposite sex-specific neurotoxic effects than during brain development.

Sex-specific effects induced by Cd - Experimental evidence:

Prenatal and perinatal exposure: In a study using C57BL/6 mice, administration of the equivalent of 10 ppm Cd via drinking water (from GD 1 to PND 10) and 0.025% methimazole, a thyroid hormone inhibitor (from GD 12 to PND 10), resulted in lower mRNA expression levels of the progesterone receptor and neurogranin (brain-specific genes known to be regulated by the thyroid hormone), and interaction between both treatments on neurogranin expression was found in females (Ishitobi et al., 2007). This was negatively correlated with activity in the open field test. However, individual exposure to Cd or methimazole alone didn't show any effect on neurogranin mRNA. This developmental exposure to Cd also differentially reduced expression of brain estrogen receptor alpha in female mice and estrogen receptor beta in males. Similar toxic interaction between hormones (progesterone; 100 mg/kg) and Cd exposure (20 μmol/kg) to generate sex-specific effects (females more adversely affected) were also seen in Fischer 344 (F344) rats in the context of hepatotoxicity (Shiraishi et al., 2002), suggesting this mechanism is not restricted to neurotoxicity.

Adolescent and adulthood exposure: A study exposing male Sprague Dawley rats to a daily dose of $CdCl_2$ (2 ppm) for 30 days starting at PND 30 (prepuberty) or PND 60 (young adulthood) found that Cd exposure results in age-dependent effects on the hypothalamic-pituitary-testicular axis of males (Lafuente et al., 2001). Thus, males may show differential toxicity to Cd due to different functionality of the hypothalamic-pituitary-testicular axis after sexual development. These studies should be replicated in females to see whether a similar age-dependent effect persists.

A recent innovative study investigated gene-environment interaction between Cd exposure and the apolipoprotein E ε4 allele (*APOE-ε4*), the variant associated with accelerated cognitive decline and an increased risk of Alzheimer's disease (Zhang et al., 2020). This study used new generation Alzheimer's disease knock-in mouse models more relevant to human genetics (*ApoE3*-KI [knock-in] & *ApoE4*-KI), and exposed them to 0.6 ppm $CdCl_2$

via drinking water for 14 weeks starting at 8 weeks of age. This level of exposure yielded blood Cd concentrations (peak blood Cd of 0.3–0.4 μg/L) that are relevant to the current general U.S. population exposure. On assessing hippocampal-dependent memory, deficits in spatial working memory (assessed using the novel object location test) were seen in all mice, starting as early as 3 weeks of exposure (in *ApoE4*-KI male mice). However, the time to reach 50% of the maximal memory impairment (used to compare the effect of Cd exposure on *ApoE3*-KI vs. *ApoE4*-KI mice) was statistically significant only in males. Further analysis showed that males had an earlier onset of memory deficits than females for both *ApoE3*-KI and *ApoE4*-KI mice.

The T-maze test (used to assess spontaneous alternation, a behavior partially due to hippocampal-dependent spatial working memory) also revealed a gene-environment interaction effect between *Apoe* and Cd; Cd-treated *ApoE4*-KI males and females showed significant decreases in spontaneous alternation compared to their respective controls, but this only appeared about 6.5 months after the end of Cd exposure (at 48 weeks, but was not apparent at 23 weeks of age). Thus, there was a slower manifestation of memory deficits in the T-maze test compared to novel object location in both sexes. The results of this study (that the gene-environment interaction between *ApoE4* and Cd is significant in males only) may seem contradictory with other evidence indicating that females are more susceptible to the effects of heavy metal exposure such as Cd. However, these effects were only observed when animals were young adults, and thus the results could be explained by the estrogen neuroprotection theory, i.e., females are safeguarded by the neuroprotective effects of estrogen and may become more susceptible to gene-environment interactions when levels of estrogen are low. Future studies ought to replicate these findings and further evaluate the influence of genetic polymorphisms, sex, and aging on the effects of heavy metal exposure and how this might differentially contribute to disease susceptibility.

Similar to the paucity of human studies investigating the sex-specific neurotoxic effects of Cd, more work is needed to fully understand the sexual dimorphism of Cd on the CNS of experimental animals. However, from animal studies, we have deduced that (1) females seem to exhibit a higher susceptibility to early life Cd exposure than males, and (2) males may be more disproportionately affected by Cd exposure than females after puberty (see Table 1 for a summary of the experimental evidence). The interaction of Cd with hormones at different life stages appears to be instrumental to its toxicity to the CNS and beyond, as well as the influence of genetic polymorphisms. The mechanisms of these interactions will require further investigation and merits the use of continued novel experimental animal studies in Cd toxicity research.

Potential Mechanisms of Cd neurotoxicity:

Cd is a well-known neurotoxicant. Although it cannot penetrate the mature BBB, it can give rise to BBB dysfunction by depleting microvascular antioxidants, inducing lipid peroxidation, and increasing BBB permeability (Paknejad et al., 2019; Shukla et al., 1996). It has also been demonstrated that Cd, as well as Pb and Hg, can accumulate in neurons via retrograde transport (Arvidson, 1994). This may be a pathway by which certain

metals bypass the BBB. However, this also means that younger individuals lacking a fully developed BBB are more vulnerable to Cd exposure.

In *in vitro* experiments, Cd exposure (0–40 μM) has been shown to dose-dependently reduce cell viability and cause cytoskeleton dysfunction in cultured neuroblastoma cells which can alter signal transduction and cell function, contributing to neurotoxicity (Ge et al., 2019). Similar results (axonal damage characterized by cytoskeleton disruption as well as mitochondrial disruption) were observed in a study examining the effects on optic nerves exposed to 200 μM Cd for 100 minutes, suggesting a common mechanism for Cd-induced white matter injury (Fern et al., 1996). While a more detailed discussion of the mechanisms of Cd neurotoxicity is discussed elsewhere (Branca et al., 2018), here we will list a few that we believe are the most important in determining special vulnerability to Cd neurotoxicity.

The antioxidant enzyme and Cd-binding protein, metallothionein, is instrumental against the neurotoxic effects of Cd exposure (Gundacker and Hengstschläger, 2012). Thus, late pregnancy may be a specifically vulnerable period to Cd exposure because metallothionein concentrations in the placenta decline at this stage of pregnancy, which could result in greater fetal Cd exposure. Cd, Pb, and Hg are all well-known to cross the placental barrier and accumulate in fetal tissue (Galicia-García et al., 1997), although trans-placental passage of Cd is more limited (Chen et al., 2014; Esteban-Vasallo et al., 2012). Other studies have also shown a protective effect of antioxidants against Cd-induced neurotoxicity *in vivo* (Gong et al., 2015; Hao et al., 2015). Pregnancy is one of the unique physiological conditions that impacts and modifies pathways involved in Cd toxicity, mainly through the induction of cell death and oxidative stress via the production of Ca^{2+} and reactive oxygen species. Thus, infants in critical developmental stages, when the BBB has not completely developed, remain most susceptible to Cd toxicity (Branca et al., 2018).

As previously mentioned, the internal Cd burden is generally higher in women compared to men due to a higher gastrointestinal absorption favored by lower Fe levels (Vahter et al., 2002b). This differential burden of Cd may explain the poor birth outcomes among pregnant women exposed to Cd and a candidate mechanism that was proposed is alteration in methylation patterns. A prospective cohort study based in Bangladesh that included 127 mother-child pairs to examine the effect of prenatal Cd exposure on DNA methylation and weight at birth found a sexual dimorphism in cord blood DNA methylation patterns that was maintained in 4.5 year-old children (Kippler et al., 2013). Cd in maternal blood collected during week 14 of pregnancy (mean = 0.38–5.4 μg/L) was used as a marker of prenatal exposure reflecting recent maternal Cd intake while maternal and child urinary Cd were used as a marker of Cd body burden reflecting long-term exposure. A sexual dimorphism in cord blood methylation was seen only when correlated with maternal blood Cd levels with effects more pronounced in boys than girls (96% vs. 29% hypermethylation, notably of genes involved in cell death). In newborn girls, Cd exposure was associated with greater hypomethylation, notably in genes involved in bone morphology and mineralization. This sexual dimorphism and its long-term maintenance might explain the high prevalence of osteoporosis associated with higher Cd in women (32% increased risk of osteoporosis associated with high dietary Cd exposure ≥ 13 μg/day) (Engström et al., 2012; Thomas et al., 2011).

In agreement with the above described human studies, a study which administered the equivalent of 10 ppm Cd (as $CdCl_2$) to female Wistar rats via drinking water throughout pregnancy found Cd-induced changes in DNA methylation that affected birth weight and size of offspring as well as the glucocorticoid system (Castillo et al., 2012). Prenatal Cd exposure was correlated with higher levels of fetal liver *de novo* methylation gene DNA methyltransferase 3 alpha (*Dnmt3a*) in male rats, but lower levels of expression in females. As a result, in males, CpG sites in the liver glucocorticoid receptor (GR) promoter region were significantly more hypermethylated than controls (185%), while females showed significantly lower methylation than controls (62%). This sex-specific effect of Cd on DNA methylation of the GR promoter region was accompanied by concomitant changes in expression levels (mRNA and protein) of fetal liver GR (i.e., exposed females had higher hepatic GR expression compared to control females, whereas exposed males had lower hepatic GR expression compared to control males). There were also corresponding changes in GR's target genes phosphoenolpyruvate carboxykinase (PEPCK) and acyl-CoA oxidase (AOX), two enzymes involved in the metabolism of carbohydrates and lipids, respectively. Lastly, prenatal Cd exposure affected birth weight and size in a sex-specific manner, with females more affected than males. Therefore, decreased levels of DNMT3A, which is particularly important for establishing DNA methylation patterns during development, may possibly explain women's increased susceptibility to Cd, as well as the role of progesterone, a female sex hormone.

Sex-specific Neurotoxic Effects of Arsenic (As):

Arsenic (As) is an element that raises a myriad of environmental and human health concerns. Although technically classified as a metalloid, having properties of both a metal and a nonmetal, As is frequently referred to as a metal and even a heavy metal due to its toxic effects and its density of 5.73 g/cm^3 which is greater than the minimal density that was defined for heavy metal classification (> 5 g/cm^3) (ATSDR, 2007). As exposure often occurs via drinking water because in some regions it is found naturally in the groundwater and most As compounds also dissolve in water. Some areas contain unusually high levels of As in the soil or water. Currently, more than 150 million people in over 70 countries are affected by elevated As levels in groundwater at levels above the World Health Organization (WHO) provisional guideline value of 10 μg/L (10 ppb) and reaching up to 5,000 μg/L (5 ppm) (Shankar et al., 2014).

Exposure to As also commonly occurs through the diet by ingestion of crops grown on As-contaminated soil or via inhalation of contaminated dust, fumes, or mists. The predominant dietary sources of As are seafood, rice/rice cereal, mushrooms, and poultry. However, the As in seafood is in an organic form called arsenobetaine that is nontoxic and not metabolized (Taylor et al., 2017). As is readily absorbed by the body and has a tendency to accumulate in the brain with the highest accumulation in the pituitary gland (Tyler and Allan, 2014). It can also cross the placenta (Punshon et al., 2015) and is a well-known teratogen. In the human body, it is primarily metabolized into monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) via methylation, a detoxification mechanism (Vahter, 2002a). However, MMA is considered more toxic than DMA and is associated with increased cancer risk, noncancer diseases, and developmental delays (Hsueh et al., 2016). Urine is the

preferred matrix for exposure assessment, given the short half-life of As in blood. Urinary As reflects predominantly recent exposure. The minimal risk level for acute duration (< 14 days) oral exposure and chronic oral exposure to inorganic As is 0.005 mg As/kg/day (i.e., 5 ppb As/day) and 0.0003 mg As/kg/day (i.e., 0.3 ppb As/day), respectively.

Chronic exposure to inorganic As is known to induce skin, lung, and bladder cancer. It is also known to cause developmental effects, diabetes, and pulmonary and cardiovascular disease (Hong et al., 2014). As is also known to interfere with oxidative phosphorylation by preventing the conversion of thiamine to thiamine pyrophosphate, inhibiting acetyl-CoA formation and causing leg weakness and unsteady gait (Abdo et al., 1989). Its neurotoxic effects include abnormal cholinergic and monoaminergic signaling, decreased glucocorticoid levels contributing to mood disorders, disrupted adult neurogenesis, and deficits in learning, memory, and locomotion (Tsuji et al., 2015; Tyler and Allan, 2014). As also has a significant effect on the white matter in the brain, inhibiting synthesis and liberation of acetylcholine in brain slices and increasing monoamine activity (Rosado et al., 2007). Multiple case studies worldwide report that high As levels can cause a Guillain-Barré-like polyneuropathy (Barton and McLean, 2013; S. Kim et al., 2012; Kishi et al., 2001; Mathew et al., 2010). Readers interested in a more detailed discussion of As neurotoxicity in humans are encouraged to read this recent review by Hitoshi Mochizuki (Mochizuki, 2019).

Sex-specific body burdens of As measured in biofluids:

When assessing the body burden of As, it is important to assess the speciated arsenicals, which can indicate sources of As exposure (inorganic vs. arsenobetaine) as well as detoxification efficiencies. Several previous studies in populations ranging from Mexico to Central Europe have found that men have higher urinary inorganic As and %MMA and lower %DMA than women (Gomez-Rubio et al., 2011; Kile et al., 2011; Lindberg et al., 2007), and also that body mass index (BMI) is associated with lower %MMA and higher %DMA (Bommarito et al., 2019). In Bangladesh, a population characterized by lower nutritional status and lower adiposity and where 19 million people are exposed to water As above the country's 50 μg/L (50 ppb) safety standard (Flanagan et al., 2012), BMI was negatively associated with inorganic As% and %MMA and is positively associated with %DMA in females, but not males. This may be due to the influence of body fat on estrogen levels that can influence one-carbon metabolism, but experimental studies are warranted to determine whether associations between BMI and As species are causal (Abuawad et al., 2021). Still, other studies have found elevated urinary As is not associated with sex. A study assessing As exposure (urinary As and DMA) by poultry consumption in the U.S. found no significant difference in the geometric mean ratios of total As or DMA by poultry intake across sex (Nigra et al., 2017). A study comparing urinary metal concentrations of older Hispanic adults of an underserved New Mexico community (Doña Ana County) to a comparable subpopulation from NHANES 2003–2010 found that males have higher levels of unadjusted geometric mean urinary As than women. This pattern was consistent across Doña Ana County, Mexican-Americans in NHANES, and the greater NHANES population (Adams et al., 2016). However, there was no association with sex in multivariable linear regression models used to estimate associations of independent variables such as sex with log-transformed, creatinine-adjusted urinary As levels. Another study comparing total As

in blood and urine of men and women aged 20–59 years in Northern France found no sex difference in blood As levels, but found that females had higher levels of creatinine-adjusted urinary As (Adams et al., 2016). Neither of these two studies examined speciated arsenicals.

Note that we did not examine As in our NHANES analysis. This was primarily because we could not evaluate As species including arsenite, arsenate, and MMA as their LODs were relatively high and the levels for these species were mostly undetectable. Furthermore, the percentage of samples below the LOD was 27% for DMA and 58% for arsenobetaine (with even higher percentages after excluding those missing urinary creatinine which would be needed for adjustment). We felt that given these considerations and smaller sample size (urinary As was measured in participants aged 3 to 5 years plus a one-third random subsample of participants ≥ 6 years of age), our analysis would not be informative.

Sex-specific As detoxification efficiencies - Epidemiological evidence:

Excessive water As exposure is a prevalent problem in Bangladesh due to the very high abundance of As found naturally in groundwater (up to 4,730 ppb) that contaminates drinking water sources (Shankar et al., 2014). Accordingly, many human studies on As toxicity and internal levels were performed on Bangladeshi populations. A randomized controlled study in Bangladesh reported that sex and age were important factors influencing As metabolism (measured by urinary As, median = 77 μg/L [ppb]) and consequently, its toxicity. Women, especially during childbearing age, were reported to have significantly higher methylation efficiency (determined by %DMA in urine) as opposed to men. This could indicate the effect of sex hormones, primarily estrogen which upregulates the synthesis of phosphatidylcholine (a major methyl donor) in metabolism (Lindberg et al., 2008). These findings are consistent with the findings from a case-control study on a central European population (Lindberg et al., 2007) indicating that higher estrogen levels, especially during adolescence, contribute to better detoxification of low dose As from the body (median urinary As = 8 μg/L). These differences may also be attributed to the higher activity of cytochrome P450 enzymes (CYPs) CYP1A2 and CYP2E1 among women compared to men (Vahter et al., 2007b). Another As exposure study on residents of San Pedro De Atacama, Chile involving As-contaminated drinking water (600–170 μg/L) showed a significantly lower urinary MMA/DMA ratio in females compared to males (30% difference at 580 μg/L and 20% difference at 60 μg/L urinary As exposure) (Hopenhayn-Rich et al., 1996). This indicates that women are better protected from As exposure and hence better protected from subsequent neurotoxic outcomes compared to males due to their increased efficiency at As metabolism.

Sex-specific effects induced by As - Epidemiological evidence:

Prenatal exposure: A study in Bangladesh assessing the effect of prenatal As exposure measured in maternal spot urine at 8 weeks (median = 81 μg/L) and 30 weeks (median = 84 μg/L) of pregnancy on 7 month old infants' problem-solving abilities and motor development (problem-solving tests, Bayley Scales of Infant Development–II) failed to detect any deficit in these prenatally exposed infants (Tofail et al., 2009). Another study in rural Bangladesh examined the association of prenatal As exposure via urinary As also at 8 and 30 weeks of pregnancy with fetal size, measured via ultrasound (Kippler et al.,

2012b). Women in this study exhibited higher As levels during pregnancy (mean urinary As at 8 and 30 weeks = 152 μg/L and 168 μg/L, respectively). This study showed a stronger statistically significant log-linear association between maternal As at early pregnancy and fetal size among boys compared to girls; As was found to affect femur length and head size, a measure positively correlated with IQ (Broekman et al., 2009). This study emphasizes the effect of window of exposure on neurologically relevant outcomes such as fetal head size.

Prenatal and postnatal exposure: As we discussed, studies of As metabolites reflective of As detoxification efficiencies suggest a lower vulnerability of females to As toxicity due to better metabolism. In contrast with this hypothesis, a study in Bangladesh assessing the effect of As exposure (via drinking water; 66 μg/L) during gestation (8 and 30 weeks) and childhood (1.5 and 5 years) via urinary As levels (80 μg/L during pregnancy; 35 μg/L at 1.5 years; 51 μg/L at 5 years) on development (using Wechsler Preschool and Primary Scale of Intelligence) at 5 years of age observed a significant negative effect of As on IQ in girls (Hamadani et al., 2011). Among boys, there was no visible effect of As exposure on IQ. However, in girls, concurrent exposure at 5 years seemed to be more influential than prenatal and early childhood exposure with an association of 100 μg/L urinary As per 1–3 point decrement.

A similar effect was observed when examining non-neurological outcomes like body weight and length. As exposure (78 μg/L) measured via urinary As at 8 and 30 weeks of pregnancy in mothers (median = 80 μg/L) and up to 2 years of age (median = 34 μg/L) in infants in a cohort in Bangladesh found a significantly greater influence of early childhood exposure as opposed to prenatal exposure on body weight and length in girls compared to boys (Saha et al., 2012). These studies underscore the importance of duration of exposure in As neurotoxicity.

Childhood exposure: An interesting study looking at elderly adults (mean age = 81 years) with “probable As intoxication” (180–8,000 mg/kg [ppm]) during infancy/early childhood exhibited declined sensory function that persisted long after the As exposure ended (Ishii et al., 2018). There were no differences in sensory function observed between males and females. There was, however, a sex difference observed in olfactory dysfunction; females who experienced “probable As exposure” during infancy and early childhood were more likely to exhibit olfactory dysfunction. However, this study has some limitations. Notably, there was a lack of comparison to age-matched healthy controls and limited biomarker collection, so the finding of sex differences associated with previous As exposure remains speculative.

In Mexico, a trial conducted on 6–8 year old children exposed to 4,000–6,000 μg/L As via groundwater looking at cognitive outcomes (Visual–Spatial Abilities with Figure Design, Peabody Picture Vocabulary Test, Wechsler Intelligence Scale for Children Revised-Mexican Version Digit Span subscale, and the Cognitive Abilities Test) and biochemical measurements observed different effects on both sexes (Rosado et al., 2007). Urinary As levels (mean = 58.1 ± 33.2 μg/L) were negatively associated with several cognitive tests in boys while only the digit span subscale test (memory evaluation test) was significantly associated with urinary As among girls.

From the above studies, which are summarized in Table 1, we can see the role sex plays in the direct (IQ, cognitive outcomes, sensory function) and indirect (methylation efficiency, CYP activity, body weight, fetal size) manifestation of neurotoxic outcomes through exposure to As. While adult women may have better methylation efficiencies, prepubescent girls are still vulnerable to the neurotoxic effects of As exposure. The two sexes may be differentially vulnerable to As neurotoxicity depending on the cognitive domain examined, but more research is needed to elucidate sex dimorphisms. Lastly, it appears that duration of exposure is an important factor in As neurotoxicity, as many studies found early childhood exposure more influential than prenatal exposure. Thus, future studies should examine sex-specific neurotoxic effects of As exposure in adulthood.

Sex-specific effects induced by As - Experimental evidence:

Prenatal exposure: The effect of As on methylation capacity and regulation of the glucocorticoid receptor (GR) system on C57BL/6 mice was studied by exposing them to 0.05 ppm sodium arsenate via drinking water 10 days prior to mating through GD 14 or GD 18, when embryos were removed (Allan et al., 2015). The study found no changes in the protein levels of GR, 11β-Hsd1, and 11β-Hsd2 (key players in glucocorticoid synthesis) in the female embryos. However, there was a significant increase in *Nr3c1* (GR promoter region) and *Hsd11b2* (corticosterone activating enzyme) gene expression at embryonic day 14 (E14) in the brain. Among males, this exposure caused a significant decrease in mRNA for *Nr3c1*, *Hsd11b1*, and *Hsd11b2* at E14 along with a decrease in protein expression of GR and 11β-Hsd1 but an increase in 11β-Hsd2. This study showed a sex-specific occurrence of translational regulation (resistance) on As exposure in females which was not seen in males. This difference was not attributed to differences in methylation/DNMT levels but to greater anti-oxidative capacity (higher reduced glutathione/oxidized glutathione ratio [GSH/GSSH] levels) within females.

Adulthood exposure: There is limited evidence in the literature reporting sex-specific neurotoxic effects of As exposure in experimental animals (see Table 1 for summary). A study assessing the effect of chronic, low-dose exposure to As (as sodium arsenate) among C57BL/6 mice (aged 3 months) observed significant hyperactivity in females in all treatment groups (0.05, 0.5, 5.0 and 50 ppm) along with a reduction in striatal and hypothalamic dopamine content (Bardullas et al., 2009). On the other hand, males exhibited hyperactivity at 0.5 ppm and hypoactivity at 50 ppm after 4 months of exposure. The study also reported a reduction in both *TH* and thioredoxin (*Trx-1*, participates in redox reactions) mRNA expression in the striatum in males and the nucleus accumbens in females, indicating a sex-specific effect of As on dopaminergic markers, locomotor activity, and antioxidant capacity of the brain. Brain As content also appeared higher in males than females within the same dosing group.

A study conducted in 6-week-old male and female Crj:CD(SD) rats exposed via oral gavage to either 0.3, 1.2, or 5.0 ppm/day of pentavalent organic arsenic diphenylarsinic acid (DPAA[V]) for 28 days observed sex differences in tissue accumulation of DPAA(V), measured by liquid chromatography-tandem mass spectrometry (Masuda et al., 2018). In the 0.3 ppm/day of DPAA(V)-exposed group, the DPAA(V) concentrations in the following

regions were significantly higher in male tissues than in the female tissues: the temporal-occipital lobe, brainstem, spinal cord, liver, kidneys, and lungs. In the 1.2 ppm/day of DPAA(V)-exposed group, the significant differences between the concentrations of male and female tissues were as follows: the DPAA(V) concentrations in the male tissues were higher in the frontal-parietal lobe and cerebellum, while the DPAA(V) concentration was higher in the kidneys of female tissue. There were no sex differences in DPAA(V) concentrations between male and female tissues among rats exposed to 5.0 ppm/day of DPAA(V). This study suggests that males and females might have different rates of As clearance across the brain. However, this study is limited by its small sample size.

Altogether, like the epidemiological evidence, the number of experimental studies investigating sexual dimorphism in As neurotoxicity is quite limited despite some very promising insights suggesting the importance of differences in clearance rates, antioxidant capacity, or expression of genes regulating dopaminergic activity and locomotion.

Potential Mechanisms of As neurotoxicity:

Arsenic is known to primarily affect the peripheral nervous system and its effects accumulate over time resulting in symmetrical peripheral neuropathy where sensory neurons with large axons are disproportionately affected compared to their counterparts. If exposure occurs during developmental stages, the CNS is most susceptible to cognitive and behavioral deficits (Luo and Shu, 2015). There seem to be sex-associated differences in the biotransformation of As. Intermediate, reduced forms of As (MMA and DMA) are highly toxic, and their differential metabolism may be responsible for differences in As-induced neurotoxicity (Vahter et al., 2007a). Sex differences might also be related to stimulated As methylation in pregnancy and these factors might explain the differential vulnerability of males and females to As neurotoxicity resulting from prenatal exposure.

From animal studies, it can be construed that As also has a varied effect on antioxidant activity, dopaminergic markers, and metabolite profiles which show differences in toxic effects to As based on sex. Results from *in vitro* and *in vivo* studies suggest that inorganic As induces oxidative stress contributing to decreased neurite outgrowth and, at higher doses (10 μM iAs), neuronal cell death (Lu et al., 2014). Yet As-induced oxidative stress can be ameliorated by co-exposure to antioxidants (Chakraborti et al., 2018; Firdaus et al., 2018; Jahanbazi Jahan-Abad et al., 2017). Even this effect can however be sex-specific due to the difference in the ratio of brain GSH/GSSH levels (Allan et al., 2015) and differences in cysteine uptake (Lavoie et al., 2002) between males and females. It is possible that this greater anti-oxidative capacity within females provides protection against low to moderate arsenate. Though there is no clear pattern or mechanism to discern the sex the most susceptible to As neurotoxicity, one can infer the importance of estrogen and the availability of methylation cofactors/methyl donors for As metabolism in contributing to sex-specific outcomes associated with As exposure, as well as the timing and duration of As exposure.

Discussion and Conclusion:

Metals are ubiquitous in the environment notably due to their constant release from both natural and anthropogenic sources and their bioaccumulative, non-biodegradable features (Ali et al., 2019). These lasting environmental pollutants have been associated with health outcomes ranging from cancer and immunotoxicity to reproductive disorders and neurotoxicity (Badr and El-Habit, 2018; Chen et al., 2016; Fu and Xi, 2020; Liu and Lewis, 2014; Marth et al., 2001). They have also been listed as contributors to the global burden of foodborne disease by the WHO resulting in more than 9 million disability-adjusted life years (DALYs) worldwide (Gibb et al., 2019). There have been several systematic reviews interpreting the health outcomes from metal toxicity (Jaishankar et al., 2014; Sall et al., 2020; Singh et al., 2018a; Wu et al., 2016), but only a few discuss the sex-specific differences in these health outcomes (Berglund et al., 2011; Skalny et al., 2018; Vahter et al., 2007a, 2002b) and none to the extent we are doing here in the context of the neurotoxicity of several ubiquitous metals.

Here, before evaluating sex-specific neurotoxic outcomes, we first examined the internal burden of Pb, Hg and MeHg, Mn, and Cd across sexes using blood metal data from NHANES (2015–2016; see Figures 2–3). Our analysis confirmed previously reported sexual dimorphisms in levels of Pb, Mn, and Cd; we found a significant interaction between sex and life stage for Pb ($p < 0.05$) and Cd ($p < 0.01$), and a significant effect of both life stage ($p < 0.001$) and sex ($p = 0.003$) for Mn with a borderline significant interaction ($p = 0.054$). However, we found no main effect of sex on Hg (total and MeHg) blood concentrations, but we did observe a significant effect of life stage on Hg concentrations ($p < 0.001$), which is expected due to its ability to bioaccumulate. This also confirms previous findings that there is no sex difference in blood levels of Hg and MeHg (perhaps one can be seen only if there are striking sex differences in fish consumption between men and women of a given population). The sex difference for Pb appeared in adolescence (12–21 years of age) and persisted across the life span, whereas sex differences in Cd and Mn were mainly apparent in adulthood (22–65 years of age) and adolescence through adulthood, respectively. Thus, we found significant main effects of categorized age and sex, as well as significant interactions, on blood metal concentrations in NHANES, indicating that sex differences in internal exposure of these metals depends on life stage.

It would have been ideal to have access to sex-specific brain metal levels data instead of blood levels that are known to not accurately reflect brain metal burden but rather recent exposures. Due to the inaccessibility of the brain, CNS metal data are unavailable in large human populations. Nevertheless, some experimental studies in mice indicated that females consistently have lower brain levels of some metals (copper and Fe) and higher levels of others (cobalt and Mn) which could play a role in their susceptibility to Alzheimer's Disease, for instance (Maynard et al., 2006). We suggest that future experimental studies measure metals in the target organ of interest in addition to measuring biomarkers of exposure employed in humans. The confirmation of this sexual dimorphism in brain metal levels in humans could be determinant in increasing our understanding of the sex-specific neurotoxic effects of certain metals.

The numerous and profound differences between men and women may stem from behavioral attributes, environmental factors, and/or biology itself. With males having a four times higher risk of developing autism as opposed to females, and females being twice as likely to develop Alzheimer's than males, the sex differences in neurological disorder incidence are hard to miss (Weiss, 2011). Unfortunately, the cellular and molecular mechanisms accounting for these differences across neurological disorders remain elusive. However, potential mechanisms that were proposed in different reviews and/or original articles are: 1) differences in metal metabolism due to hormonal influences (sexual maturation and menopause/andropause) (Zhu et al., 2021); 2) sex differences in anatomy and neurochemistry; 3) sex differences in redox homeostasis (Ruszkiewicz et al., 2019; Wang et al., 2020); 4) sex differences in inflammation, gliosis, and immune response (Zhu et al., 2021); 5) sex differences in behavioral and executive reserve (Illán-Gala et al., 2021); and 6) potential interactions between sex and genes (e.g., polymorphisms/genetic background) determining susceptibility to metals (Maynard et al., 2006; Singh et al., 2018b). Here, we will discuss those we found the most relevant in the context of our review.

Sex differentiation begins in the embryo under genetic and hormonal control and affects exposure, susceptibility, risk, and health throughout life leading to differences in many aspects of vulnerability to xenobiotics and other stressors. Differential toxicokinetics and toxicodynamics are only one of the many factors affecting this sexual dimorphism. For instance, CYP2J5 (a cytochrome P450 enzyme), which metabolizes arachidonic acid to epoxyeicosatrienoic acids in the mouse kidney, is upregulated by testosterone and downregulated by estrogen (Ma et al., 2004). Organ mass differences, physiologic differences, as well as varied water and food intake also affect metabolism.

From a neurobiological aspect, there are several differences in the neuroanatomy as well as the neurochemistry of sexes ranging from the sexually dimorphic nucleus of the preoptic area to varied sensitivity to cholinergic stimulation or serotonergic system (Ngun et al., 2011). The X and Y chromosomes contain several genes related to brain structure and function which directly influence brain structure by their expression in brain cells and indirectly by the action of the sex hormones (Arnold, 2004). Studies have shown that brain development is not entirely hormone-dependent but a combination of both hormones as well as genes independent of hormone status (Guilarte,et al., 2006b). The special vulnerability of the nervous system during its long period of development is therefore a critical issue for neurotoxicology. The importance of windows of vulnerability and susceptibility in the sex-specific neurotoxicity of some metals is illustrated by exposure to Pb. Males exhibit adverse neurological consequences following early prenatal/perinatal Pb exposure while females show deficits following exposure to Pb at later developmental periods both in humans and animals (Burns et al., 1999; Joo et al., 2018). The best evidence we have of sex-specific effects is primarily in infants and young children. Studies conducted in adults (though limited) indicate that the mature adult brain is less susceptible to metal-induced neurotoxicity, further highlighting the importance of critical windows of exposure for toxicity. The differential neurotoxicity of heavy metals on adult men and women is a clear knowledge gap that future studies need to address.

Overall, as summarized in Table 1, epidemiological and experimental studies indicate that males are most of the time more vulnerable to the neurotoxicity of some metals such as Pb and Hg. However, for other metals such as Mn, Cd, and As, the picture is more nuanced. Multiple studies and cohorts around the world have shown that males have higher body burdens of Pb and are more susceptible to its neurotoxic effects, including decreased cognitive development, attention deficits, and behavior problems. Males show higher internal Pb levels than females even in experimental studies where rodents are dosed with equal amounts of Pb, suggesting a biological mechanism accounting for the higher Pb body burden observed in males in human populations (rather than solely behavior factors that increase their exposure). Furthermore, even when females are administered higher doses of Pb to achieve an internal Pb burden similar to males, they can remain unaffected while males display behavioral deficits at the same doses, confirming that males have an increased susceptibility to Pb concentrations that is not a result of their increased internal Pb exposure.

Males appear more vulnerable to prenatal and early postnatal Pb exposure, and the adverse effects of such early life exposure may even persist into adulthood (suggested by some epidemiological studies and numerous experimental studies). While it appears that in general males are more susceptible to the neurotoxic effects of Pb exposure, some epidemiological and experimental studies have suggested that females are more susceptible to the neurotoxic effects of Pb (e.g., IQ loss, behavior problems, associative learning and memory) at higher Pb levels or Pb exposure measured in later developmental periods (i.e., childhood and adolescence). Thus, Pb may have a differential neurotoxic impact on the two sexes depending on the window of exposure. Inconsistent results in sex-specific effects could also be explained by Pb's complex interactions with estrogen. Lastly, it is difficult to compare the *in vivo* experimental literature due to the various dosing and exposure paradigms implemented, different species and outcomes assessed, and the timing of assessment.

Multiple studies conducted in cohorts around the world (French Guiana, Canada, China, Brazil) and in multiple species of experimental animals have consistently reported that males are more susceptible to the neurotoxic effects of inorganic Hg and MeHg than females. There is limited evidence of sex differences in internal Hg and MeHg exposure across sexes. The adult brain appears less susceptible to Hg-induced toxicity, although sex differences in MeHg toxicity (adversely affecting males) in adults have been reported at high levels of exposure. Females may be less susceptible due to the neuroprotective effect of estrogen and through sex differences in antioxidant activity such as glutathione metabolism (Ruszkiewicz et al., 2019; Wang et al., 2020). Providing a plausible mechanism for the higher vulnerability of male brains to Hg, multiple significant interactions between metallothionein polymorphisms and various neurological outcomes were evidenced in boys exposed to Hg (urinary Hg levels) but not in girls of the same age range (8–12 years old) (Woods et al., 2013). Finally, different types of polymorphisms were associated with susceptibility to Hg exposure in the context of autism spectrum disorder, which affects boys more than girls (Lozano et al., 2021; Rahbar et al., 2021).

Our survey of the literature indicates that despite discrepancies and inconsistent findings in many studies, overall, girls appear more susceptible to adverse effects (including cognitive, neurodevelopmental, and behavioral effects) following various levels of Mn exposure.

Females tend to have higher blood Mn concentrations and have been reported to show higher brain Mn accumulation in animal studies. This sex difference in blood Mn levels appears during puberty, suggesting sex differences in internal Mn exposure may be due in part to differences in sex hormones. However, as is the case with Pb, their increased susceptibility may not be due to increased internal Mn burden, as a study showed that at similar hair Mn levels across sexes, adverse effects were observed in girls only (Menezes-Filho et al., 2014). However, in adults, it appears that men may be more susceptible, as shown by an epidemiological study and a study in mice, possibly indicating a protective effect of estrogen in women. Sex-specific effects of Mn exposure in adult men and women is a sizable knowledge gap.

Some studies have reported beneficial effects following Mn exposure. Unlike other heavy metals, Mn is an essential nutrient and needs to be absorbed in minimal quantities, which could explain some of these findings. Furthermore, the beneficial effects were mostly observed in boys only, again indicating that females are less likely to benefit from Mn exposure. Differences in Mn neurotoxicity could be due to sex differences in Mn brain accumulation, different nutritional demands in males versus females (e.g., low Fe status leads to increased Mn accumulation), interactions with hormones (e.g., estrogen) and neurotransmitters, and differential regulation of oxidative stress. Polymorphisms in Mn transporter genes can also confer greater susceptibility to or protection from Mn-induced neurotoxicity (Broberg et al., 2019).

Like Mn, females generally exhibit a higher internal Cd burden compared to males, which can be due to increased gastrointestinal absorption of Cd when Fe levels are low. The number of studies evaluating sex-specific neurotoxic effects of Cd exposure are limited. However, existing literature suggests that girls are more adversely affected when exposure occurs during development or early in life whereas males are more adversely affected following later exposure (e.g., after puberty). There is agreement in some epidemiological and *in vivo* rodent studies examining similar outcomes; human and rat studies have both demonstrated that prenatal Cd exposure affects female birth weight and fetal size more than males. Future experimental studies should seek to confirm sex-specific effects reported in the epidemiological literature. In addition, further research is needed to assess neurotoxic effects of Cd at different windows of exposure (such as later in life). Overall, research should also evaluate the effect of genetic polymorphisms in addition to sex and age in evaluating heavy metal induced neurotoxicity.

Studies suggest that males have higher levels of urinary As. Women may have lower As levels due to increased efficiency at metabolizing As to less toxic species. In particular, estrogen upregulates the synthesis of a major methyl donor that is involved in As metabolism, granting women protection from As exposure. There is very limited evidence of sex-specific neurotoxic effects of As exposure, making inferences about sex differences inconclusive. Future studies examining sex differences in As neurotoxicity should consider the influence of sex hormones such as estrogen, sex differences in As metabolism, clearance rates and antioxidant capacity, the timing and duration of As exposure, and sex differences in methylation and expression of key genes regulating targets of As toxicity.

We are only starting to recognize and unravel how toxic metal exposures produce a wide variety of sex-specific effects. Timing of exposure is often a relevant factor but strikingly the neurotoxic outcomes are distinct between males and females. Metals like MeHg and Pb are toxic at every age, but their toxicity is exacerbated during the developmental period. This is due in part to the insufficiently developed BBB during that stage, which enhances the neurotoxicity of these metals. Although metals' toxicity on brain development is more dramatic during fetal life when most cell division and migration occurs, it can still have prominent consequences for years postnatally. This can be due for example to altered methylation and gene expression affecting antioxidant capabilities and metal biotransformation and clearance. Also, myelination and neuronal network pruning during the postnatal period can lead to the manifestation of relatively sensitive effects that were latent or not apparent at earlier stages of development.

There is an interplay of a variety of factors ranging from molecular, hormonal, and epigenetic mechanisms affecting gene regulation, expression, and function to differences in absorption and metabolism which influence the sexual dimorphism seen with metal-associated neurotoxicity. Damages to the endothelial structure of the BBB can also be a factor, knowing that at least nine metals have been found to accumulate locally and injure the BBB (Zheng et al., 2003). This is a fundamental cause of leakage of blood-borne materials to surrounding brain parenchyma leading to neurotoxicity. Based on the current evidence, another source of sexual dimorphism in metal neurotoxicity reported in the epidemiologic literature is the differential vulnerability of exposure to certain kinds of toxicants across sex due to differences in occupation, time spent indoors, community, or workplace environment. Within animals, sexual dimorphism may occur via different feeding niches and differences in locomotor and exploratory behavior (Gochfeld, 2007). The impact of this differential exposure is further affected by the neurobiological and neurogenetic differences between the sexes.

While the precise mechanisms underlying each metal's neurotoxicity remain unclear, oxidative stress, competition with essential metals such as Fe and zinc, and dysregulation of gene expression have been supported by many studies as common fundamental processes implicated in metal toxicity. Sex differences affecting these mechanisms such as differences in accumulation, antioxidant capacity, nutrition requirements, sex hormones, and methylation/gene expression can result in sex-specific neurotoxic effects. For instance, an increasing body burden of heavy metals like Pb and Hg seems to sex-specifically impair general intelligence, cognitive ability, development, and behavioral problems due to differences in neuroendocrine, epigenetic, as well as antioxidant activity among the two sexes. On the other hand, sexual dimorphism due to metals like Mn and Cd occurs due to the differential uptake, absorption, and clearance of the metal, which can depend on nutritional status, antioxidant enzymes, and genetic polymorphisms in metal transporter genes. Differences in biotransformation of metals like As are likely responsible for the sex-associated variation in neurotoxicity. The evidence of sex-specific effects of heavy metal exposure continues to remain inconsistent; while the evidence does not support a clear pattern on sex differences in neurotoxicity, it does prove their very existence.

These inconsistencies may stem from differences in study design, sample size, variation in effect parameters, biomarkers of exposure and period of exposure, and confounders and effect modifiers such as socioeconomic status or social norms that are difficult to control for. Aside from this, factors such as low statistical power (particularly limited power to assess interactions), dropouts, failure to correct for multiple comparisons, and the retrospective nature of most epidemiological studies introduce heterogeneity in the results making it difficult to compare effects across different cohorts. This discrepancy is also seen in experimental studies but to a lesser extent due to the ability to control sample size, study protocols, animal care, and dosing and timing of exposure. Hence, for evaluating sexual dimorphism in terms of heavy metal neurotoxicity, the genetic background, epigenetic modifications, diet, physio-pathological condition, individual behavior, and the co-exposure to other xenobiotics must be considered. Other important covariates including socioeconomic factors, risky behaviors, and age are also important. There is a need to identify these factors that confer differential susceptibility across sexes and developmental stages and integrate them into a mechanistic framework to discern how sex influences neurotoxicity, for the purpose of revising recommendations for exposure and to inform interventions and therapeutics.

Study limitations:

We are limited by a handful of epidemiological and experimental studies that have independently analyzed outcomes from both sexes, restricting the scope of this paper. For controlled experimental studies, we indicate studies that clearly specify whether the reported exposure dose was properly adjusted for the metal formulation (such as a metal salt or metal hydrate) by stating that the dose was “equivalent to” the given concentration of the metal. However, many studies did not specify whether the reported dose was the equivalent dose of the metal itself. Thus, the specific metal exposure dose is uncertain in some cases. We call on researchers to clearly indicate whether or not their reported exposure doses are concentrations of the metal itself versus the metal formulation to ease comparison of results. Also, as just mentioned in the Discussion, due to differences in biomarkers of exposure and varied outcome measures across studies, low sample size (and its effect on power), and potential confounding interactions with other uncontrollable factors, it is hard to conclude with certainty if heavy metal neurotoxicity prefers a particular sex. Lastly, because the assessment of neurobehavioral, cognitive, and neuropsychological outcomes is done on a spectrum of life stages when the brain is undergoing different stages of development between sexes, drawing inference on sex-specific vulnerabilities could sometimes be seen as not perfectly accurate. The neurotoxicity observed could also be confounded by multiple factors not always accounted for, such as dietary habits, drug intake, competing diseases, and geography, influencing the phenotype (toxicity) which makes it highly unlikely that one single element will function as disease determinant.

Furthermore, this review does not discuss the effect of metal mixtures or co-exposure to other chemicals which are more commonly present in the environment and may have a synergistic or an additive effect on neurotoxicity. This is due to the limited data currently available for metal mixture exposure and their associated sex-specific neurotoxicity. Another factor is the lack of fluency of traditional epidemiologists in the complexity of statistical

analysis surrounding metal mixtures. However, one should note that the evaluation of health effects of exposure to a single trace metal may underestimate the true effects of environmental neurotoxicity due to possible interactions with other metals (Claus Henn et al., 2014). A review by von Stackelberg and colleagues presents a detailed assessment of metal mixtures and neurodevelopmental outcomes (von Stackelberg et al., 2015). Metal mixtures have more recently been shown to cause sex-specific effects on IQ (Guo et al., 2020) and visuospatial learning (Rechtman et al., 2020). Interestingly, Rechtman and colleagues only saw this effect when metals were investigated as a mixture, but not when each metal was investigated individually.

This review also does not discuss the neurotoxicity of other ubiquitous metals such as chromium (Cr), copper (Cu), and nickel (Ni) and only focuses on the five most commonly occurring metals known to be associated with neurotoxicity. Lastly, since we were looking at both experimental and epidemiological evidence, we did not include studies that investigate gender or gender-specific behavior or discuss differences in gender-specific behaviors that could underlie sex-specific effects (such as the interaction between betel nut use, more common in men, and As toxicity) for the sake of consistency. While compelling evidence supports the physiological and clinical bases of sexual dimorphism, emerging evidence also point to the importance of differences in behavior, occupation, social norms, and attitudes associated with gender (Krista Conger, 2017). Therefore, it can be predicted that future research should establish that gender and sex are intertwined characteristics governing an organism's biology and susceptibility to metal neurotoxicity.

Future Scope:

There is a dire need for translational or integrational research in toxicity assessment which takes sex into consideration. Sexual dimorphism in neurotoxicity adds another dimension to the interplay of mechanisms and pathways of metal toxicity and lends new insight into the possible preventive and therapeutic interventions surrounding metal toxicity and sex-specific neurological disorders. As Ruszkiewicz and Aschner rightfully mention, in the age of precision medicine, consideration of individual differences in susceptibility arising from genetic predispositions and environmental factors are vital for a personalized approach to neurotoxicology (Ruszkiewicz and Aschner, 2019).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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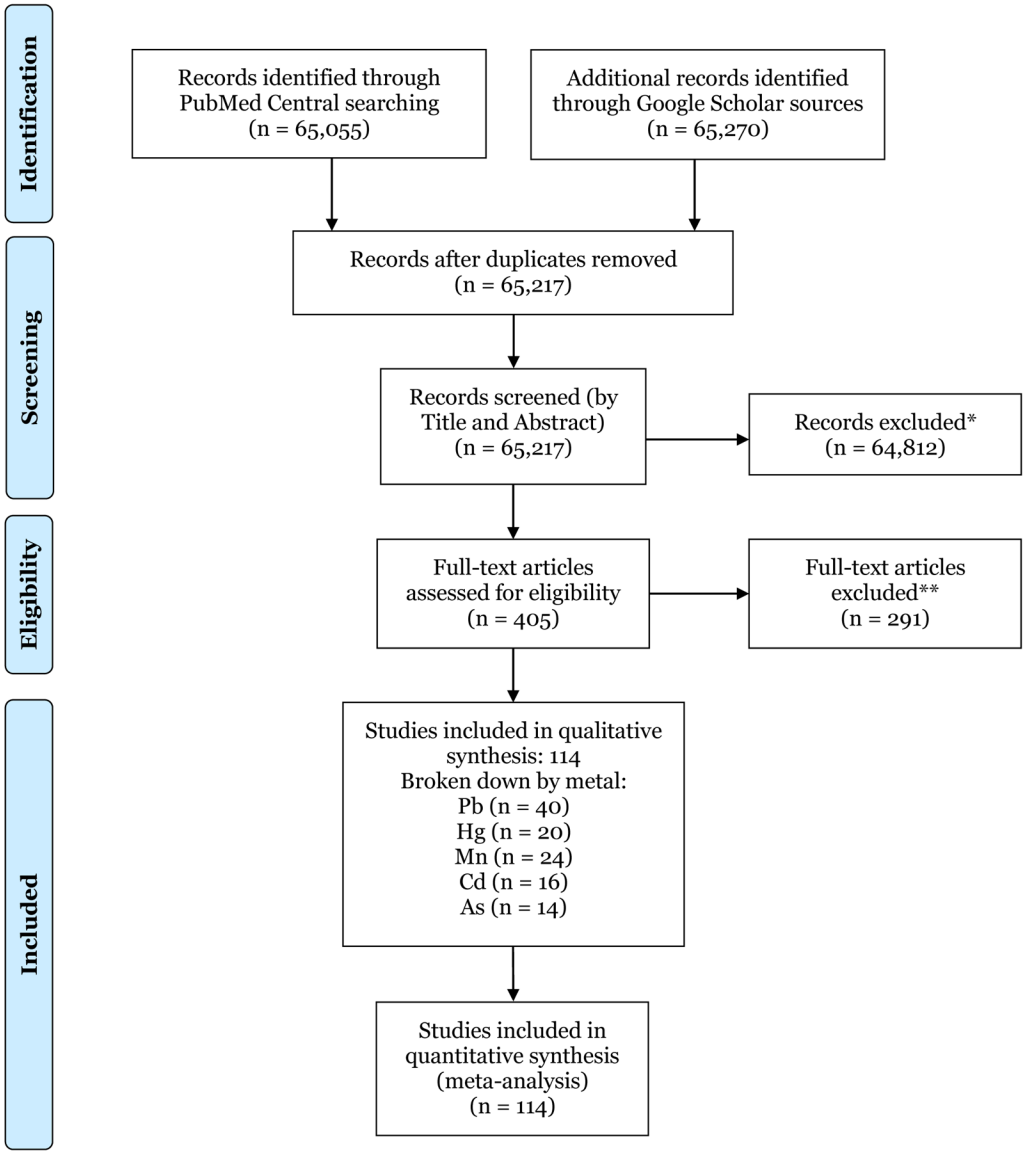

Figure 1. Flow diagram for inclusion and exclusion of studies.

*Records excluded after initial screening of all titles and abstracts

**Records excluded if 1) missing information regarding: dose of exposure, period of exposure, biomarker of interest, domain assessed; 2) conducted *in vivo* in a non-rodent species; 3) published in languages other than English, except for one study for Cd written in Chinese

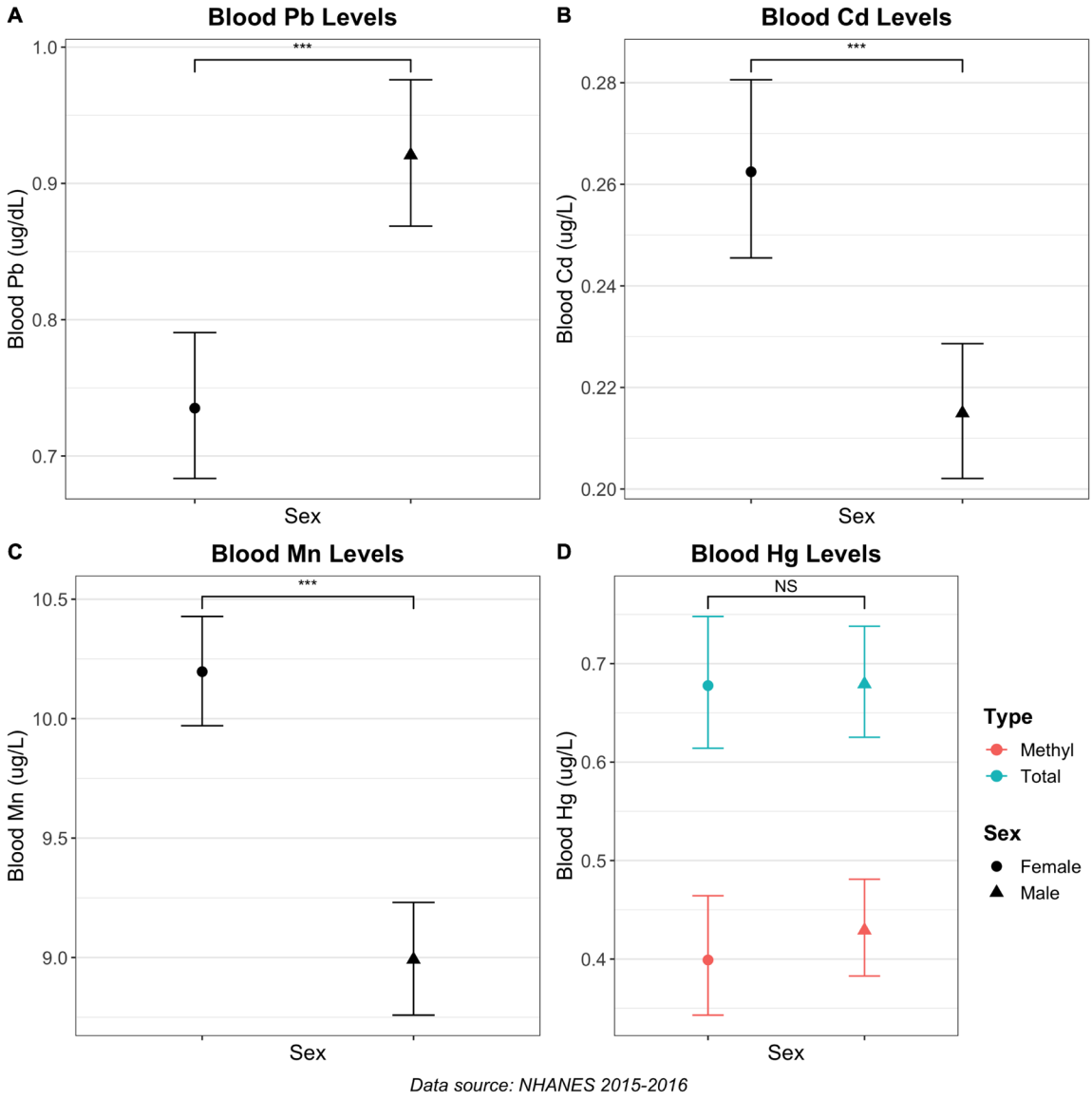

Figure 2. Geometric means (95% confidence intervals, CIs) of whole blood concentrations of Pb, Cd, Mn, and Hg (total Hg and MeHg) stratified by sex, NHANES 2015–2016.

Symbols represent the unadjusted geometric means and bars represent 95% CIs. ***p < 0.001, NS: not significant. (A) Blood lead, Pb; (B) Blood cadmium, Cd; (C) Blood manganese, Mn; (D) Blood mercury, Hg (total Hg and methyl mercury, MeHg). N = 4,987 for Pb, Cd, Mn, and total Hg; N = 4,937 for MeHg. See Supplementary Table 3 for exact values.

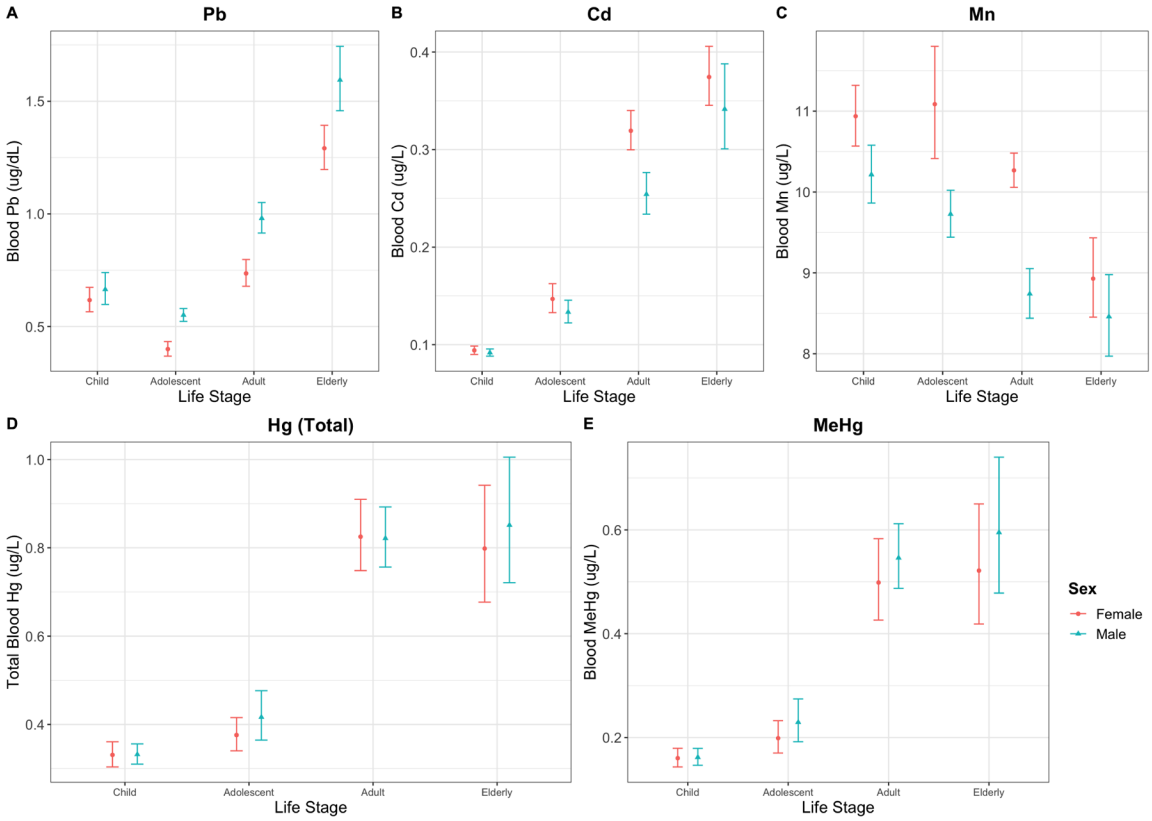

Figure 3. Geometric means (95% confidence intervals, CIs) of whole blood concentrations of Pb, Cd, Mn, and Hg (total Hg and MeHg) stratified by life stage and sex, NHANES 2015–2016.

Symbols represent unadjusted geometric means and bars represent 95% CIs. (A) Lead, Pb; (B) Cadmium, Cd; (C) Manganese, Mn; (D) Total Mercury, Hg; (E) Methyl mercury, MeHg. N = 4,987 for Pb, Cd, Mn, and total Hg; N = 4,937 for MeHg. See Supplemental Table 4 for exact values. The life stages are defined as follows: child, age < 12 years; adolescent, age 12–21 years; adult, age 22–65 years; elderly, age > 65 years.

Table 1:

Sex-specific body burdens of Pb, Cd, Mn, Hg, and As measured in biofluids and summary of epidemiological and experimental evidence of sex-specific neurological outcomes for Pb, Cd, Mn, Hg, and As.

Access Table 1 via this link: https://meethila-gade.shinyapps.io/Sex_specific_neurotox/. Note that 1 μg/dL is equivalent to 10 ppb (0.01 ppm); 1 μg/L is equivalent to 1 ppb (0.001 ppm); 1 μg/g, 1 mg/kg, and 1 mg/L are each equivalent to 1 ppm; and 1 mg/g is equivalent to 1,000 ppm.

Glossary of terms:

ADHD: Attention Deficit Hyperactivity Disorder;

ALS: Amyotrophic Lateral Sclerosis;

ALSPAC: Avon Longitudinal Study of Parents and Children;

ASHRAM: Arsenic Health Risk Assessment and Molecular Epidemiology;

AUC: area under the curve;

BASC-2: Behavior Assessment System for Children, 2nd edition;

BDNF: Brain derived neurotrophic factor;

B-MDI: Bayley Mental Development Index;

BSID: Bayley Scales of Infant Development;

BSID-II: Bayley Scales of Infant Development-II;

Ca: Calcium;

CASS: Conners' Adolescent Self-Report Scale;

CAVLT-II: Children's Auditory Verbal Learning Test, 2nd edition;

$CdCl_2$: Cadmium chloride;

CHAMACOS: Center for the Health Assessment of Mothers and Children of Salinas;

CPRS: Conners' Parents Rating Scales;

CPT-II: Conners' Continuous Performance Test II;

CSF: Cerebrospinal fluid;

DMA: Dimethylarsinic acid;

DNMT1: DNA methyltransferase 1;

DNMT1a: DNA methyltransferase 1 alpha;

DNMT3A: DNA methyltransferase 3 alpha;

DPAA(V): Arsenic diphenylarsinic acid;

DSM-IV: Diagnostic and Statistical Manual of Mental Disorders, 4th Edition;

F: Females;

GD: Gestational day;

GM: Geometric Mean;

GPx1: Glutathione peroxidase 1;

GR: Glucocorticoid receptor;

GS: Glutamine synthetase;

GSH: Glutathione;

GSSH: Glutathione disfulfide (reduced form of glutathione);

GW: Gestational Week;

$HgCl_2$: Mercuric chloride;

Glossary of terms:

HPA: Hypothalamic-pituitary-adrenal;

Hsd: Hydroxysteroid dehydrogenase;

INMA: INfancia y Medio Ambiente (“Environment and Childhood”);

IP: Intraperitoneal;

IQ: Intelligence Quotient;

IV: Intravenous;

M: Males;

MAO: monoamine oxidase;

MeCP2: Methyl-CpG binding protein 2;

MeHg: Methyl mercury;

MDI: Mental Development Index;

MINIMat: Maternal and Infant Nutrition Interventions of Matlab;

MMA: Methylarsonic acid;

MMT: methylcyclopentadienyl manganese tricarbonyl;

$MnCl_2$: Manganese chloride;

$MnSO_4$: Manganese sulfate;

MS: Multiple Sclerosis;

MSCA: McCarthy Scales of Children's Abilities;

NA: Not available;

NHANES: National Health and Nutrition Examination Survey;

PDI: Psychomotor Development Index;

PHIME: Public Health Impact of Manganese Exposure in susceptible populations;

PND: Postnatal day;

ppb: Parts per billion;

ppm: Parts per million;

qRT-PCR: Real-time quantitative reverse transcription PCR;

REPRO_PL: Polish Mother and Child Cohort;

SC: Subcutaneous;

SMBCS: Sheyang Mini Birth Cohort Study;

Trx-1: Thioredoxin;

TrxR1: Thioredoxin;

TNFα: Tumor necrosis factor alpha;

WASI: Wechsler Abbreviated Intelligence Scale for Children;

WISC-IV: Wechsler Intelligence Scale for Children, 4th edition;

WISC-RM: Wechsler Intelligence Scale for Children Revised-Mexican Version;

WRAVMA: Wide Range Assessment of Visual Motor Ability;

yrs: years.