Methylmercury and Measures of Attention Deficits in the ELEMENT Cohort

By

Rebecca Lynne Tutino

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Doctoral Committee:

Professor Karen E. Peterson, Chair
Associate Professor Niladri Basu, McGill University
Professor Howard Hu, University of Toronto
Assistant Professor Sung Kyun Park
Associate Professor Brisa N. Sanchez
Associate Professor Marc Weisskopf, Harvard University

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ADHD	Attention deficit/hyperactivity disorder
ANKK1	Ankyrin repeat and kinase domain containing 1
ASR	Acoustic startle reflex
CI	Confidence interval
COMT	Catechol-O-methyltransferase
CPT-II	Conners' Continuous Performance Test II
CRS-R	Conners' Rating Scales-Revised
DA	Dopamine
DAT1	Dopamine transporter 1
DRD2	Dopamine receptor 2
DRD4	Dopamine receptor 4
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders, 4th Edition
ELEMENT	Early Life Exposure in Mexico to Environmental Toxicants
EMG	Electromyography
Hg	Mercury
ICPMS	Inductively coupled plasma- mass spectrometer
IHg	Inorganic mercury
ITI	Intertrial interval
LOD	Limit of detection

MeHg	Methylmercury
MPH	Methylphenidate
Pb	Lead
PPI	Prepulse inhibition
SLC6A3	Solute carrier family 6
SNP	Single nucleotide polymorphism

Abstract

Attention-deficit/hyperactivity disorder (ADHD) is one of the most common psychiatric disorders in school-age children and is the cause of multiple burdens related to healthcare costs, academic performance, and later employment. As such, its etiology represents a major public health concern. While genetics play a large role in the etiology of ADHD, multiple environmental exposures may contribute to risk. Here, we investigate the role of one toxicant, methylmercury (MeHg), measured in hair and blood, and its possible interactions with both other toxicants (lead, Pb) and a series of candidate genes. Additionally, we examined possible associations of MeHg exposure with the acoustic startle reflex (ASR) and the sensorimotor gating process, prepulse inhibition (PPI), in which deficits have been observed in individuals with attention deficits. Participants were recruited from the Early Life Exposure in Mexico to Environmental Toxicants (ELEMENT) study, a longitudinal birth cohort that began in the 1990s. ADHD symptoms were screened using the Conners' Continuous Performance Test II (CPTII) and the Conners' Rating Scales-Revised (CRS-R). We found no significant associations between prenatal and postnatal MeHg exposures and ADHD screening scores. No interaction was seen for concurrent exposures to MeHg and Pb. For prenatal exposure, generally, interactions between the two metals corresponded to increasing attention deficits scores in trimester 1, while interactions between the two corresponded to decreasing attention deficits scores in trimester 2, and no pattern was seen in trimester 3. Additionally, we found that associations between hair Hg concentration and attention measures differed by dopamine receptor D4 (DRD4) and catechol-O-methyltransferase (COMT) genotype. ASR without a prepulse was non-linearly associated with

MeHg exposure. For ASR response magnitudes with prepulses, higher MeHg generally corresponded to higher ASR magnitudes, especially in the right tail of their respective distributions. No significant associations were seen between MeHg and PPI. This research adds to our understanding of how environmental influences like MeHg can play a role in the development of attention deficits in children and adolescents.

Introduction

Attention-deficit/ hyperactivity disorder (ADHD), affects approximately 8-10% of school aged-children worldwide (Escobar et al. 2005; Rowland et al. 2002). It is the most common psychological disorder in that age group (Escobar et al. 2005; Rowland et al. 2002). Although commonly assumed by the public to be a disorder of childhood, it can persist into adulthood with an estimated prevalence of in adults of 4.4% (Kessler et al. 2006). It is responsible for an estimated $31.6 billion in excess healthcare costs (Birnbaum et al. 2005). A diagnosis of ADHD is also associated with multiple deficits in academic performance (DeShazo Barry et al. 2002; Merrel and Tymms 2001; Henker and Whalen 1989) and increases in number of work absences, short-term disability, and worker compensation claims during later employment (Secnik et al. 2005). The costs and quality of life deficits associated with this and other mental health disorders present a pressing public health concern.

While multiple possible environmental risk factors for ADHD have been identified, these are not always well understood (Norman et al. 2013). Environmental exposures are complex and interact with each other, as well as individual characteristics. An exposure may only have an effect in a specific window of susceptibility, which could contribute to a condition that may not manifest clinically until much later in life (Landrigan and Etzel 2013). Understanding how environmental exposures, especially those that are widespread and present chronically at low doses, contribute to ADHD risk is imperative.

Overview of ADHD

ADHD is defined in the Diagnostic and Statistical Manual as a "persistent pattern of inattention and/or hyperactivity-impulsivity that interferes with functioning or development." (American Psychiatric Association 2013) It is classed as a neurodevelopmental disorder, with symptom onset occurring before 12 years of age. A diagnosis in children and adolescents requires an individual to exhibit at least six *inattention* symptoms and/or at least six *hyperactivity/impulsivity* symptoms in two or more settings and must be shown to interfere with the individual's functioning (American Psychiatric Association 2013).

Inattention is characterized by distraction, outside of an obvious source, and an inability to focus. Inattention symptoms would include inability to listen in class, making careless mistakes on assignments, and poor time management, among many others (American Psychiatric Association 2013). Hyperactivity is defined as "excessive motor activity," while impulsivity is defined as "hasty actions which occur in the moment without forethought and that have a high potential for harm to the individual." Hyperactivity and impulsivity symptoms would include, among others fidgeting, feeling restless, talking excessively and interrupting (American Psychiatric Association 2013). Based on which type of symptom predominates, an individual case can be specified as inattentive presentation, hyperactive/impulsive presentation, or combined presentation (American Psychiatric Association 2013).

While ADHD is most commonly identified after a child begins schooling, some symptoms are identifiable in early life. It is developmentally appropriate for preschoolers to exhibit behaviors associated with ADHD, but cases can present with hyperactivity and impulsivity beyond developmental norms (Cherkasova et al. 2013). However, compared to studies of ADHD in older children, there are relatively few studies in this age group (Egger et al.

2006). While ADHD was initially thought to present exclusively in children and adolescents, it is now know that symptoms can persist into adulthood (Haavik et al. 2010).

ADHD symptoms indicate deficits in behavioral control, motor control, and executive functions. Imaging studies suggest abnormalities in the fronto-striato-thalamic circuitry of individuals with ADHD, which could provide a basis for these deficits (Cherkasova and Hechtman 2013). Individuals with ADHD have volumetric reductions, especially in the right hemisphere (Cherkasova and Hechtman 2013). These are specifically volume reductions in the dorsolateral prefrontal cortex (PFC), caudate, pallidum, corpus callosum, and cerebellum (Seidman et al. 2005). Interestingly, as the brain matures, the brains of children with ADHD follow the same developmental patterns as controls, but are delayed. Studies in adults show similar, but less pronounced structural changes (Cherkasova and Hechtman 2013). Additionally, studies of executive function find reduced activity in the anterior cingulate cortex (ACC), multiple areas of the PFC, thalamus, basal ganglia, and the parietal cortex, as well as increased activity in several other areas (Cherkasova and Hechtman 2013).

Limitations of Environmental Epidemiology Studies of ADHD

There are limitations when studying environmental exposures and ADHD. While ADHD is common, it is difficult to find a large enough number of cases in a population cohort to yield meaningful results. Thus, rather than identifying participants with clinically diagnosable ADHD, many assess behavioral outcomes on a continuous scale, such as the Conner's Rating Scales-Revised (CRS-R) and the Behavior Assessment System for Children (BASC), which produce quantitative results based on a profile of clinically relevant symptoms (Symeonides et al. 2013). Treating ADHD as a continuous set of behaviors rather than a discrete outcome can improve the statistical power of a study and can allow for examination of subclinical symptoms. However,

this method does assume that the same factors will be relevant across the continuum of symptoms (Symeonides et al. 2013).

Additionally, studies can examine executive function deficits related to ADHD. However, these deficits can be heterogeneous between individuals in a sample, observed trends might be more easily confounded. Furthermore, because ADHD diagnosed is based on behavioral criteria, executive function deficits may not be sufficiently predictive (Symeonides et al. 2013).

There are also concerns about the effects of reporter differences. A study from the early 1980s found that adults with ADHD symptoms often under-report their symptom severity (Wender et al. 1981). A more recent study found marked differences between self-report data from adolescents and parent-report data (Rohde et al. 2001). Evidence indicates that school age children should be able to reliably report on their own health and that using a proxy reporter may introduce bias. These factors could create inconsistencies between studies that use different report methodologies (Riley 2004).

Role of Other Assessment Methods in Addressing Limitations

Because of these issues, efforts have been made to identify potential sets of biomarkers of ADHD to help support diagnostic methods. Potential biomarkers have included genetic markers, biochemical features, and physical changes in the brain (Faraone et al. 2014). While some of the suggested biomarkers, including those related to endophenotypes, may be less practical for use in diagnostic practices (Faraone et al. 2014), they could be useful in the context of environmental epidemiology studies to support findings observed using more traditional screening methods.

One physiological measure of interest is prepulse inhibition (PPI). PPI is a measure of sensorimotor gating and can be observed in the acoustic startle reflex (ASR). In PPI, exposure to

a brief pre-stimulus (or pre-pulse) ahead of a startling stimulus results in a dampened response (Li et al. 2009). Alterations in this process have been observed in a number of psychiatric and neurological conditions, including ADHD (Pålsson et al. 2011; Schulz-Juergensen et al. 2014), although it is not always observed in ADHD (Feifel et al. 2009; Kohl et al. 2013). In the case of attention deficits, deficits in this process are particularly pronounced during tasks which require selective attention (Hawk et al. 2003; Scholes and Martin-Iverson 2010). Further, deficits in PPI in ADHD cases have been observed to be remediated with methylphenidate treatment (Schulz-Juergensen et al. 2014; Hawk et al. 2003) and it has been suggested that PPI might be useful as a measure of dopaminergic function (Swerdlow et al. 2003).

Etiology of ADHD

Multiple factors are thought to be related to the development of ADHD. Among these, genetic factors feature most prominently. Heritability of ADHD is estimated to be 71-90%, based on twin studies. Adoption studies also suggest a strong genetic component, although they do not completely rule out interactions with the environment (Thapar et al. 2013). Candidate genes for ADHD risk are primarily dopaminergic and serotonergic. While the risk associated with these genes is generally small and cannot individually explain the entirety of a person's risk, these findings, especially related to the dopaminergic genes, have been consistent (Thapar et al. 2013; Swanson et al. 2007).

Deficits in dopaminergic signaling are a particularly promising mechanism. Methylphenidate (MPH, Ritalin), which is frequently used to treat ADHD, appears to act by stimulating the release of stored presynaptic dopamine and blocking reuptake. (Scahill et al. 2004; Chadchankar et al. 2012) The candidate genes associated with risk include dopamine (DA) transporter (DAT1). Imaging studies have shown variable levels of DAT1 expression in the

striatum and mesencephalon. Initial studies showed a dramatic increase in DAT1 expression in ADHD cases compared to controls, while later studies showed no differences or lower levels. A recent review of these imaging studies suggested that these variable findings might be a result of DAT1 expression regulation by DA levels. That is, that lower DA levels may result in lower DAT1 density. MPH treatment increases DA levels, and DAT1 expression would thus be higher as well (Swanson et al. 2007)

Exposures Associated with ADHD

Although genetic factors likely play a large role in ADHD risk, environmental factors and gene-environment interactions may also play in the role in the development of ADHD. Most likely, if an exposure contributes to ADHD, it could occur during specific windows of susceptibility. In many of the existing studies, pre- and peri-natal exposures are of particular interest. However, associations with exposures in childhood have also been found. Nevertheless, it is still unclear whether any of these possible risk factors are causally related to ADHD.

For example, maternal smoking during pregnancy has been repeatedly associated with increased rates of ADHD. This has been consistent through methodological differences- using diagnosed ADHD versus symptoms and self-reported cigarette use versus the biomarker cotinine- and a dose-response relationship which had been previously observed (Thapar et al. 2013). It is possible, however, that this association is confounded by genetic factors (Thapar et al. 2013). Additionally, a study of gene-environment interactions found interactions between maternal smoking during pregnancy and DA gene polymorphisms among a group of children with ADHD- combined type (Neuman et al. 2007).

Lead exposure has also been associated with ADHD in multiple studies. Young children are often exposed to lead contaminated dust and soil during hand-to-mouth behaviors (Thornton

et al. 1990; Lanphear et al. 1998). Additionally, resorption of the maternal skeleton during pregnancy can mobilize lead and expose the developing fetus (Rothenberg et al. 2000). Low-level lead exposure in childhood has most notably been associated with reductions in measured intelligence (Koller et al. 2004; Lanphear et al. 2005), as has prenatal exposure (Schnaas et al. 2006; Gomaa et al. 2002). Similarly, a number of studies have found associations between deficits in attention measures and exposure to lead during childhood (Nigg et al. 2008; Calderon et al. 2001; Nigg et al. 2010; Braun et al. 2006). Fewer studies have associated exposure during prenatal development and attention deficits (Plusquellec et al. 2007). Multiple studies noted that lead exposure was associated with hyperactivity and impulsivity symptoms, but not inattention (Nigg et al. 2008; Boucher et al. 2012; Sioen et al. 2013), which could indicate that the associations between lead and attention might be mediated by lead's cognitive effects. That is, that cognitive deficits lead to poorer behavioral control (Nigg et al. 2008). In vivo studies suggest that lead exposure during synaptogenesis decreases levels of the proteins synaptophysin and synaptobrevin, resulting in impaired vesicular release (Neal et al. 2010).

Multiple other environmental risk factors for ADHD have been proposed. These include maternal medication use during gestation, maternal obesity, essential nutrient deficiency, a number of psychosocial conditions, and additional metals, such as mercury. (Thapar et al. 2013; Froehlich et al. 2011). There are still many gaps in the available literature regarding how these factors might interact with each other, how they interact with genetic susceptibilities, and the role of epigenetic processes (Froehlich et al. 2011; Mill and Petronis 2008).

A Focus on Organic Mercury

Methylmercury (MeHg) is an organic form of mercury (Hg) and a known neurotoxicant, included in top three of the Agency for Toxic Substances and Disease Registry's priority list of

hazardous substances (ATSDR 2011). Gaseous elemental Hg generally enters the atmosphere via emissions from coal combustion (Sherman et al. 2012). Elemental Hg then deposits in aquatic environments during precipitation events (Sherman et al. 2012). Bacteria in river sediments can then add a methyl group to the elemental Hg to form MeHg (Yu et al. 2012; Gilmour et al. 2013).

Once MeHg is produced, it is prone to bioaccumulation and biomagnification. *Bioaccumulation* is defined as the uptake of pollutants by organisms from a media, including dietary sources. The result of this process are tissue concentrations of the pollutant higher than what was found in the original media (IUPAC 1993). *Biomagnification* refers to the increasing tissue concentration of a pollutant associated with increasing trophic level. That is, an organism higher on the food chain would have a higher tissue concentration of a pollutant than its prey (IUPAC 1996). Human exposure to MeHg is frequently via consumption of fish. The level of exposure is dependent on the trophic level of the fish, the level of contamination where the fish was caught or raised, and the amount consumed (Clarkson and Magos 2006). As of 2010, 81% of fish advisories in the United States were due at least in to part MeHg levels (U.S. EPA 2011). In the U.S., low-level exposure is widespread and roughly 3% of women of child-bearing age have exposure levels above the CDC's level of concern (5.8µg/L) (U.S. EPA 2013). This is of note because MeHg has been shown to cross the placental barrier to expose the developing fetus (Mergler et al. 2007).

A number of epidemiological studies have examined the relationship between MeHg exposures and later behavioral and cognitive effects. Studies examining concurrent MeHg exposure generally do not find an association with attention deficits (Ha et al. 2009; Nicolescu et al. 2010). More studies have found associations with prenatal exposure. A 1997 study in the

Faroe Islands found attention deficits related to high cord blood levels of MeHg (Grandjean et al. 1997). A more recent study by Grandjean et al. and a study by Oken et al. found similar results (Grandjean et al. 2012; Oken et al. 2005). However, a similar cohort in the Seychelles has consistently failed to find deficits (Myers et al. 2003; Davidson et al. 2010).

The existing literature on MeHg exposure and brain structure and function changes consists primarily of animal studies and observations of poisoning cases. Autopsies of individuals affected by MeHg contamination in Minamata showed a number of pathological changes. Lesions in the cerebrum tended to form selectively in the calcarine sulcus, transverse temporal gyrus, pre-central gyrus, and post-central gyrus. In the cerebellum, there was granule cell loss, while other cells were unaffected (Eto 2000). Fetal autopsies from Minamata showed, in several cases, characteristics of cerebral palsy, as well as diffuse neuronal hypoplasia, rather than localized cell destruction (Eto 2000).

There are three mechanisms by which MeHg might cause cytotoxicity. These include disruption of intracellular Ca^{2+}, induction of oxidative stress, or forming complexes with thiol-containing compounds (Ceccatelli et al. 2010). Neuronal cell types are differentially susceptible to these mechanisms of cytotoxicity. For example, cytotoxicity of cerebellar granule cells initiates with Ca^{2+} disruption (Ceccatelli et al. 2010). Similar observations have been made in animal studies. In chick embryos, selective loss of cerebellar granule cells was observed, as well as poorer cell development (Bertossi et al. 2004). Multiple studies in developing rats found similar results, in addition to degeneration in the neostriatum (Sakamoto et al. 2002; Sakamoto et al. 1998; Kakita et al. 2000).

A number of studies have examined changes to catecholamine signaling and processing after MeHg exposure. A 1997 study by Faro et al. found that chronic MeHg exposure in rats

resulted in increased striatal release of DA (Faro et al. 1997). Other studies found prenatally exposed rats had decreased monoamine oxidase (MAO) activity (Chakrabarti et al. 1998; Beyrouty et al. 2006). More recently, a Tiernan et al. study found that MeHg-induced DA release was associated with increases in DA synthesis, tyrosine hydroxylase activity, and intracellular levels of DA (Tiernan et al. 2013).

There are currently few studies of gene-environment interactions examining MeHg, genes and behavioral outcomes. However, there are related studies which can be informative. One recent study examined inorganic Hg from dental amalgams and variants of metallothionein (MT). MT is a protein involved in the prevention of metal toxicity. This study found that while no associations were seen for genetic variants or exposure alone, boys who had both a genetic variant and exposure had pronounced negative associations with multiple domains of neurobehavior (Woods et al. 2013). Another study found that interactions between elevated blood Hg and variants of glutathione S-transferases (GSTs), another detoxification enzyme, were associated with reduced birth weight (Lee et al. 2010). This suggests that, in addition to interactions with DA related genes, genes related to metals detoxification should be considered.

There continue to be questions about MeHg's possible contribution to attention deficits that demand further study. While many studies have examined MeHg at different time points, the window of susceptibility remains unclear. For instance, associations with pre-natal exposure have been observed, but these studies generally only measure exposure near parturition or during pregnancy overall. Perhaps risk is most elevated during a specific point in pregnancy, as is the case with lead and cognition (Hu et al. 2006).

Objectives of this Work

There are many gaps in the literature regarding the role of environmental exposures in the etiology of ADHD and attention deficits more generally. Our long-term goal is to better understand the relationship between mercury and attention deficits. This is approached via our central hypothesis that MeHg contributes to ADHD via disruption of dopaminergic pathways. The objective for this work is thus to determine if there are associations between multiple measures of attentions deficits and explore the relevant windows of exposure, using the previously established ELEMENT (Early Life Exposure in Mexico to Environmental Toxicants) study.

Here, we examine three specific aims:

1. **Explore the relationship between Hg exposure in multiple windows of exposure and screening instrument scores.** This can be further broken down into several sub-aims
    i. Explore the relationship between concurrent MeHg and inorganic Hg (IHg) and screening instrument scores. Our working hypothesis is that scores indicating attention deficits will be weakly associated with concurrent exposure.
    ii. Explore the relationship between prenatal MeHg (Trimesters 1, 2, 3 and delivery) and screening instrument scores. Our working hypothesis is that scores indicating attention deficits will be associated with high levels of exposure in the first two trimesters.
    iii. Explore the possible interactions between MeHg and Pb at the above time points. Our working hypothesis is that MeHg and Pb will act synergistically and be associated with further increases in attention deficits as measured by screening instruments.

2. **Examine potential disruptions in dopaminergic pathways as a mechanism for MeHg effects on attention processes via study of genetic polymorphisms**. Our working hypothesis is that genetic variants will be associated with attention deficits and that the nature of these associations will be modified by considering MeHg exposure.
3. **Explore the relationship between concurrent MeHg exposure and acoustic startle reflex (ASR) and prepulse inhibition (PPI).** Our working hypothesis is that increased ASR and PPI deficits will be associated with high concurrent MeHg exposure, as suggested by previous animal studies

This work is expected to narrow the relevant window of exposure for the previously observed association between MeHg exposure and attention deficits. It is also the first study of PPI deficits and MeHg exposure in humans. Additionally, this will further our understanding of dopaminergic signaling as a target of MeHg neurotoxicity and contributor to attention deficits.

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Chapter 1

Mercury, Lead-Mercury Interactions, and Attention Deficits in Children

Abstract

Attention deficit-hyperactivity disorder (ADHD) is one of the most common neurological disorders in school-aged children. Multiple exposures have been investigated as possible ADHD risk factors. Prenatal exposure to the neurotoxicant methylmercury (MeHg) has been previously associated with ADHD symptoms, but there is limited evidence for how postnatal MeHg exposure, inorganic Hg exposure, timing of prenatal MeHg exposure, or mixtures of neurotoxic metals might be relevant. Here, we examine the relationships between attention deficits, as measured by the Conners' Continuous Performance Test II (CPT-II) and Conners' Rating Scales-Revised (CRS-R), and several time points of mercury exposure, and interactions with lead (Pb). Prenatal (Trimesters 1, 2, 3, and birth) and postnatal (ages 6-12) exposure to Hg and Pb were measured in participants from the Early Life Exposure in Mexico to Environmental Toxicants (ELEMENT) study. Concurrent and prenatal exposures were not significantly associated with CPT-II or CRS-R outcomes, although higher exposure generally corresponded to higher scores. A possible negative, but not statistically significant, interaction between Hg and Pb was seen. Interactions between the two metals related to increasing attention deficit scores in trimester 1, while interactions between the two related to decreasing scores in trimester 2. Several of these were statistically significant. No pattern was seen in later pregnancy or between prenatal Hg and concurrent Pb exposure. These findings suggest that MeHg is not associated with ADHD, except when considered in mixtures, although additional research is needed to confirm this.

Introduction

Attention deficit/hyperactivity disorder (ADHD) is characterized by persistent impulsivity, inattention, and hyperactivity that is present in multiple contexts and impairs functioning (American Psychiatric Association 2013). ADHD is one of the most common neurological disorder in school-aged children worldwide (Escobar et al. 2005; Polanczyk et al. 2015). Measures of the worldwide prevalence of ADHD vary widely, but it is estimated at 7.2% worldwide (Polanczyk et al. 2014; Thomas et al. 2015). The CDC estimates a prevalence of 9.5% in children in the U.S. (Bloom et al. 2013).

The existing literature suggests that ADHD is highly heritable. While the heritability is estimated to be 76%, the interactions between the genes involved are complex (Faraone and Mick 2010). Further, there is heterogeneity in the disorder which could potentially involve environmental influences or gene-environment interactions (Archer et al. 2011). These include environmental exposures such metals, including methylmercury (Karagas et al. 2012; Goodlad et al. 2013; Sanders et al. 2015).

Methylmercury (MeHg), the organic form of mercury (Hg) and an established neurodevelopmental toxicant (Clarkson and Magos 2006), exposure has recently been linked with attention and cognition deficits (Karagas et al. 2012). Exposure to MeHg is primarily via fish and seafood consumption and is thus ubiquitous amongst populations that consume fish (Driscoll et al. 2013). A growing number of studies have investigated prenatal MeHg exposure and attention deficits. A study of 917 children in the Faroe Islands, aged approximately 7 years, found deficits in attention, as measured by the NES Continuous Performance Test, could be associated with cord blood mercury levels (Grandjean et al. 1997). Another study of 135 pregnant mothers in the U.S. found associations between maternal hair mercury levels and

maternal fish consumption during pregnancy and lower cognitive scores in their 6-month old children (Oken et al. 2005). There is animal data in support of the aforementioned epidemiological studies. Mercury compounds have been shown to disrupt the function of several neurotransmitters implicated in the etiology of deficits in attention and cognition (Faraone and Mick 2010)

Lead exposure has been associated previously with many of the same outcomes as MeHg exposure, including cognitive deficits (Lanphear et al. 2000), behavioral deficits (Rice 2000), and diagnosed ADHD. Furthermore, both toxicants have been suggested to disrupt dopaminergic signaling (Tavakoli-Nezhad et al. 2001). A previous study by our group found that higher lead levels are associated with hyperactivity and impulsivity symptoms (Huang et al. 2015). Thus, clarifying how the two may interact is imperative to understanding their potential impact on the development of attention deficits.

While there is evidence in support of an association between MeHg exposure and deficits in attention and cognition, outstanding questions remain. Some studies have revealed no association between MeHg exposure and symptoms of ADHD, as measured using an ADHD/DSM-IV related scale (Ha et al. 2009; Nicolescu et al. 2010), or measured attention via the German test battery for attention performance of children (Nicolescu et al. 2010) or finger-tapping (Myers et al. 2003; van Wijngaarden et al. 2013). Gestation has been suggested as a susceptible period by several studies, but it is not clear if some periods of pregnancy are more susceptible. Though a recent paper by a panel of experts concludes that low-level exposure to MeHg may be associated with neurocognitive effects in children, they note that consideration needs to be given to better characterizing and resolving several factors. These include the timing and windows of exposure, the accuracy of the biomarker and exposure assessment, sex

differences, and the types of outcome measures (Karagas et al. 2012). Tackling such questions is particularly important given that fish consumption, while a major source of MeHg exposure, is a key source of dietary protein worldwide and also linked to beneficial neurological outcomes (Mahaffey et al. 2011).

The objective of the current study was to address some of the limitations highlighted by Karagas et al (Karagas et al. 2012), and to further increase our understanding of whether MeHg-exposure is associated with ADHD symptoms and measures of attention. Specifically, we aim to explore the relationship between Hg exposure in multiple windows of exposure and attention deficits, as measured by two screening instruments (Connors Continuous Performance Test and Conners' Rating Scales-Revised). This includes, first, exploring the relationship between concurrent MeHg, inorganic mercury (IHg), and attention, where we hypothesize that scores indicating attention deficits will be at most weakly associated with exposure. This is based on the existing conflicting results, even in high exposure populations (Karagas et al. 2012). Second, we will examine the relationship between prenatal MeHg (Trimesters 1, 2, 3 and delivery) and attention, where we hypothesize that scores indicating deficits will be associated with high levels of exposure in the first two trimesters. We suspect the first two trimesters as the susceptible period based on animal studies which find deficits in the equivalent developmental period (Cagiano et al. 1990; Eccles and Annau 1982; Maier et al. 1997). Third, we will examine potential interactions between MeHg and Pb at the available time points, where we hypothesize that MeHg and Pb will act synergistically and be associated with further deficits. These aims capitalized upon the rich resources of a sequentially enrolled epidemiologic birth cohort series running since 1994 called the Early Life Exposures in Mexico to Environmental Toxicants (ELEMENT) study (Afeiche et al. 2011).

Methods

ELEMENT Cohort

The ELEMENT study, consisting of three sequentially enrolled cohorts, was initially designed to research the influence of maternal lead exposure on offspring neurodevelopment. Pertinent details of ELEMENT, such as inclusion and exclusion criteria, collection methods, and demographics can be found elsewhere (Afeiche et al. 2011; Tellez-Rojo et al. 2006). In brief, Cohort 1 subjects were recruited 1994-1995, Cohort 2 subjects were recruited 1997-2001, Cohort 3 subjects were recruited 2001-2004 (Afeiche et al. 2011). In 2006 participants were recruited from all three cohorts for follow-up visits regarding behavioral outcomes. For analysis of concurrent exposures, children were included if their mothers were recruited into Cohorts 2 and 3, had at least one mercury exposure value and at least one attention measure from the same visit. For analysis of prenatal exposure, mothers and children were included if the mother had at least one mercury exposure value from pregnancy or delivery and her child had at least one of the attention measures from the follow-up visits. Behavior outcomes at these visits were linked to exposure measures taken at the same time as the outcomes (i.e. concurrent exposures), as well as prenatal exposures obtained from the ELEMENT biorepository.

The research protocol was approved by the ethics and research committees of the partnering institutions, including the National Institute of Public Health of Mexico, the Harvard School of Public Health, the Brigham and Women's Hospital, the University of Michigan School of Public Health, the University of Toronto, and the participating hospitals.

Human Biospecimens & Biomarker Analysis

Blood, hair and urine samples were collected from the participating children at the follow-up visit. Venous whole blood samples were collected into vials certified for trace metals

analysis and stored at 4^{o}C until analysis. Spot (second morning void) urine samples were collected and stored frozen until analysis. Scalp hair samples were obtained from each participant using stainless steel scissors and the proximal end was designated. Prenatal samples were collected from participating mothers at visits during the first, second and third trimesters, as well as at delivery. Venous whole blood samples were collected in the same manner as for childhood visits at the trimester visits. Cord blood was collected at delivery. Prenatal samples were frozen at -80^{o}C until analysis.

Mercury was analyzed in all samples as described elsewhere (Basu et al. 2014). Briefly, total mercury content was carried out using a Direct Mercury Analyzer 80 (DMA-80, Milestone Inc., CT). Daily instrument calibration, procedural blanks, replicates, and several certified reference materials were analyzed. Reference materials included CRM #13 for hair (National Institute for Environmental Studies, Japan), DOLT-4 (dogfish liver; National Research Council, Canada), and QMEQAS for blood and urine (Institut National de Santé Publique du Québec). Recoveries of the reference materials ranged from 80 to 110%. The analytical detection limit was less than 1.0 ng mercury.

Lead was analyzed in whole blood samples collected from participants. The majority of the available blood samples (a subset of 342) were analyzed using Inductively Coupled Plasma Mass Spectrometry (ICPMS) (Agilent 7500c, Agilent Technologies, Palo Alto, CA) in an ISO-designated clean room to minimize contamination as we have previously detailed (Huang et al. 2015). As with mercury, procedural blanks, replicates, and several certified reference materials were analyzed. For quality control and assurance, accuracy and precision were estimated through the use of certified reference materials (Institut National de Santé du Québec, INSPQ, QMEQAS09), as well as replicated samples. The average detection limit was 0.03 μg/dL. Based

on the recoveries of the reference materials, the overall accuracy was 88.2% (SD= 9.5%). The remaining 70 blood samples were analyzed at the Michigan Department of Community Health using a similar ICPMS approach with a detection limit of 1.3 μg/dL and all values above the limit of detection (LOD). The overall accuracy was 102.4% (SD=2.0%) We performed cross-validation between the two laboratories in 64 samples. These were 96.2% consistent after removal of two outliers.

Attention Measures

Two sets of instruments were used: the Connors Continuous Performance Test (CPT, 2nd Edition) and the parent-responses from the Conners' Rating Scales-Revised (CRS-R). The CPT is a computer-administered task that produces multiple scores, including those for omission errors, commission errors, and mean and standard deviation of reaction time. All outcomes are scaled such that a higher score indicates greater attention problems. Participants were seated at a computer while presented with a task comprised of a series of images over approximately 15 minutes. Participants were instructed to press the space bar in response to a target image, while ignoring non-target images. There is not a great deal of existing literature examining this test in Hispanic and Latino populations, but there is evidence that suggests ethnicity may not act as a major confounder for CPT assessments, particularly if images are used rather than text(Leany et al. 2012).

The CRS-R uses parent-completed questionnaires that provide information about the extent to which a child's difficulties managing attention are manifested as dysfunctions in everyday life. Each item of the questionnaire is linked to the DSM-IV criteria for ADHD-inattentive subtype, ADHD-hyperactive-impulsive subtype, and ADHD-combined subtype. The CRS-R produces index scores for hyperactivity, inattention, and ADHD overall, as well as other

behaviors, such as perfectionism, opposition, and somatization. The Spanish version of the CRS-R has previously been used in Hispanic and Latino populations (Ortiz-Luna and Acle-Tomasini 2006; Montiel et al. 2008).

Statistical Analysis

Data were analyzed using R x64 3.0.1. Univariate descriptive statistics and graphical displays were obtained for all variables. Outliers were detected using the ExtremeValues package for R, which uses a distribution based method for identifying outliers (van der Loo 2010). Spearman correlations were used to assess association among all biomarkers. Bivariate analyses were used to relate mercury biomarker values with demographic characteristics. Descriptive statistics are reported as mean (standard deviation), unless otherwise indicated.

Linear models were constructed which included a number of covariates selected a priori for potential relevance to either the exposure or the analyzed attention measures. Maternal IQ was calculated based on the mothers' scores on the Spanish Wechsler Adult Intelligence Scale (Tellez-Rojo et al. 2004; Wechsler 1968). Maternal education was the cumulative number of years that the mother attended school at time of recruitment. Information about smoking during pregnancy (yes/no) was obtained from a questionnaire administered to the mother during pregnancy. Mothers who responded "yes" at any point during pregnancy were excluded from analyses. As previously described (Fortenberry et al. 2014), direct questions about income were deemed too intrusive within this cohort. Thus, a continuous measure of socioeconomic status based on reported possessions and household assets was used instead. Maternal age and marital status at recruitment were also included, as were child age at the follow-up visit and child sex.

The exposure measures were not normally distributed and were highly skewed. Because of this, they were log-transformed prior to entering into the models. Further, model diagnostics

revealed that CPT-II Omission Errors, CPT-II Commission Errors, CRS-R Distractibility Score, CRS-R Hyperactivity Score, CRS-R ADHD Index, and DSM-IV Inattention Symptoms did not meet normality and were highly skewed. Accordingly, these outcomes were also log-transformed. Given these transformations, the actual beta coefficients are presented in tables and figures. However, in the text, we also provide interpretations that consider these transformations. Specifically, we calculated the difference in log-transformed outcomes for a 10% higher Hg concentration as $\Delta Outcome = \left(e^{\beta_{exposure} * \ln(1.10)} - 1\right) * 100$. For outcomes not requiring log-transformation (CPT-II Hit Reaction Time, DSM-IV Hyperactivity/Impulsivity Symptoms, and DSM-IV Total Symptoms) we used $\Delta Outcome = \beta_{exposure} * \ln(1.10)$.

Models were also constructed to assess possible interactions between MeHg and Pb exposures. These models included MeHg, Pb, and their cross-product. All exposures were log-transformed and centered, so that single effect coefficients in the models could be interpreted as the effect when the other metal was held constant at the geometric mean. Once again, the actual beta coefficients are presented in tables and figures, but interpretations which take transformations into account are presented in the text. These included models that looked for interactions between MeHg exposure and Pb exposure at the same time point and models assessing interactions between concurrent Pb exposure and prenatal MeHg. The latter was meant to replicate previous studies of Pb and MeHg interactions at these time points (Boucher et al. 2012a).

Results

Population Characteristics

Overall, 466 Cohort 2 and 3 children with complete demographic information participated in the follow-up visit (Table 1.1). A slight majority of the child participants were

male. A majority of mothers had been married at the time they were recruited. Sixteen mothers reported smoking during pregnancy. Given the small proportion of our sample and the existing literature suggesting an association between maternal smoking status in pregnancy and later attention problems (Braun et al. 2006; Froehlich et al. 2011; Neuman et al. 2007), these mothers, and their children, were omitted from subsequent analyses. For any given psychometric outcome, a maximum of 17 outliers were removed. (Table 1.1)

Exposure data for this cohort was previously reported for mercury (Basu et al. 2014) and lead (Huang et al. 2015; Zhang et al. 2012) and briefly summarized here. Concurrent mercury and lead exposure data is available for 72.8-96.4% of the children, depending on the biomarker. Blood, hair and urine mercury levels of participating children were 1.8 ± 1.3 μg/L, 0.60 ± 0.47 μg/g, and 0.82 ± 0.93 μg/L respectively (Table 1.1). Across children, mercury levels in blood, hair and urine were correlated. Blood and hair mercury levels of the same individuals were most correlated ($r=0.69$, $p<<0.001$), while urine mercury levels were moderately correlated to both blood ($r=0.39$, $p<<0.001$) and hair ($r=0.37$, $p<<0.001$) mercury levels. Blood lead levels of participating children averaged 3.1 ± 1.8 μg/dL. Blood lead and blood mercury levels were not correlated ($r=0.08$, $p=0.35$).

Prenatal exposure data is only available for participants from two of the three study cohorts. (Table 1.1) Mercury exposure levels were found to be 3.1 ± 1.8 μg/L in the first trimester, 3.1 ± 1.8 μg/L in the second, and 3.4 ± 2.6 μg/L in the third. Cord blood mercury levels were 4.4 ± 2.3 μg/L. Correlations between the individual trimester exposures are detailed in our previous work on exposure assessment in this population (Basu et al. 2014). Lead exposure levels were found to be 6.2 ± 4.4 μg/dL in the first trimester, 5.3 ± 3.3 μg/dL in the second, and 5.7 ± 3.3 μg/dL in the third. Cord blood lead levels were 4.9 ± 3.2 μg/dL. The lead

levels for all three trimesters were correlated ($r_{Tri1\text{-}Tri2}$=0.74, p<<0.001; $r_{Tri1\text{-}Tri3}$=0.67, p<<0.001; $r_{Tri2\text{-}Tri3}$=0.77, p<<0.001). Cord blood levels and trimester exposure levels were also correlated. Trimester 3 and cord blood were most strongly correlated (r=0.72, p<<0.001), followed by trimester 2 (r=0.54, p<0.001), and then trimester 1 (r=0.40, p<0.001). Prenatal lead and mercury levels were not correlated to each other.

Table 1.1 Demographic Characteristics, Exposure Assessment, and Psychological Testing

DEMOGRAPHIC CHARACTERISTICS	**N**	**Mean(SD)**	**Median**	**Range**
Child Age	466	9.1 (1.3)	9.3	(6.9, 12.5)
Household SES Level	466	6.7 (2.5)	6.5	(1, 14)
Maternal Age at Recruitment	466	26.0 (5.5)	26	(14, 44)
Maternal Education Level at Recruitment	466	10.9 (2.6)	11	(2, 20)
Maternal IQ	466	92.6 (18.5)	91	(60, 182)
	Total N	**N(%)**		
Sex of Child (Male)	466	237 (50.9%)		
Maternal Smoking During Pregnancy ("Ever Smoked")	466	16 (3.4%)		
Maternal Marital Status ("Married")	466	343 (73.6%)		
EXPOSURE ASSESSMENT				
Exposure at Follow-up Visit	**N**	**Mean(SD)**	**Median**	**Range**
Mercury (µg/L or µg/g)				
Blood (µg/L)	314	1.8 (1.3)	1.5	(0.23, 8.5)
Hair (µg/g)	435	0.60 (0.47)	0.46	(0.06, 3.1)
Urine (µg/L)	436	0.82 (0.93)	0.50	(0.02, 7.0)
Lead (µg/dL)				
Blood	320	3.1 (1.8)	2.4	(0.47, 11.0)
Prenatal Exposure	**N**	**Mean(SD)**	**Median**	**Range**
Mercury (µg/L)				
Trimester 1 Blood	123	3.1 (1.8)	2.7	(0.82, 10.5)
Trimester 2 Blood	168	3.1 (1.8)	2.8	(0.68, 10.7)
Trimester 3 Blood	147	3.4 (2.6)	2.7	(0.46, 14.6)
Cord Blood	86	4.4 (2.3)	3.9	(1.4, 12.7)
Lead (µg/dL)				
Trimester 1 Blood	249	6.2 (4.4)	5.4	(1.2, 23.3)
Trimester 2 Blood	260	5.3 (3.3)	4.5	(0.9, 20.3)
Trimester 3 Blood	246	5.7 (3.3)	5.0	(1.1, 7.3)
Cord Blood	211	4.9 (3.2)	4.1	(0.90, 20.0)
PSYCHOLOGICAL TESTING	**N**	**Mean (SD)**	**Median**	**Range**
CRS-R Scores	443			
Cognitive Problems/Distraction	438	53.4 (9.7)	52.0	(40, 88)
Hyperactivity	432	55.0 (9.5)	53.0	(42, 83)
ADHD Index	440	53.8 (9.8)	52.0	(40, 86)
DSM IV Inattention	438	52.8 (9.3)	51.5	(40, 84)
DSM IV Hyperactivity-Impulsivity	428	56.4 (9.3)	54.0	(41, 81)
DSM IV Total	435	54.9 (9.3)	53.0	(40, 81)
CPT-II Scores	439			
Omission Errors	431	51.8 (8.7)	49.3	(38.5, 83.7)
Commission Errors	422	51.6 (8.2)	52.7	(31.7, 67.6)
Hit Reaction Time	433	49.7 (10.0)	50.0	(21.6, 76.7)

Linear Models

Concurrent Mercury Exposure Associations

Concurrent exposure was not significantly associated with CPT-II or CRS-R outcome measures. In Figure 1.1, the top series depicts the results of models where outcome measures were log-transformed. Thus, a 10% higher blood Hg concentration corresponds to a 0.14 unit higher (95% CI: -0.09, 0.37) CPT-II Omissions score (calculated as $0.14 = \left(e^{0.015*\ln(1.10)} - 1\right) * 100$). The same increase in hair Hg concentration corresponded to a 0.06 unit higher (95% CI: -0.12, 0.25) score for that outcome, while a 10% higher urine Hg concentration was related to a 0.13 unit higher (95% CI: -0.01, 0.28) score. This indicates higher Hg levels tended to show higher likelihood to fail to respond to targets during the CPT-II, although none of these were statistically significant. Among this group of models (Figure 1.1), higher exposures generally corresponded to increasing scores. Increasing scores represent greater attention deficit symptoms. A notable exception to this was CPT-II commission scores, where a 10% higher blood or hair Hg concentration was related to a 0.16 unit lower (95% CI: -0.47, 0.16) or a 0.10 unit lower (95% CI: -0.33, 0.12) score, respectively. Similarly, a 10% higher blood or urine Hg concentration was related to a 0.12 unit lower (95% CI: -0.38, 0.14) or a 0.04 unit lower (95% CI: -0.21, 0.13) CRS-R Hyperactivity score (Figure 1.1).

Additionally, the lower series in Figure 1.1 depicts the models where log transformation was not necessary. For example, a 10% higher blood Hg concentration corresponded to a 0.01 unit higher (95% CI: -0.15, 0.17) DSM-IV Hyperactivity/Impulsivity score (calculated as $0.01 = 0.094 * \ln(1.10)$). The same increase in hair Hg concentration corresponded to a 0.07 unit higher (95% CI: -0.06, 0.19) score for that outcome, while a 10% higher urine Hg concentration corresponded to a 0.03 unit higher (95% CI: -0.07, 0.12) score. Among this group

of models, higher exposures generally corresponded to increasing scores, which represent greater attention deficit symptoms (Figure 1.1).

Prenatal Mercury Exposure Associations

Prenatal exposure was not significantly associated with CPT-II or CRS-R outcome measures. In Figure 1.2, as with Figure 1.1, the top series depicts the results of models where outcome measures were log-transformed. Thus, a 10% higher trimester 1 blood Hg concentration corresponded to a 0.24 unit higher (95% CI: -0.41, 0.89) CRS-R Distractibility score. The same increase in trimester 2 blood Hg concentration corresponded to a 0.34 unit higher (95% CI: -0.16, 0.85) score for that outcome, while a 10% higher trimester 3 Hg concentration corresponded to a 0.10 unit higher (95% CI: -0.37, 0.57) score. This indicates participants with higher Hg levels tended to have slightly higher distractibility symptoms, although none of these were statistically significant. The magnitude of these relationships was largest for trimester 2 exposure. This pattern was also observed for CPT-II omissions, while CRS-R Hyperactivity, ADHD and DSM-IV Inattention scores had the greatest unit increase for a 10% higher exposure in trimester 1. The magnitude of these relationships was progressively lower in trimesters 2 and 3. However, for CPT-II commission scores, a 10% higher trimester 1 blood Hg concentration corresponded to a 0.02 unit decrease (95% CI: -0.58, 0.57) in that score. For the same increase in trimester 2 blood Hg concentration, this was a 0.34 unit lower (95% CI: -0.86, 0.19) score, while that increase in trimester 3 Hg concentration corresponded to a 0.13 unit lower (95% CI: -0.60, 0.36) score (Figure 1.2).

Again, the lower series in Figure 1.2 depicts the models where log transformation was not necessary. Here, a 10% higher trimester 1 blood Hg concentration corresponded to a 0.22 unit higher (95% CI: -0.15, 0.58) DSM-IV Hyperactivity/Impulsivity score. The same increase in

trimester 2 blood Hg concentration corresponded to a 0.15 unit higher (95% CI: -0.16, 0.45) score for that outcome, while a 10% higher trimester 3 Hg concentration was related to a 0.08 unit higher (95% CI: -0.23, 0.38) score. This indicates participants with higher Hg levels tended to show higher levels of distractibility symptoms, with the largest magnitude of difference observed in trimester 1 and then progressively smaller magnitudes in trimesters 2 and 3. However, once again none of these were statistically significant. This pattern was also observed for DSM-IV Total scores. For CPT-II hit reaction time, however, a 10% higher trimester 1 blood Hg concentration corresponded to a 0.29 unit decrease (95% CI: -0.64, 0.06) in that score. For the same increase in trimester 2 blood Hg concentration and trimester 3 Hg concentration, there was a 0.05 unit higher (95% CI: -0.23, 0.32) and a 0.12 unit higher (95% CI: -0.13, 0.36) score, respectively (Figure 1.2).

When associations with cord blood Hg concentrations were considered, the patterns observed across the trimesters for CPT-II commissions, CRS-R Distractibility scores, ADHD Index, DSM-IV Inattention score, CPT-II Hit Reaction Time, DSM-IV Hyperactivity/Impulsivity score, and DSM-IV Total score were continued. However, for CPT-II Omissions score a 10% higher cord blood Hg concentration corresponded to a 0.26 unit higher (95% CI: -0.35, 0.88) score, a greater magnitude than the observed peak at trimester 2. For CRS-R Hyperactivity score, a 10% higher cord blood Hg concentration was associated with a 0.17 unit higher (95% CI: -0.51, 0.84) score, a reversal of the observed pattern across the trimesters. However, once again, none of these were statistically significant (Figure 1.3).

Concurrent Lead-Mercury Interactions

In models that consider potential interactions between Hg and Pb, a negative relationship was generally observed, although this was not statistically significant (Figure 1.4). In several

cases, although higher Hg and Pb concentrations were individually related to higher attention scores while the other metal is held constant at the geometric mean, higher levels of both exposures together were associated with lower scores. For example, a 10% higher Hg concentration while Pb is held constant at the geometric mean corresponded to a 0.03 unit higher (95% CI: -0.35, 0.42) hyperactivity score, while the same increase in Pb while Hg is held constant corresponded to a 0.21 unit higher (95% CI: -0.35, 0.42) score. However, a 10% higher concentration of both metals corresponded to a 0.08 unit lower (95% CI: -0.78, 0.61) score. A similar relationship was observed for ADHD index, DSM-IV inattention, and DSM-IV total symptoms.

Additionally, for distractibility and commission errors, higher levels of one metal corresponded to higher scores while the other is held constant, while higher levels of the other corresponded to lower scores, but higher levels of both corresponded to an even further lower score of interest. For example, a 10% higher Hg concentration while Pb concentration is held constant at the geometric mean corresponded to a 0.21 unit higher (95% CI: -0.22, 0.65) distractibility score, while the same increase in Pb while Hg is held constant was related to a 0.15 unit decrease (95% CI: -0.65, 0.35). A 10% higher level of both metals the corresponded to a further 0.20 unit lower (95% CI: -0.96, 0.57) score. None of these associations were statistically significant (Figure 1.4).

However, for three of the measures, higher levels of both metals corresponded to higher attention scores. For example, a 10% higher Hg concentration while Pb is held constant corresponded to a 0.28 unit higher (95% CI: -0.09, 0.65) omission error score, while the same increase in Pb concentration while Hg is held constant corresponded to a 0.14 unit higher (95% CI: -0.28, 0.56) score. A 10% higher level of both metals corresponded to a further 0.09 unit

lower (95% CI: -0.54, 0.74) score. The same pattern was observed for hit reaction time. For DSM-IV hyperactivity-impulsivity, a 10% higher Hg levels while Pb is held constant corresponded to a 0.09 unit higher (95% CI: -0.14, 0.33) score, while the same increase in Pb levels corresponded to a 0.01 unit lower (95% CI: -0.28, 0.26) score. However, a 10% higher level of both metals then corresponded to a 0.06 unit higher (95% CI: -0.36, 0.47) score. Again, none of these associations were statistically significant (Figure 1.4).

Prenatal Lead-Mercury Interactions

When examining potential interactions of prenatal exposure to these metals, in general, an increasing relationship was seen for the interactions in trimester 1, a decreasing relationship was seen in trimester 2, and no pattern was seen in trimester 3 (Figure 1.5). In trimester 1, among the CRS-R outcomes, higher levels of individual concentrations of both metals, while the other is held constant at the geometric mean, consistently correspond to higher scores, while higher levels of both metals corresponded to an even higher score. Two of these were statistically significant and several others had a p-value less than 0.10. For example, a 10% higher Hg concentration while Pb is held constant corresponded to a 0.38 unit higher (95% CI: -0.02, 0.79) DSM-IV total symptoms and a 10% higher Pb concentration while Hg is held constant corresponded to a 0.03 unit higher (95% CI: -0.54, 0.60) score. The same increase in both metals then corresponded to a further 1.28 unit higher (95% CI: 0.11, 2.44) score. Notably, the increase in the interaction term is often much larger in magnitude than that for the individual metals. This could potentially indicate a synergistic relationship, but due to the design of this study this cannot be determined conclusively.

Among the CPT outcomes, there was not a single pattern, but a similar relationship with exposure to both metals was observed. For omission errors, a 10% higher Hg concentration while

Pb is held constant corresponded to a 0.01 unit higher (95% CI: -0.56, 0.59), while a 10% higher Pb concentration while Hg is held constant corresponded to a 0.56 unit lower (95% CI: -1.36, 0.21) score. A 10% higher level of both then corresponded to an additional 0.19 unit higher (95% CI: -1.44, 1.85) omission errors. A similar pattern was observed for commission errors, although the direction of the relationships for higher individual levels of Hg and Pb was reversed. In the case of hit reaction time, a 10% higher Hg concentration while Pb was held constant corresponded to a 0.28 unit lower (95% CI: -0.65, 0.09) score and a 10% higher Pb concentration while Hg was held constant corresponded to a 0.49 unit lower (95% CI: -1.00, 0.02). However, a 10% higher level of both then corresponded to a 0.27 unit higher (95% CI: -0.79, 1.32) hit reaction time score. None of these, however, were statistically significant (Figure 1.5).

In the second trimester, a similarly consistent pattern was observed, but in the opposite direction as trimester 1 for lead and the interaction term. For example, a 10% higher level of Hg while Pb is held constant corresponded to a 0.09 unit higher (95% CI: -0.32, 0.49) DSM-IV hyperactivity-impulsivity score, while a 10% higher level of Pb while Hg is held constant corresponded to a 0.24 unit lower (95% CI: -0.73, 0.24) score. A 10% higher level of both exposures then corresponded to an additionally 0.85 unit lower (95% CI: -1.64, -0.05) score. A similar relationship was observed for distractibility, hyperactivity, DSM-IV inattention, DSM-IV total symptom score, omission errors, and hit reaction time. For ADHD index, a higher level of Hg or Pb individually corresponded to a lower score, while higher level of both corresponds to an additionally lower score. The interaction associations for all of the CRS-R outcomes were at least marginally statistically significant, but none of the CPT outcomes were (Figure 1.5).

In the third trimester, as with the first trimester, higher levels of the individual metals were generally associated with higher attention scores, but the overall pattern was less clear cut.

For example, a 10% higher level of Hg while Pb was held constant corresponded to a 0.60 unit lower (95% CI: -1.18, -0.02) score and a 10% higher level of Pb while Hg was held constant corresponded to a 0.32 unit lower (95% CI: -1.11, 0.46) hyperactivity score. However, a 10% higher level of both exposures then corresponded to a 0.20 unit higher (95% CI: -0.98, 1.40) score. A similar pattern was observed for ADHD index, DSM-IV inattention, DSM-IV hyperactivity-impulsivity, DSM-IV total symptoms, and omission errors. For distractibility, a higher level of Hg corresponded to lower scores, while higher levels of Pb corresponded to higher scores. Higher levels of Hg and Pb then corresponded to an additionally higher score. However, one of the associations for interactions was statistically significant. Additionally, higher individual levels of each metal corresponded to higher hit reaction time scores. An increase in both then corresponded to an additionally higher score (Figure 1.5).

The major exception to this pattern was for commission errors. There, a 10% higher level of Hg while Pb was held constant corresponded to a 0.03 unit lower (95% CI: -0.56, 0.51) score and a 10% higher level of Pb while Hg was held constant corresponded to a 0.48 unit lower (95% CI: -1.21, 0.25) commission error score. A 10% higher level of both metals was then corresponded to a further 0.15 unit lower (95% CI: -1.26, 0.97) score. (Figure 1.5)

Prenatal Mercury-Concurrent Lead Interactions

Several models were constructed to model potential interactions between prenatal Hg exposure and concurrent Pb exposure (Figure 1.6). However, in these models, there was not as consistent of a pattern as what was seen in the prenatal exposure interactions. In trimester 1, a higher level of Hg and Pb levels alone corresponded to higher scores, while a higher level of both corresponded to lower scores for several of the hyperactivity related CRS-R outcomes (hyperactivity, ADHD index, DSM-IV hyperactivity-impulsivity, and DSM-IV total symptoms).

In the second trimester, higher Hg levels corresponded to lower attention scores, higher Pb levels corresponded to higher attention scores, and higher levels of both corresponded to an additionally higher score for all three of the DSM-IV symptom scales and hit reaction time. Also, across all three trimesters, individual increases in Hg and Pb exposures were related to higher omission error scores, and an increase in both was related to an additional increase in that score. However, none of these associations were statistically significant.

Discussion

The aim of the current study was to characterize the association between prenatal (Trimesters 1, 2, 3 and delivery) and postnatal (ages 6-12 years) mercury exposures and CPT-II and CRS-R scores, and in general we found no significant relationships. In our cohort, we found that concurrent exposure to Hg, as measured in blood, hair, and urine, generally corresponded to higher CRS-R and CPT-II scores, although this relationship was not significant (Figure 1.1). Prenatal Hg exposure typically corresponded to higher CRS-R and CPT-II scores, although these relationships were not statistically significant (Figure 1.2). A negative but not statistically significant relationship was seen for postnatal MeHg and Pb interactions.

In our cohort, girls were more likely to have greater attention deficit symptoms in all but one measure. This was significant for measures related to inattention symptoms: distractibility, ADHD index, DSM-IV inattention and total symptoms, and CPT-II hit reaction time. They also had significantly fewer errors of commission on the CPT-II, which is a measure related to impulsivity. Although it was thought that ADHD predominantly affected boys, more recent research suggests that boys are more likely to be referred for treatment (Bruchmüller et al. 2012; Biederman et al. 2005). Other studies have suggested that girls with ADHD inattention symptoms have greater internalizing symptoms and perceived peer deviation (Becker et al. 2013;

Cardoos et al. 2012), which could have a compounding effect on symptoms over time. The finding that girls had less impulsivity on the CPT replicates previous work (Hasson and Fine 2012).

Additionally, maternal age, maternal education, and marital status were consistently associated with lower attention deficit symptoms. The association with maternal age was consistent with previous work, where the offspring of younger mothers had the greatest risk for ADHD later in life (Chang et al. 2014; Sagiv et al. 2013). Previous work has also reported that low maternal education may be a risk factor for attention deficits (Sagiv et al. 2013; Gurevitz et al. 2014), as was marital status (Sagiv et al. 2013).

Our cohort represents an intermediate exposure level compared to many existing studies that included postnatal exposure (Basu et al. 2014). This is particularly true of blood and urine, where our exposure levels were higher than those in several other studies, although our population's exposure was still low compared to populations like that in the Faroe Islands and the Seychelles, where exposure is substantially higher (Figure 1.1.D). For studies using hair as a biomarker, we represent a comparatively low exposure group to several of the existing studies, which again include the Faroe Islands and the Seychelles (Figure 1.1.D). Notably, exposure is generally higher in the Seychelles than the Faroe Islands, but the deficits consistently observed in the Faroe Islands, but not in the Seychelles (Karagas et al. 2012; Myers et al. 2003; Debes et al. 2006). Sources of MeHg exposure and resulting nutritional differences related to the consumption of fish have been suggested as a possible reason for this (Davidson et al. 2008; Stokes-Riner et al. 2011).

We represent a much lower level of exposure than other studies which have previously used cord blood to assess prenatal exposure. Venous blood levels during the trimesters are not

shown on this comparison, because to our knowledge, we are the first study to examine exposure during each trimester using maternal venous blood Hg concentrations. Previous studies have also used maternal hair Hg levels collected near delivery. Depending on the length of hair used, this can represent a longer period of pregnancy, but often represents exposure during the third trimester (Grandjean et al. 2005; Sakamoto et al. 2007).

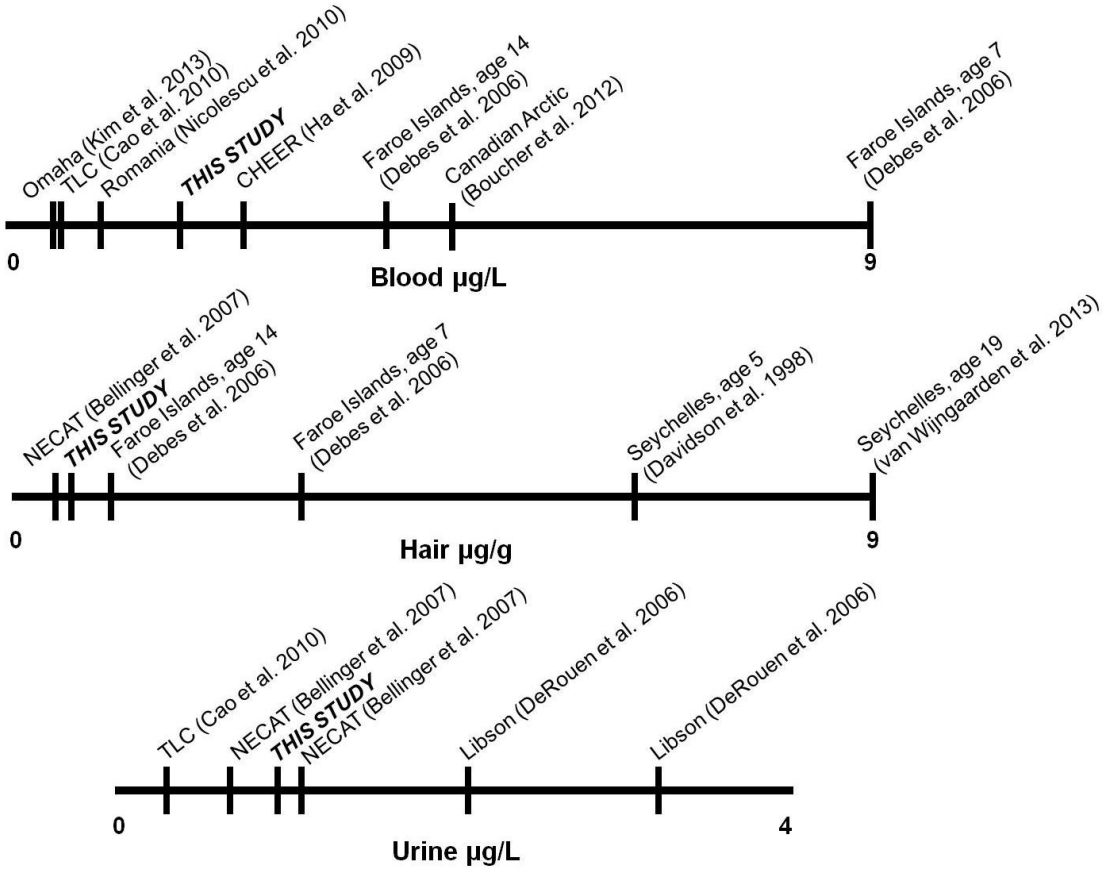

Figure 1.1.D Relative exposure levels of a selection of existing studies of postnatal exposure and ADHD symptoms (Ha et al. 2009; Nicolescu et al. 2010; van Wijngaarden et al. 2013; Boucher et al. 2012a; Debes et al. 2006; Bellinger et al. 2007; DeRouen et al. 2006; Kim et al. 2013; Cao et al. 2010; Davidson et al. 1998)

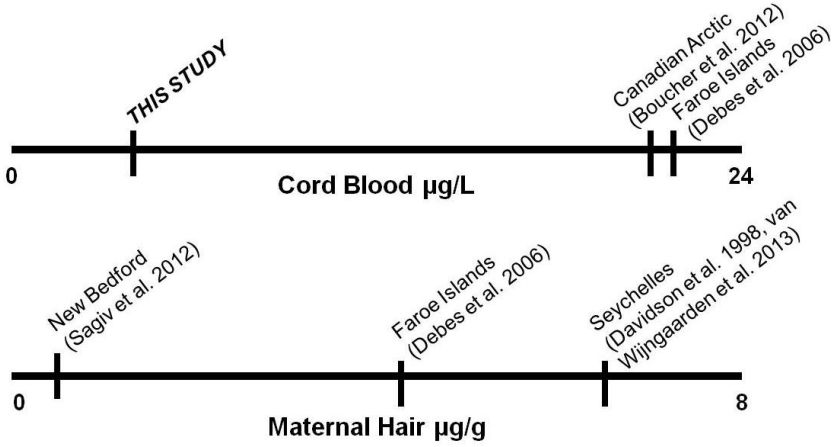

Figure 1.2.D Relative exposure levels of a selection of existing studies of prenatal exposure and ADHD symptoms (van Wijngaarden et al. 2013; Boucher et al. 2012a; Debes et al. 2006; Davidson et al. 1998; Sagiv et al. 2012)

A few previous studies have examined postnatal Hg exposure and various attention measures. In a cohort of Faroese children at age 14 years with exposure levels 1.5-2.0 times that observed in our cohort, postnatal exposure measured in blood and hair was found to only be weakly related to increasing levels of attention deficits. Similarly to our study, they also observed a number of associations in the opposite direction of what was anticipated (Debes et al. 2006). Of their battery of tests, which included a continuous performance test, a significant association was only observed for one test (the NES2 finger tapping test) and hair Hg, although this was no longer significant and smaller in magnitude after adjustment with covariates (Debes et al. 2006).

A few previous studies have examined associations between inorganic Hg and neurobehavioral outcomes, but have failed to find a significant difference based on exposure (Bellinger et al. 2007; DeRouen et al. 2006). The New England Children's Amalgam Trial compared urinary Hg levels and a battery of neuropsychological tests in two groups of 6-10 year old children. They found that there was no difference in test performance between their two study groups. The mean urinary Hg level in our study was intermediate to the mean levels of

each of their study groups (Bellinger et al. 2007). Another randomized control trial looking at dental amalgams in 8-10 year old children in Lisbon also examined urinary Hg and neurobehavioral outcomes over a series of several years. Mean urinary Hg levels were approximately 1.5-4.0 times those in our population. Although there were observable differences in the urinary Hg in their study groups, they too observed no statistically significant differences in attention between the groups (DeRouen et al. 2006). Our study found that urinary Hg levels generally corresponded to higher attention scores, but that this was not statistically significant. One association, between urinary Hg and omission errors approached but did not meet statistical significance ($p=0.076$).

The greatest magnitude of increase in those scores was generally observed in Trimester 1 (hyperactivity, ADHD index, all three DSM-IV symptom scales) and Trimester 2 (distractibility and omission errors). (Figure 1.2) Previous studies have assessed prenatal Hg exposure via total Hg concentrations in maternal hair (van Wijngaarden et al. 2013; Sagiv et al. 2012; Oken et al. 2005) or cord blood (Grandjean et al. 1999). Studies using hair to assess exposure during pregnancy generally either took a long length of hair cut to correspond to each trimester (Cernichiari et al. 1995) or used the few centimeters closed to the scalp, representing exposure in the final trimester and at delivery (Oken et al. 2005). Here, we use whole blood collected from participating mothers during each trimester of pregnancy, in addition to cord blood.

The Faroe Islands studies of cognition at 7 and 14 years examined associations with cord blood exposure and found that increasing cord blood Hg was associated with increasing CPT hit reaction time. Mean cord blood exposure in this population was approximately 5 times that observed in our cohort (Grandjean et al. 1997; Debes et al. 2006). We also observed such a relationship, although it was not statistically significant (Figure 1.2, 1.3). Sagiv et al. examined a

cohort of children and mothers from New Bedford, Massachusetts. They found that Hg concentrations in maternal hair collected postpartum were associated with ADHD-related behaviors, as measured using CRS-R and a CPT. In particular, they saw higher risk with higher Hg exposure and DSM-IV inattention and hyperactivity/impulsivity scores (Sagiv et al. 2012). Our results for DSM-IV hyperactivity/impulsivity score at trimester 3 were consistent, although not statistically significant, with their findings, although our results for cord blood and that outcome were not consistent. Our results for DSM-IV inattention were not consistent with their findings (Figure 1.2, 1.3). However, they did also find some non-linearity and a protective effect of maternal fish consumption during pregnancy (Sagiv et al. 2012). The Seychelles Child Development Study, which follows mothers and children similarly to our study, has previously reported no adverse effects with prenatal MeHg exposure (van Wijngaarden et al. 2013; Davidson et al. 2008). Additionally, because we did not examine maternal fish intake or related nutritional factors at this time, any deficits in relation to MeHg exposure could be lessened via negative confounding. (Davidson et al. 2008; Stokes-Riner et al. 2011).

Most studies of concurrent Hg exposure, as measured in blood or hair, have found no statistically significant associations between exposure and attention related outcomes, while observing associations between lead exposure and outcome measures (Ha et al. 2009; Nicolescu et al. 2010; Kim et al. 2013). In a case-control study in Omaha, NE, Kim et al found that postnatal Pb, where our levels are 1.5-3.0 times those in their population, was associated with case status, but Hg, where our levels were 4.5 times those in their population, was not. They did not directly examine interaction terms, but stratified by location within a "lead investigation area" (Kim et al. 2013). Both Ha et al. and Nicolescu et al. used a methodology similar to what

we used here and also found that lead, but not Hg levels were associated with ADHD related symptoms (Ha et al. 2009; Nicolescu et al. 2010).

While this study did not examine lead individually in models, but rather as a covariate in our Hg-Pb interaction models, a previous study in our group found that low levels of concurrent blood lead (<5 µg/dL) was associated with increased hyperactivity and impulsivity behaviors. This paper used a slightly different methodology than we did here. The associations they observed were non-linear and only present at certain exposure levels and were not present in linear models (Huang et al. 2015). Thus, our results are generally consistent with the existing understanding of concurrent metals exposure and attention deficits. Additional analyses with our data that incorporates lead as a binary covariate, using 5 µg/dL, the level at which associations were previously observed in our population, as a cut point, may yield results like those observed by Huang et al.

We also examined potential interactions between Hg exposure and Pb exposure in our cohort. Concurrent exposures to both metals generally corresponded to inhibitory or antagonistic interactions, wherein one or both metals individually corresponded to higher attention deficit scores, but higher exposure to both corresponded to lower attention deficit scores. However, none of these relationships were statistically significant (Figure 1.4). Prenatal exposures to both metals, though, did have a strong pattern of associations, particularly in relation to CRS-R outcomes (Figure 1.5). In Trimester 1, increases in both metals were consistently related to higher attention scores. This was statistically significant for two of the CRS-R outcomes (hyperactivity and DSM-IV total symptoms) and marginally statistically significant for two others (distractibility and ADHD index). In Trimester 2, increases in one or both metals corresponded with lower scores. These were statistically significant for four of the CRS-R

outcomes (ADHD index and the three DSM-IV symptom scales) and marginally significant for two others (distractibility and hyperactivity). Trimester 3 was similar to the first trimester, but much less clear cut.

A few studies have examined interactions between lead and mercury, generally using concurrent and cord blood levels. A study by Boucher et al found associations between cognitive deficits and both concurrent lead and prenatal lead, as measured in cord blood, but no association with MeHg at either of those respective time points. However, they did observe that associations between behavioral outcomes were more pronounced for concurrent Pb and lower levels of concurrent MeHg and for cord Pb and higher levels of cord MeHg (Boucher et al. 2012b). Another study suggested that the interaction of prenatal lead and methylmercury, as measured in cord blood, was sub-additive (Yorifuji et al. 2011).

An additional study by Boucher et al examined ADHD-type behaviors in relation to prenatal MeHg exposure and postnatal Pb exposure. They found that MeHg was associated with greater teacher-reported ADHD behaviors and Pb was associated with greater hyperactivity and impulsivity (Boucher et al. 2012a). We examined prenatal MeHg, by trimester, and concurrent Pb levels (Figure 1.6). However, there were no consistently observed associations in those analyses.

There were several limitations to this study. Trimester blood samples and cord blood samples were only available for a small subset of the overall cohort, limiting the sample sizes in those analyses. We used observations from mother-child pairs where there was at least one prenatal exposure measure and attention measures at the follow-up visit. It is possible that this could lead to selection bias. Individuals may not have completed follow-up visits for many reasons, but it is possible that individuals with particularly high exposure or more severe deficits

did not participate. We used parent reports for the CRS-R, but not teacher reports. Previous studies examining correlation between parent-reported and teacher-reported ADHD symptoms have shown that there can be considerable variation between the two sources (Lavigne et al. 2012). Additionally, our procedure for outliers made sure that data was representative of the majority for the available data and was not unduly influenced by outliers, but this may have led to the exclusion of real, but extreme, values.

While our study yielded few significant results, it still adds to the existing body of knowledge regarding the potential role of MeHg in the development of ADHD. It supports the suggestion that childhood exposure likely plays little or no role in the development of the disorder. Additional analyses examining possible negative confounding due to fish consumption could help clarify the observed relationships, particularly those with prenatal exposure.

Figures

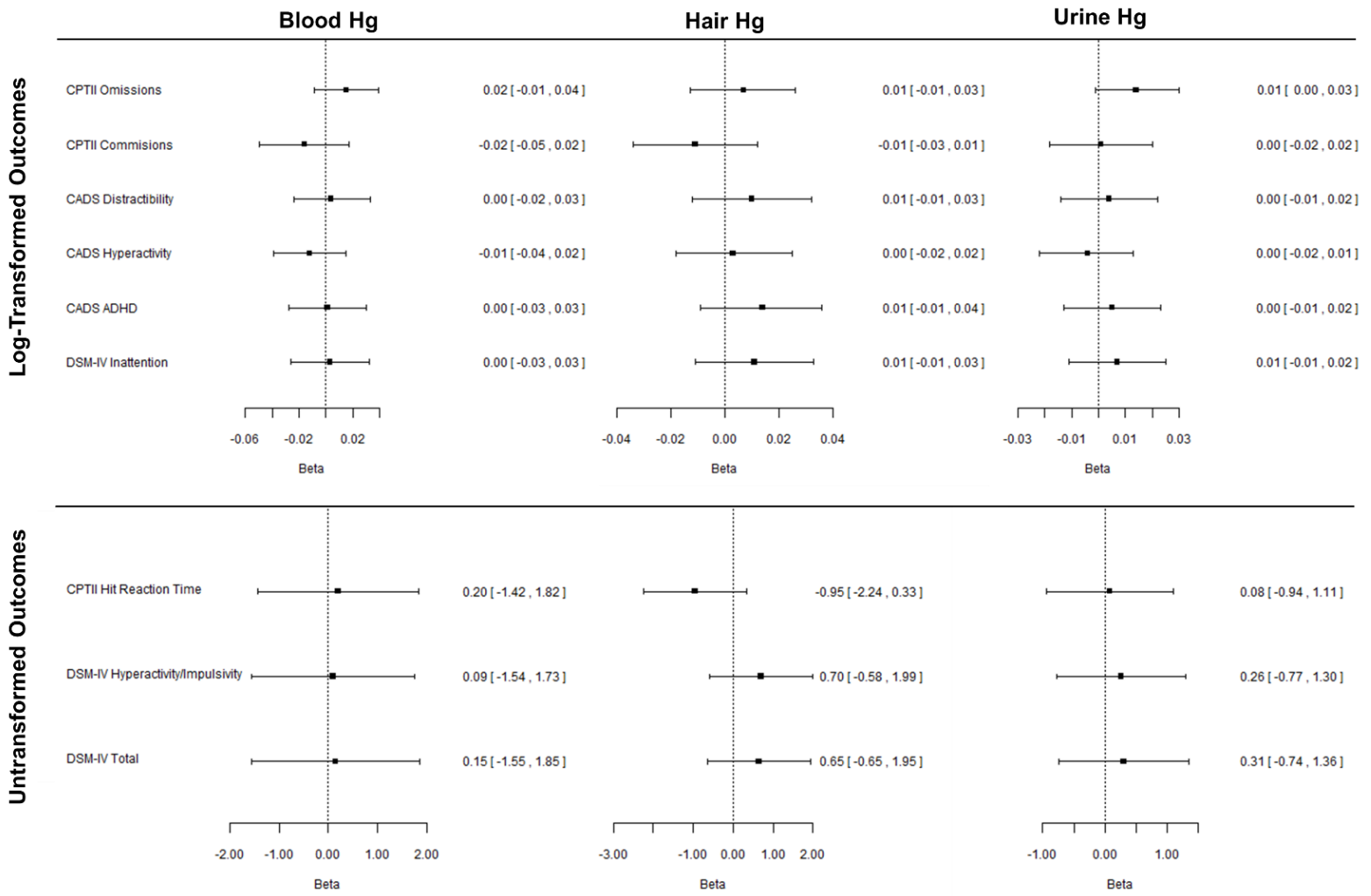

Figure 1.1 Results from linear regressions of CPT and CRS-R outcomes with exposures concurrent with the follow-up visit. In the top series, points and brackets represent beta coefficients and 95% confidence intervals for associations between the log-transformed CPT and CRS-R outcomes and log-transformed Hg concentration. In the bottom series, points and brackets represent beta coefficients and 95% confidence intervals for associations between the CPT and CRS-R outcome measures and log-transformed Hg concentration.

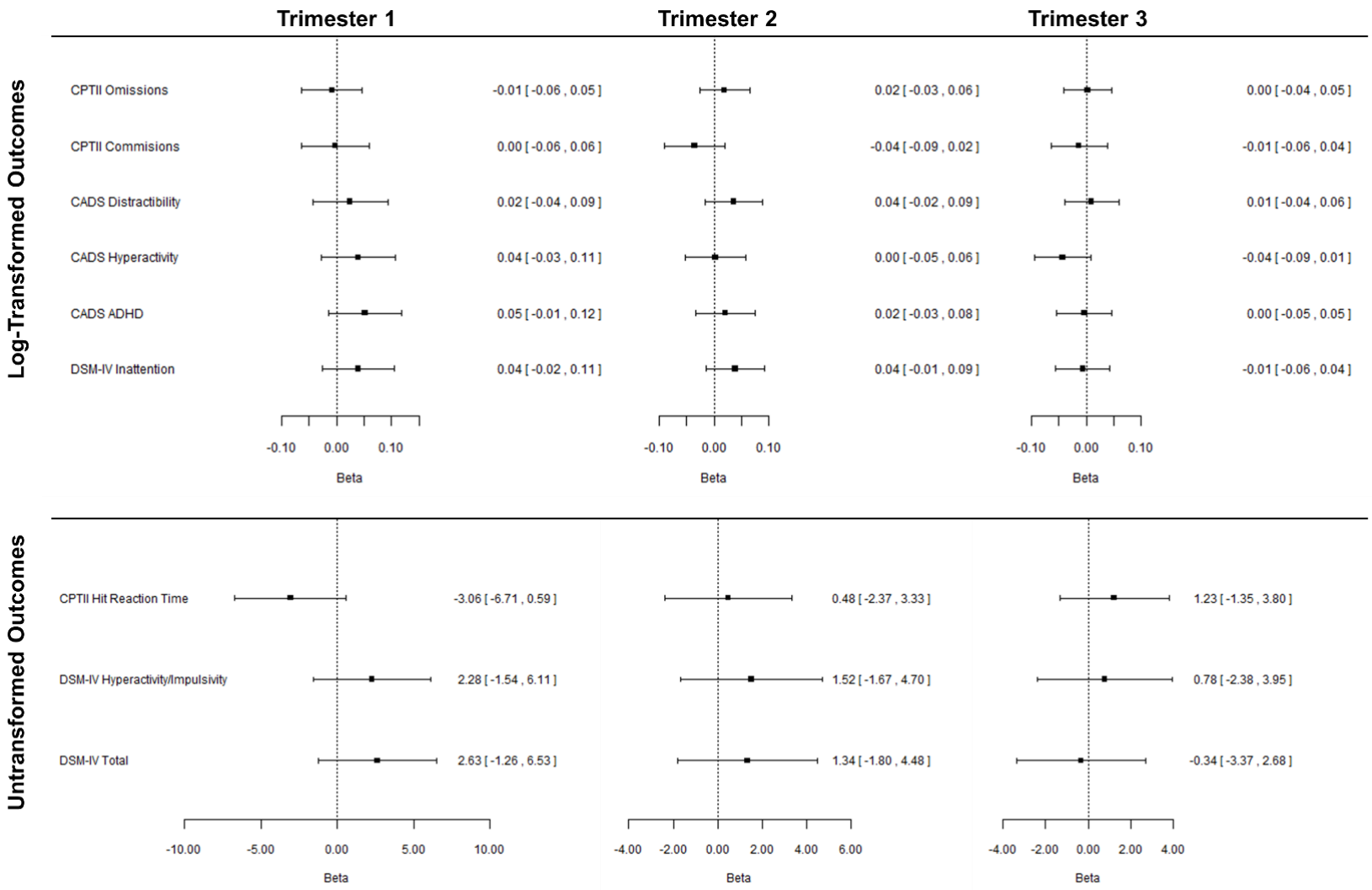

Figure 1.2 Results from linear regressions of CPT and CRS-R outcomes with prenatal exposures. In the top series, points and brackets represent beta coefficients and 95% confidence intervals for associations between the log-transformed CPT and CRS-R outcomes and log-transformed Hg concentration. In the bottom series, points and brackets represent beta coefficients and 95% confidence intervals for associations between the CPT and CRS-R outcome measures and log-transformed Hg concentration.

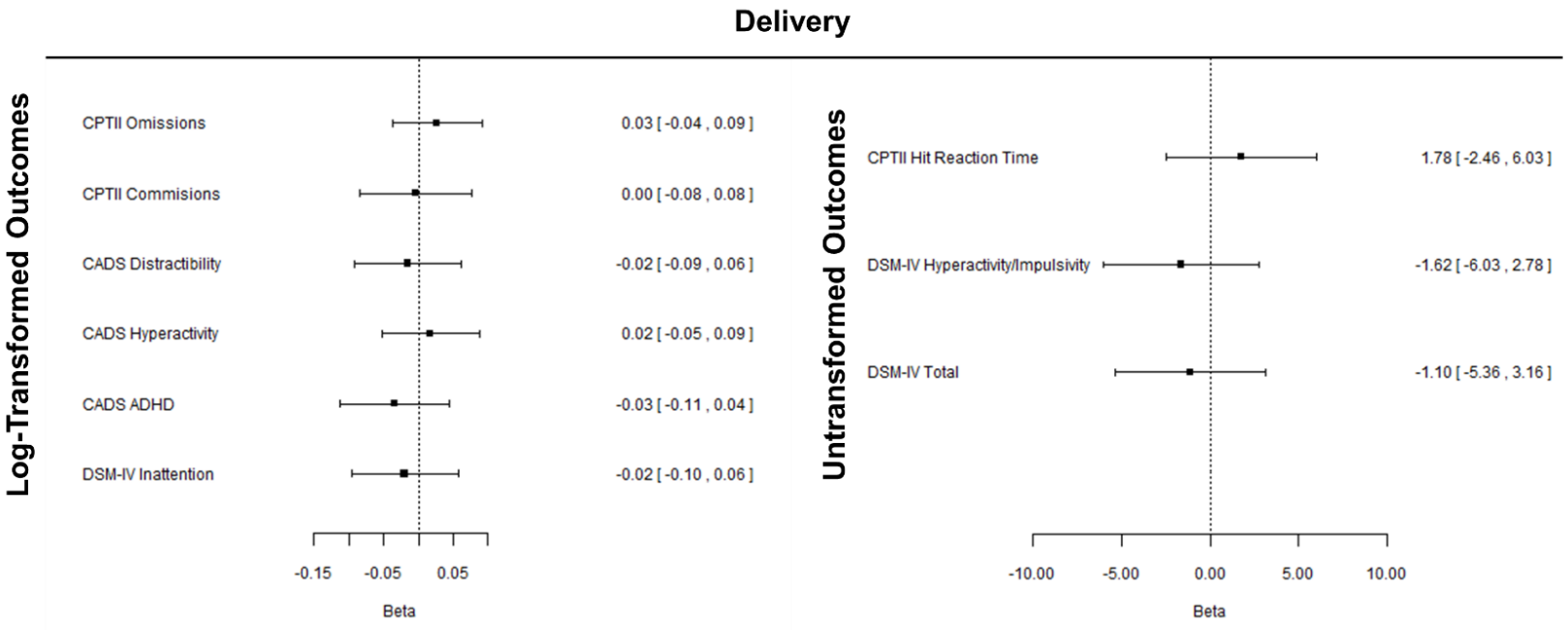

Figure 1.3 Results from linear regressions of CPT and CRS-R outcomes with cord blood mercury level. In the right column, points and brackets represent beta coefficients and 95% confidence intervals for associations between the log-transformed CPT and CRS-R outcomes and log-transformed Hg concentration. In the left column, points and brackets represent beta coefficients and 95% confidence intervals for associations between the CPT and CRS-R outcome measures and log-transformed Hg concentration.

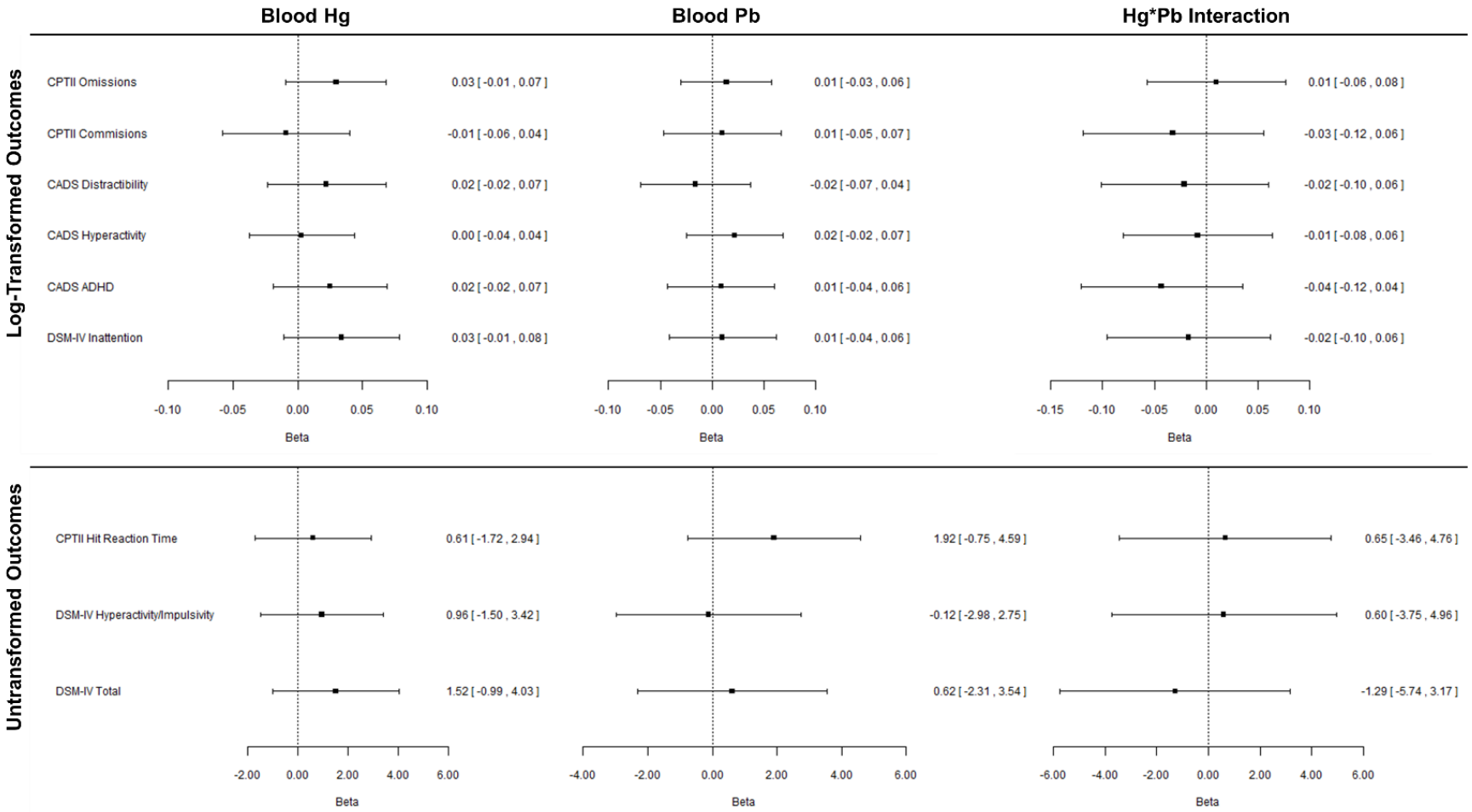

Figure 1.4 Results from interaction models of CPT and CRS-R outcomes with exposures concurrent with the follow-up visit. In the top series, points and brackets represent beta coefficients and 95% confidence intervals for associations between the log-transformed CPT and CRS-R outcomes and geometric mean of Hg exposure, geometric mean of Pb exposure, and the interaction between the two. In the bottom series, points and brackets represent beta coefficients and 95% confidence intervals for associations between the CPT and CRS-R outcome measures and geometric mean of Hg exposure, geometric mean of Pb exposure, and the interaction between the two.

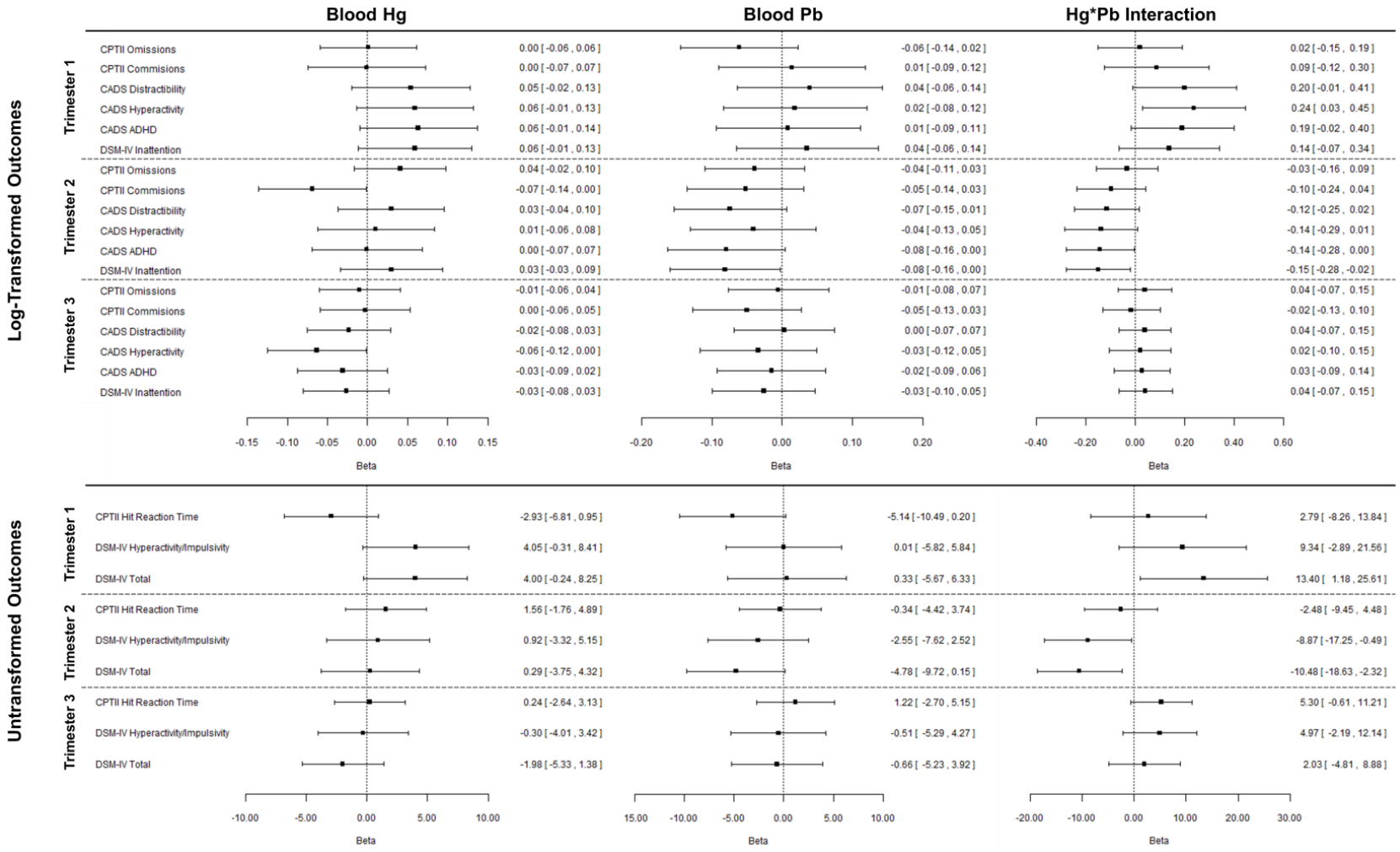

Figure 1.5 Results from interaction models of CPT and CRS-R outcomes with prenatal mercury and lead exposure. In the top series, points and brackets represent beta coefficients and 95% confidence intervals for associations between the log-transformed outcomes and geometric mean of Hg exposure, geometric mean of Pb exposure, and the interaction between the two. In the bottom series, points and brackets represent beta coefficients and 95% confidence intervals for associations between the CPT and CRS-R outcome measures and geometric mean of Hg exposure, geometric mean of Pb exposure, and the interaction between the two.

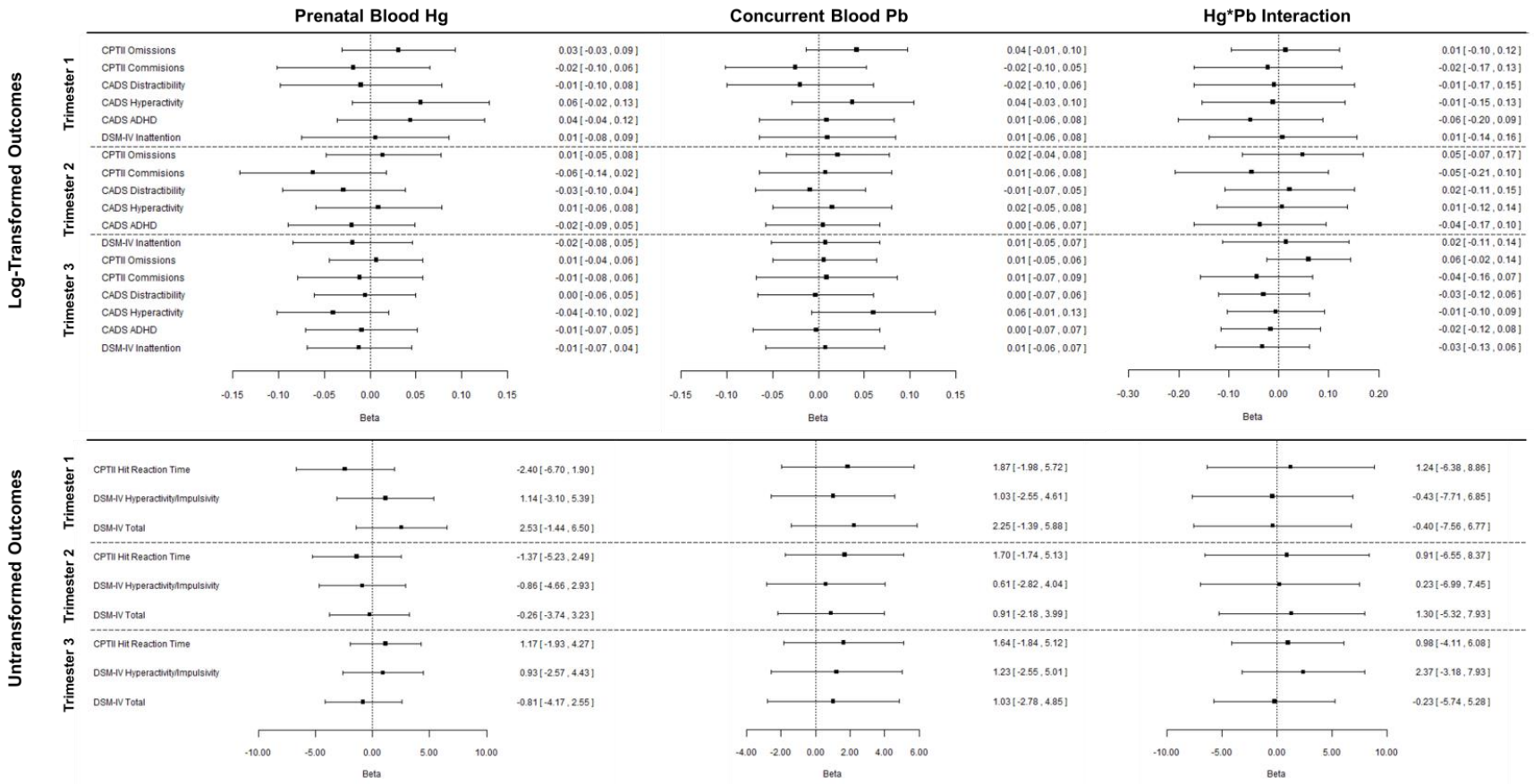

Figure1.6 Results from interaction models of CPT and CRS-R outcomes with prenatal mercury and concurrent lead exposure. In the top series, points and brackets represent beta coefficients and 95% confidence intervals for associations between the log-transformed CPT and CRS-R outcomes and geometric mean of prenatal Hg exposure, geometric mean of concurrent Pb exposure, and the interaction between the two. In the bottom series, points and brackets represent beta coefficients and 95% confidence intervals for associations between the CPT and CRS-R outcome measures and geometric mean of prenatal Hg exposure, geometric mean of concurrent Pb exposure, and the interaction between the two.

Appendix 1

Supplemental Tables

Table S1.1.1 Full models for CPT and CRS-R outcomes and concurrent blood Hg exposure. Darker shades of blue indicate larger positive betas, while darker shades of red indicate larger negative betas.

	Blood									
	log(Distractibility) (N=298)		log(Hyperactivity) (N=298)		log(ADHD) (N=298)		log(DSM Inattention) (N=298)		DSM Hyperactivty-Impulsivity (N=298)	
	Beta	P-value	Beta	P-value	Beta	P-value	Beta	P-value	Beta	P-value
Model		NS		0.044		NS		0.088		0.040
Intercept	4.105	<<0.001	3.992	<<0.001	4.011	<<0.001	3.908	<<0.001	54.85	<<0.001
log (Exposure)	0.004		-0.012		0.001		0.003		0.094	
Sex of Child: Female	0.057	0.004	0.012		0.043	0.036	0.053	0.010	1.716	
Age of Child at Visit	-0.002		0.011		0.003		0.013		0.675	
Cohort: 2a	0.028		0.001		-0.004		-0.009		-0.741	
Cohort: 3	0.002		0.014		-0.011		0.030		0.803	
SES Level	-2.7E-04		-0.006		-0.002		-7.5E-04		-0.296	
Maternal Age	-0.004	0.065	-0.003	0.069	-0.004	0.052	-0.005	0.018	-0.245	0.028
Maternal Education	-0.004		-0.011	0.006	-0.008	0.065	-0.005		-0.599	0.016
Maternal IQ	-2.8E-04		0.001	0.028	0.001	0.068	8.4E-04		0.096	0.010
Maternal Marital Status: Married	-0.015		0.020		8.8E-04		-0.010		0.931	

	DSM Total (N=298)		log(Omissions) (N=298)		log(Commisions) (N=300)		Hit Reaction Time (N=300)	
	Beta	P-value	Beta	P-value	Beta	P-value	Beta	P-value
Model		0.031		0.001		NS		<<0.001
Intercept	55.97	<<0.001	3.911	<<0.001	3.675	<<0.001	68.60	<<0.001
log (Exposure)	0.152		0.015		-0.016		0.197	
Sex of Child: Female	3.329	0.005	0.007		-0.049	0.038	2.659	0.020
Age of Child at Visit	0.446		0.004		0.013		-1.061	
Cohort: 2a	-0.253		-0.028		-0.026		1.684	
Cohort: 3	0.034		0.086	0.011	0.067		2.827	
SES Level	-0.261		0.002		0.005		-0.304	
Maternal Age	-0.244	0.035	-3.3E-04		-2.8E-04		-0.172	
Maternal Education	-0.481	0.063	-0.002		-0.006		0.231	
Maternal IQ	0.070	0.074	-4.1E-04		0.002	0.029	-0.066	0.073
Maternal Marital Status: Married	0.687		-0.004		0.018		-3.192	0.016

Table S1.1.2 Full models for CPT and CRS-R outcomes and concurrent hair Hg exposure. Darker shades of blue indicate larger positive betas, while darker shades of red indicate larger negative betas.

	Hair									
	log(Distractibility) (N=423)		log(Hyperactivity) (N=422)		log(ADHD) (N=426)		log(DSM Inattention) (N=425)		DSM Hyperactivty-Impulsivity (N=425)	
	Beta	P-value	Beta	P-value	Beta	P-value	Beta	P-value	Beta	P-value
Model		0.037		NS		NS		NS		NS
Intercept	4.100	<<0.001	4.064	<<0.001	4.119	<<0.001	4.007	<<0.001	60.67	<<0.001
log (Exposure)	0.010		0.003		0.014		0.011		0.702	
Sex of Child: Female	0.046	0.006	0.003		0.030	0.076	0.037	0.029	0.602	
Age of Child at Visit	-0.002		5.5E-04		-0.006		0.002		-0.050	
Cohort: 2a	0.006		0.006		-8.4E-04		-0.010		-0.154	
Cohort: 3	-0.018		-0.014		-0.041		-0.008		-1.815	
SES Level	9.6E-04		-0.004		2.0E-04		6.3E-04		-0.252	
Maternal Age	-1.9E-04		-3.3E-04		-8.3E-04		-6.7E-04		-0.039	
Maternal Education	-0.006		-0.012	0.002	-0.009	0.024	-0.006		-0.492	0.027
Maternal IQ	-4.6E-04		0.001	0.060	5.4E-04		2.5E-04		0.064	0.044
Maternal Marital Status: Married	-0.037	0.053	2.3E-04		-0.018		-0.026		-0.286	

	DSM Total (N=425)		log(Omissions) (N=416)		log(Commisions) (N=416)		Hit Reaction Time (N=425)	
	Beta	P-value	Beta	P-value	Beta	P-value	Beta	P-value
Model		0.073		<<0.001		NS		<<0.001
Intercept	61.71	<<0.001	4.091	<<0.001	3.777	<<0.001	70.77	<<0.001
log (Exposure)	0.646		0.007		-0.011		-0.953	
Sex of Child: Female	2.359	0.017	0.007		-0.036	0.043	2.107	0.030
Age of Child at Visit	-0.269		-0.011		0.006		-1.508	0.032
Cohort: 2a	0.149		-0.007		-3.8E-04		0.714	
Cohort: 3	-2.340		0.068	0.016	0.044		2.278	
SES Level	-0.135		8.8E-04		0.003		-0.245	
Maternal Age	-0.040		-6.6E-04		-7.2E-04		-0.092	
Maternal Education	-0.511	0.023	-0.002		-0.006		0.174	
Maternal IQ	0.048		-4.9E-04		0.001	0.013	-0.055	0.075
Maternal Marital Status: Married	-0.688		-0.011		0.023		-3.611	0.001

Table S1.1.3 Full models for CPT and CRS-R outcomes and concurrent urine Hg exposure. Darker shades of blue indicate larger positive betas, while darker shades of red indicate larger negative betas.

	Urine									
	log(Distractibility) (N=423)		log(Hyperactivity) (N=422)		log(ADHD) (N=426)		log(DSM Inattention) (N=425)		DSM Hyperactivty-Impulsivity (N=425)	
	Beta	P-value	Beta	P-value	Beta	P-value	Beta	P-value	Beta	P-value
Model		0.035		NS		NS		NS		NS
Intercept	4.093	<<0.001	4.046	<<0.001	4.082	<<0.001	3.976	<<0.001	58.46	<<0.001
log (Exposure)	0.004		-0.004		0.005		0.007		0.263	
Sex of Child: Female	0.046	0.006	0.003		0.031	0.068	0.037	0.030	0.738	
Age of Child at Visit	-7.7E-04		-1.3E-04		-0.004		0.005		0.013	
Cohort: 2a	0.001		0.004		-0.003		-0.012		-0.486	
Cohort: 3	-0.018		-0.019		-0.034		0.002		-1.683	
SES Level	0.001		-0.003		5.9E-04		7.4E-04		-0.233	
Maternal Age	-2.5E-04		-1.0E-04		-8.0E-04		-8.2E-04		-0.014	
Maternal Education	-0.005		-0.012	0.001	-0.009	0.026	-0.005		-0.504	0.021
Maternal IQ	-5.9E-04		0.001	0.015	5.8E-04		1.9E-04		0.072	0.022
Maternal Marital Status: Married	-0.041	0.034	-0.005		-0.023		-0.029		-0.447	

	DSM Total (N=425)		log(Omissions) (N=413)		log(Commisions) (N=414)		Hit Reaction Time (N=422)	
	Beta	P-value	Beta	P-value	Beta	P-value	Beta	P-value
Model		0.079		<<0.001		NS		<<0.001
Intercept	59.84	<<0.001	4.048	<<0.001	3.797	<<0.001	70.07	<<0.001
log (Exposure)	0.306		0.014	0.076	0.001		0.085	
Sex of Child: Female	2.420	0.015	0.012		-0.037	0.0384	1.817	0.060
Age of Child at Visit	-0.149		-0.007		0.005		-1.426	0.040
Cohort: 2a	-0.035		-0.010		-0.007		1.355	
Cohort: 3	-2.007		0.079	0.005	0.049		2.498	
SES Level	-0.121		7.5E-04		0.003		-0.285	
Maternal Age	-0.034		-3.3E-05		-5.7E-04		-0.069	
Maternal Education	-0.492	0.027	-0.001		-0.004		0.142	
Maternal IQ	0.049		-6.2E-04		0.001	0.0476	-0.047	
Maternal Marital Status: Married	-0.880		-0.011		0.025		-3.914	<0.001

Table S1.1.4 Full models for concurrent blood Hg and Pb exposure interactions. Darker shades of blue indicate larger positive betas, while darker shades of red indicate larger negative betas. "centhg" refers to centered, log-transformed mercury concentration and "centpb" refers to centered, log-transformed lead concentration.

	Concurrent Blood									
	log(Distractibility) (N=150)		log(Hyperactivity) (N=149)		log(ADHD) (N=151)		log(DSM Inattention) (N=151)		DSM Hyperactivty-Impulsivity (N=149)	
	Beta	P-value	Beta	P-value	Beta	P-value	Beta	P-value	Beta	P-value
Model		NS		NS		NS		NS		NS
Intercept	4.228	<<0.001	3.980	<<0.001	4.177	<<0.001	3.838	<<0.001	55.205	<0.001
centhg	0.022		0.003		0.025		0.034		0.959	
centpb	-0.016		0.022		0.009		0.010		-0.118	
Sex of Child: Female	0.077	0.016	0.026		0.058	0.060	0.073	0.019	2.259	
Age of Child at Visit	-0.011		0.016		-0.009		0.017		0.830	
Cohort: 3	-0.069		-0.014		-0.070		0.020		0.862	
SES Level	1.9E-04		-0.007		-0.001		0.001		-0.333	
Maternal Age	-0.003		-0.004		-0.004		-0.004		-0.266	0.080
Maternal Education	-0.002		-0.012	0.052	-0.009		-0.004		-0.850	0.022
Maternal IQ	-3.2E-04		0.002	0.054	0.001		0.001		0.116	0.037
Maternal Marital Status: Married	-0.030		-0.029		-0.027		-0.038		0.119	
centhg*centpb	-0.021		-0.008		-0.043		-0.017		0.601	

	DSM Total (N=152)		log(Omissions) (N=146)		log(Commisions) (N=150)		Hit Reaction Time (N=151)	
	Beta	P-value	Beta	P-value	Beta	P-value	Beta	P-value
Model		NS		0.070		NS		0.006
Intercept	59.885	<0.001	3.805	<<0.001	3.871	<<0.001	73.329	<<0.001
centhg	1.520		0.030		-0.009		0.611	
centpb	0.618		0.014		0.010		1.917	
Sex of Child: Female	4.033	0.022	0.009		-0.001		-0.683	
Age of Child at Visit	0.261		0.012		-0.005		-1.462	
Cohort: 3	-2.243		0.125	0.029	0.039		-0.199	
SES Level	-0.352		0.002		0.004		-0.417	
Maternal Age	-0.268	0.086	-0.001		-0.003		-0.013	
Maternal Education	-0.538		-0.006		-0.014	0.064	0.841	0.016
Maternal IQ	0.093		2.20E-04		0.003	0.025	-0.130	0.014
Maternal Marital Status: Married	-1.281		-3.50E-04		0.048		-4.948	0.006
centhg*centpb	-1.287		0.010		-0.032		0.652	

Table S1.2.1 Full models for CPT and CRS-R outcomes and first trimester blood Hg exposure. Darker shades of blue indicate larger positive betas, while darker shades of red indicate larger negative betas.

	Trimester 1									
	log(Distractibility) (N=118)		log(Hyperactivity) (N=118)		log(ADHD) (N=119)		log(DSM Inattention) (N=118)		DSM Hyperactivty-Impulsivity (N=116)	
	Beta	P-value	Beta	P-value	Beta	P-value	Beta	P-value	Beta	P-value
Model		NS		NS		NS		0.084		NS
Intercept	4.084	<<0.001	4.112	<<0.001	4.071	<<0.001	3.754	<<0.001	57.94	<0.001
log (Exposure)	0.025		0.040		0.052		0.040		2.284	
Sex of Child: Female	0.042		-0.003		0.031		0.024		0.317	
Age of Child at Visit	7.2E-04		-0.001		-7.6E-04		0.023		0.161	
Cohort: 3	-0.010		-0.033		-0.006		0.061		0.552	
SES Level	3.4E-04		-0.008		5.5E-05		-5.3E-04		-0.407	
Maternal Age	0.002		2.3E-04		-2.3E-05		0.001		0.013	
Maternal Education	-0.002		-0.017	0.063	-0.012		-0.008		-0.758	
Maternal IQ	-0.001		0.002		3.6E-04		4.3E-04		0.073	
Maternal Marital Status: Married	-0.087	0.037	-0.059		-0.087	0.030	-0.116	0.004	-2.118	

	DSM Total (N=120)		log(Omissions) (N=118)		log(Commisions) (N=113)		Hit Reaction Time (N=119)	
	Beta	P-value	Beta	P-value	Beta	P-value	Beta	P-value
Model		NS		0.001		NS		0.003
Intercept	66.32	<<0.001	4.110	<<0.001	4.041	<<0.001	80.12	<<0.001
log (Exposure)	2.632		-0.008		-0.002		-3.059	
Sex of Child: Female	1.886		0.043		-0.013		-0.348	
Age of Child at Visit	-0.426		-0.008		-0.015		-1.730	
Cohort: 3	-2.608		0.112	0.075	0.003		2.479	
SES Level	-0.420		-0.004		0.005		-0.338	
Maternal Age	0.058		-0.002		-0.001		-0.109	
Maternal Education	-0.782		0.004		-0.001		0.835	0.080
Maternal IQ	0.044		-0.001		4.2E-04		-0.149	0.030
Maternal Marital Status: Married	-5.054	0.033	-0.027		0.054		-3.181	

Table S1.2.2 Full models for CPT and CRS-R outcomes and second trimester blood Hg exposure. Darker shades of blue indicate larger positive betas, while darker shades of red indicate larger negative betas.

	Trimester 2									
	log(Distractibility) (N=163)		log(Hyperactivity) (N=163)		log(ADHD) (N=164)		log(DSM Inattention) (N=163)		DSM Hyperactivty-Impulsivity (N=163)	
	Beta	P-value	Beta	P-value	Beta	P-value	Beta	P-value	Beta	P-value
Model		NS		NS		NS		NS		NS
Intercept	3.886	<<0.001	3.996	<<0.001	3.942	<<0.001	3.667	<<0.001	48.36	0.001
log (Exposure)	0.036		0.003		0.021		0.039		1.518	
Sex of Child: Female	0.041		0.005		0.030		0.019		1.490	
Age of Child at Visit	0.008		0.008		0.003		0.025		0.904	
Cohort: 3	0.030		0.007		0.008		0.095		2.558	
SES Level	0.001		-0.003		0.004		0.001		-0.176	
Maternal Age	-0.001		-0.001		-0.003		-0.001		-0.178	
Maternal Education	0.003		-0.007		-0.003		-0.002		-0.189	
Maternal IQ	-8.4E-04		8.3E-04		7.3E-04		3.3E-04		0.063	
Maternal Marital Status: Married	-0.027		-0.041		-0.031		-0.045		-1.510	

	DSM Total (N=165)		log(Omissions) (N=159)		log(Commisions) (N=159)		Hit Reaction Time (N=163)	
	Beta	P-value	Beta	P-value	Beta	P-value	Beta	P-value
Model		NS		0.007		NS		0.033
Intercept	56.42	<0.001	4.058	<<0.001	3.962	<<0.001	62.38	<<0.001
log (Exposure)	1.340		0.019		-0.035		0.478	
Sex of Child: Female	1.786		0.007		-0.033		1.033	
Age of Child at Visit	0.200		-0.012		-0.007		-0.817	
Cohort: 3	0.108		0.088		0.031		2.350	
SES Level	-0.138		-0.002		-0.002		-0.441	
Maternal Age	-0.118		-0.001		1.1E-04		-0.056	
Maternal Education	-0.340		0.003		-0.005		0.625	0.089
Maternal IQ	0.041		-6.7E-04		0.001		-0.079	
Maternal Marital Status: Married	-2.197		0.007		0.035		-2.306	

Table S1.2.3 Full models for CPT and CRS-R outcomes and third trimester blood Hg exposure. Darker shades of blue indicate larger positive betas, while darker shades of red indicate larger negative betas.

	Trimester 3									
	log(Distractibility) (N=141)		log(Hyperactivity) (N=140)		log(ADHD) (N=142)		log(DSM Inattention) (N=141)		DSM Hyperactivty-Impulsivity (N=143)	
	Beta	P-value	Beta	P-value	Beta	P-value	Beta	P-value	Beta	P-value
Model		NS		NS		NS		0.045		NS
Intercept	3.716	<<0.001	3.762	<<0.001	3.770	<<0.001	3.399	<<0.001	48.38	0.004
log (Exposure)	0.010		-0.043		-0.003		-0.006		0.784	
Sex of Child: Female	0.074	0.019	0.019		0.054	0.093	0.049		1.980	
Age of Child at Visit	0.020		0.028		0.017		0.043	0.059	0.989	
Cohort: 3	0.032		0.043		0.011		0.116		1.440	
SES Level	0.005		0.002		0.006		0.008		-0.056	
Maternal Age	-8.8E-04		-0.002		-0.002		-9.0E-04		-0.175	
Maternal Education	-9.5E-04		-0.011		-0.011		-0.007		-0.579	
Maternal IQ	4.0E-04		0.002	0.049	0.002	0.091	0.002	0.076	0.108	
Maternal Marital Status: Married	-0.048		-0.068	0.070	-0.042		-0.069	0.055	-1.669	

	DSM Total (N=143)		log(Omissions) (N=140)		log(Commisions) (N=138)		Hit Reaction Time (N=142)	
	Beta	P-value	Beta	P-value	Beta	P-value	Beta	P-value
Model		NS		<<0.001		NS		0.004
Intercept	48.79	0.002	3.860	<<0.001	3.741	<<0.001	69.97	<<0.001
log (Exposure)	-0.344		0.003		-0.013		1.225	
Sex of Child: Female	3.276	0.087	0.015		9.3E-04		-1.085	
Age of Child at Visit	0.794		0.004		0.011		-1.873	
Cohort: 3	0.235		0.172	0.005	0.092		1.364	
SES Level	0.109		0.004		-0.002		-0.199	
Maternal Age	-0.108		0.003		3.4E-04		-0.061	
Maternal Education	-0.602		-0.002		-0.005		0.642	0.081
Maternal IQ	0.101		-0.001		9.8E-04		-0.062	
Maternal Marital Status: Married	-3.240		-0.005		0.034		-2.348	

Table S1.2.4 Full models for CPT and CRS-R outcomes and cord blood Hg exposure. Darker shades of blue indicate larger positive betas, while darker shades of red indicate larger negative betas.

	Delivery									
	log(Distractibility) (N=87)		log(Hyperactivity) (N=84)		log(ADHD) (N=87)		log(DSM Inattention) (N=87)		DSM Hyperactivty-Impulsivity (N=86)	
	Beta	P-value	Beta	P-value	Beta	P-value	Beta	P-value	Beta	P-value
Model		NS		NS		NS		NS		NS
Intercept	3.905	<<0.001	3.701	<<0.001	3.845	<<0.001	3.956	<<0.001	36.35	0.075
log (Exposure)	-0.015		0.017		-0.034		-0.020		-1.623	
Sex of Child: Female	0.053		-0.002		0.010		0.033		1.861	
Age of Child at Visit	0.019		0.052	0.075	0.021		0.014		3.238	0.073
Cohort: 2a	0.086		0.050		0.056		0.091		-1.258	
Cohort: 3	0.103		0.174	0.020	0.094		0.115		8.796	0.056
SES Level	0.002		-0.004		-0.001		0.002		-0.343	
Maternal Age	-0.004		-0.004		-0.002		-0.003		-0.159	
Maternal Education	0.007		-0.002		0.004		0.004		-0.190	
Maternal IQ	-0.002		-0.001		-6.3E-04		-0.002		-0.010	
Maternal Marital Status: Married	-0.044		-0.047		-0.036		-0.052		-0.556	

	DSM Total (N=87)		log(Omissions) (N=83)		log(Commisions) (N=80)		Hit Reaction Time (N=83)	
	Beta	P-value	Beta	P-value	Beta	P-value	Beta	P-value
Model		NS		NS		NS		NS
Intercept	40.67	0.035	3.325	<<0.001	2.886	<<0.001	55.33	0.005
log (Exposure)	-1.100		0.027		-0.004		1.785	
Sex of Child: Female	2.306		-0.027		-0.050		1.792	
Age of Child at Visit	2.308		0.026		0.079	0.0211	-1.028	
Cohort: 2a	2.486		0.014		-0.102		1.113	
Cohort: 3	8.293	0.061	0.137	0.051	0.123		4.139	
SES Level	-0.071		-0.003		-0.007		-0.131	
Maternal Age	-0.170		0.006	0.084	0.003		-0.170	
Maternal Education	-0.133		0.002		0.001		0.137	
Maternal IQ	-0.038		0.002		0.003	0.0818	0.025	
Maternal Marital Status: Married	-1.299		-0.008		0.018		1.063	

Table S1.3.1 Full models for first trimester Hg and Pb exposure interactions. Darker shades of blue indicate larger positive betas, while darker shades of red indicate larger negative betas. "centhg" refers to centered, log-transformed mercury concentration and "centpb" refers to centered, log-transformed lead concentration.

	Trimester 1									
	log(Distractibility) (N=88)		log(Hyperactivity) (N=88)		log(ADHD) (N=89)		log(DSM Inattention) (N=88)		DSM Hyperactivty-Impulsivity (N=86)	
	Beta	P-value	Beta	P-value	Beta	P-value	Beta	P-value	Beta	P-value
Model		NS		0.042		NS		0.066		NS
Intercept	4.108		4.185		4.179		3.667		64.891	0.001
centhg	0.054		0.059		0.063	0.094	0.059		4.052	0.073
centpb	0.039		0.018		0.008		0.035		0.010	
Sex of Child: Female	0.036		-0.033		0.022		0.018		0.752	
Age of Child at Visit	-0.005		-0.002		-0.006		0.026		-0.122	
Cohort: 3	-0.043		-0.069		-0.034		0.060		-1.757	
SES Level	0.006		0.002		0.011		0.009		0.091	
Maternal Age	0.003		-0.001		-3.3E-04		0.002		-0.119	
Maternal Education	-0.005		-0.030	0.004	-0.020	0.052	-0.009		-1.317	0.030
Maternal IQ	-0.001		0.004	0.015	0.001		0.001		0.149	0.080
Maternal Marital Status: Married	-0.089	0.067	-0.105	0.027	-0.106	0.027	-0.135	0.005	-3.590	
centhg*centpb	0.201	0.065	0.238	0.027	0.191	0.077	0.138		9.335	

	DSM Total (N=90)		log(Omissions) (N=89)		log(Commisions) (N=83)		Hit Reaction Time (N=89)	
	Beta	P-value	Beta	P-value	Beta	P-value	Beta	P-value
Model		0.042		0.003		NS		0.006
Intercept	72.078	<0.001	4.029	<<0.001	4.081	<<0.001	79.173	<<0.001
centhg	4.003	0.068	0.001		-0.001		-2.929	
centpb	0.330		-0.061		0.014		-5.144	0.063
Sex of Child: Female	1.237		0.064	0.066	-0.023		-0.619	
Age of Child at Visit	-0.732		-0.004		-0.013		-1.926	
Cohort: 3	-5.386		0.112		0.031		-0.017	
SES Level	0.158		-0.006		0.009		-0.241	
Maternal Age	-0.004		7.5E-04		-0.003		-0.033	
Maternal Education	-1.349	0.028	0.012		-0.008		1.045	0.055
Maternal IQ	0.121		-0.002	0.046	4.6E-04		-0.190	0.015
Maternal Marital Status: Married	-6.578	0.018	-0.028		0.066		-3.245	
centhg*centpb	13.398	0.035	0.020		0.087		2.790	

Table S1.3.2 Full models for second trimester Hg and Pb exposure interactions. Darker shades of blue indicate larger positive betas, while darker shades of red indicate larger negative betas. "centhg" refers to centered, log-transformed mercury concentration and "centpb" refers to centered, log-transformed lead concentration.

	Trimester 2									
	log(Distractibility) (N=112)		log(Hyperactivity) (N=112)		log(ADHD) (N=113)		log(DSM Inattention) (N=112)		DSM Hyperactivty-Impulsivity (N=112)	
	Beta	P-value	Beta	P-value	Beta	P-value	Beta	P-value	Beta	P-value
Model		NS		NS		NS		0.098		NS
Intercept	3.879		3.834		3.907		3.587		43.856	0.011
centhg	0.030		0.010		-0.001		0.030		0.915	
centpb	-0.074	0.072	-0.041		-0.079	0.065	-0.081	0.046	-2.552	
Sex of Child: Female	0.045		0.024		0.048		0.034		2.880	
Age of Child at Visit	0.006		0.021		0.004		0.027		1.321	
Cohort: 3	-0.017		0.001		-0.041		0.038		2.197	
SES Level	0.005		0.003		0.010		0.009		0.123	
Maternal Age	0.001		-0.003		-0.001		0.001		-0.206	
Maternal Education	0.007		-0.008		1.6E-04		0.005		-0.033	
Maternal IQ	-0.001		0.002		2.6E-05		-1.6E-04		0.062	
Maternal Marital Status: Married	-0.039		-0.055		-0.035		-0.066	0.091	-1.375	
centhg*centpb	-0.115	0.091	-0.138	0.068	-0.142	0.046	-0.149	0.025	-8.869	0.041

	DSM Total (N=112)		log(Omissions) (N=110)		log(Commisions) (N=111)		Hit Reaction Time (N=113)	
	Beta	P-value	Beta	P-value	Beta	P-value	Beta	P-value
Model		NS		0.056		NS		0.013
Intercept	52.383	0.002	4.047	<<0.001	3.938	<<0.001	70.158	<<0.001
centhg	0.286		0.041		-0.069	0.048	1.562	
centpb	-4.783	0.060	-0.039		-0.052		-0.340	
Sex of Child: Female	2.957		0.012		-0.033		-0.052	
Age of Child at Visit	0.364		-0.004		-0.011		-1.193	
Cohort: 3	-3.046		0.107		-0.012		3.400	
SES Level	0.214		-0.004		-0.006		-0.637	0.076
Maternal Age	-0.027		5.6E-04		0.001		0.066	
Maternal Education	-0.069		0.002		-7.4E-04		0.563	
Maternal IQ	0.026		-0.001		0.001		-0.131	0.027
Maternal Marital Status: Married	-3.382		0.013		0.029		-2.453	
centhg*centpb	-10.476	0.013	-0.033		-0.096		-2.483	

Table S1.3.3 Full models for third trimester Hg and Pb exposure interactions. Darker shades of blue indicate larger positive betas, while darker shades of red indicate larger negative betas. "centhg" refers to centered, log-transformed mercury concentration and "centpb" refers to centered, log-transformed lead concentration.

	Trimester 3									
	log(Distractibility) (N=113)		log(Hyperactivity) (N=112)		log(ADHD) (N=114)		log(DSM Inattention) (N=114)		DSM Hyperactivty-Impulsivity (N=111)	
	Beta	P-value	Beta	P-value	Beta	P-value	Beta	P-value	Beta	P-value
Model		NS		NS		NS		0.035		NS
Intercept	3.410		3.435	<<0.001	3.558		3.316		34.723	0.098
centhg	-0.023		-0.063	0.046	-0.031		-0.026		-0.295	
centpb	0.003		-0.034		-0.015		-0.026		-0.511	
Sex of Child: Female	0.069	0.034	0.041		0.060	0.087	0.068	0.043	2.756	
Age of Child at Visit	0.040	0.085	0.043		0.030		0.043	0.073	1.710	
Cohort: 3	0.085		0.065		0.047		0.087		4.188	
SES Level	0.010		0.010		0.012	0.088	0.013	0.048	0.555	
Maternal Age	0.001		-0.001		-0.001		4.2E-04		-0.177	
Maternal Education	-0.001		-0.012		-0.009		-0.002		-0.660	
Maternal IQ	0.001		0.003	0.028	0.002		0.002		0.141	0.081
Maternal Marital Status: Married	-0.077	0.047	-0.097	0.034	-0.067		-0.105	0.009	-1.644	
centhg*centpb	0.040		0.021		0.028		0.042		4.973	

	DSM Total (N=115)		log(Omissions) (N=113)		log(Commisions) (N=111)		Hit Reaction Time (N=115)	
	Beta	P-value	Beta	P-value	Beta	P-value	Beta	P-value
Model		0.097		0.002		NS		0.006
Intercept	26.416		3.739		3.631		78.588	<0.001
centhg	-1.975		-0.010		-0.003		0.244	
centpb	-0.659		-0.006		-0.050		1.221	
Sex of Child: Female	4.080	0.051	0.018		-0.031		-1.495	
Age of Child at Visit	2.179		0.010		0.022		-2.544	0.049
Cohort: 3	3.646		0.187	0.013	0.129		0.524	
SES Level	0.604		0.005		-0.004		-0.036	
Maternal Age	-0.055		0.004		-0.001		-0.029	
Maternal Education	-0.589		0.002		-0.002		0.542	
Maternal IQ	0.147	0.056	-0.001		0.001		-0.079	
Maternal Marital Status: Married	-5.187	0.035	-0.036		0.028		-2.560	
centhg*centpb	2.033		0.039		-0.016		5.303	0.082

Table S1.4.1 Full models for Trimester 1 Hg and concurrent Pb exposure interactions. Darker shades of blue indicate larger positive betas, while darker shades of red indicate larger negative betas. "centhg" refers to centered, log-transformed mercury concentration and "centpb" refers to centered, log-transformed lead concentration.

	Trimester 1 Hg*Concurrent Pb									
	log(Distractibility) (N=83)		log(Hyperactivity) (N=80)		log(ADHD) (N=82)		log(DSM Inattention) (N=82)		DSM Hyperactivty-Impulsivity (N=79)	
	Beta	P-value	Beta	P-value	Beta	P-value	Beta	P-value	Beta	P-value
Model		NS		NS		NS		NS		NS
Intercept	4.077	<<0.001	4.089	<<0.001	4.094	<<0.001	3.497	<<0.001	61.915	0.001
centhg	-0.010		0.055		0.044		0.006		1.143	
centpb	-0.020		0.037		0.009		0.010		1.032	
Sex of Child: Female	0.038		-0.002		0.021		0.014		0.310	
Age of Child at Visit	-0.001		0.016		0.005		0.037		0.537	
Cohort: 3	-0.012		0.005		-0.005		0.095		1.126	
SES Level	0.001		-0.012		-0.003		0.006		-0.563	
Maternal Age	-0.002		-0.007	0.060	-0.005		-0.003		-0.273	
Maternal Education	-0.005		-0.017	0.087	-0.009		-0.013		-1.049	0.054
Maternal IQ	0.001		0.003	0.051	0.002		0.004	0.037	0.146	0.073
Maternal Marital Status: Married	-0.095	0.088	-0.074		-0.096	0.058	-0.115	0.025	-2.840	
centhg*centpb	-0.009		-0.011		-0.056		0.008		-0.433	

	DSM Total (N=80)		log(Omissions) (N=80)		log(Commisions) (N=82)		Hit Reaction Time (N=82)	
	Beta	P-value	Beta	P-value	Beta	P-value	Beta	P-value
Model		NS		0.047		NS		0.032
Intercept	35.336	0.052	4.106	<<0.001	3.950	<<0.001	80.165	<0.001
centhg	2.532		0.031		-0.018		-2.398	
centpb	2.246		0.042		-0.025		1.870	
Sex of Child: Female	0.949		0.033		-0.024		-2.740	
Age of Child at Visit	2.064		0.005		0.012		-1.801	
Cohort: 3	2.782		0.117		0.095		0.514	
SES Level	-0.080		-0.009		-0.002		-0.534	
Maternal Age	-0.130		-0.003		-0.007	0.071	-0.110	
Maternal Education	-0.857		0.009		-0.015		1.253	0.034
Maternal IQ	0.174	0.042	-0.003	0.043	0.002		-0.179	0.046
Maternal Marital Status: Married	-5.710	0.023	0.005		0.050		-4.368	0.094
centhg*centpb	-0.397		0.014		-0.022		1.243	

Table S1.4.2 Full models for Trimester 2 Hg and concurrent Pb exposure interactions. Darker shades of blue indicate larger positive betas, while darker shades of red indicate larger negative betas. "centhg" refers to centered, log-transformed mercury concentration and "centpb" refers to centered, log-transformed lead concentration.

	Trimester 2 Hg*Concurrent Pb									
	log(Distractibility) (N=102)		log(Hyperactivity) (N=102)		log(ADHD) (N=103)		log(DSM Inattention) (N=102)		DSM Hyperactivty-Impulsivity (N=101)	
	Beta	P-value	Beta	P-value	Beta	P-value	Beta	P-value	Beta	P-value
Model		NS		NS		NS		NS		NS
Intercept	3.802	<<0.001	3.961	<<0.001	4.123	<<0.001	3.742	<<0.001	61.706	<0.001
centhg	-0.029		0.009		-0.020		-0.019		-0.865	
centpb	-0.009		0.015		0.005		0.008		0.612	
Sex of Child: Female	0.022		0.011		0.015		0.012		0.350	
Age of Child at Visit	0.016		0.020		-0.010		0.019		0.227	
Cohort: 3	0.011		0.020		-0.078		0.010		-2.000	
SES Level	0.006		-0.006		0.003		0.006		-0.189	
Maternal Age	-0.002		-0.007	0.026	-0.006	0.095	-0.003		-0.301	0.099
Maternal Education	0.002		-0.009		-0.003		-0.004		-0.582	
Maternal IQ	9.6E-05		0.002		0.001		0.002		0.111	
Maternal Marital Status: Married	-0.051		-0.040		-0.038		-0.070	0.071	-2.081	
centhg*centpb	0.022		0.007		-0.037		0.015		0.230	

	DSM Total (N=109)		log(Omissions) (N=100)		log(Commisions) (N=104)		Hit Reaction Time (N=104)	
	Beta	P-value	Beta	P-value	Beta	P-value	Beta	P-value
Model		NS		NS		NS		NS
Intercept	27.970	0.099	4.163	<<0.001	4.000	<<0.001	66.439	<0.001
centhg	-0.255		0.014		-0.062		-1.366	
centpb	0.907		0.021		0.008		1.696	
Sex of Child: Female	1.580		-0.004		-0.040		-0.953	
Age of Child at Visit	2.490	0.076	-0.015		-0.009		-1.095	
Cohort: 3	5.939		0.041		-0.017		-1.479	
SES Level	0.244		-0.006		-0.005		-0.595	
Maternal Age	-0.217		-4.2E-04		-0.002		-0.069	
Maternal Education	-0.451		0.008		-0.012		0.977	0.027
Maternal IQ	0.116	0.098	-0.001		0.003	0.096	-0.076	
Maternal Marital Status: Married	-2.960		-0.017		0.027		-4.220	0.058
centhg*centpb	1.301		0.048		-0.054		0.907	

Table S1.4.3 Full models for Trimester 3 Hg and concurrent Pb exposure interactions. Darker shades of blue indicate larger positive betas, while darker shades of red indicate larger negative betas. "centhg" refers to centered, log-transformed mercury concentration and "centpb" refers to centered, log-transformed lead concentration.

Trimester 3 Hg*Concurrent Pb

	log(Distractibility) (N=95)		log(Hyperactivity) (N=96)		log(ADHD) (N=97)		log(DSM Inattention) (N=96)		DSM Hyperactivty-Impulsivity (N=94)	
	Beta	P-value	Beta	P-value	Beta	P-value	Beta	P-value	Beta	P-value
Model		NS		0.045		NS		0.063		NS
Intercept	3.543	<<0.001	3.715	<<0.001	3.959	<<0.001	3.358	<<0.001	48.514	0.011
centhg	-0.005		-0.040		-0.009		-0.012		0.929	
centpb	-0.003		0.060	0.083	-0.002		0.008		1.227	
Sex of Child: Female	0.057		0.016		0.028		0.062	0.097	0.571	
Age of Child at Visit	0.034		0.037		0.004		0.040		1.555	
Cohort: 3	0.057		0.034		-0.042		0.076		2.987	
SES Level	0.008		-0.011		0.001		0.009		-0.447	
Maternal Age	-0.003		-0.009	0.019	-0.006	0.093	-0.004		-0.460	0.024
Maternal Education	-0.004		-0.009		-0.007		-0.008		-0.743	
Maternal IQ	0.002		0.004	0.005	0.003	0.062	0.004	0.007	0.179	0.026
Maternal Marital Status: Married	-0.082	0.046	-0.069		-0.042		-0.087	0.038	-0.966	
centhg*centpb	-0.030		-0.006		-0.016		-0.033		2.371	

	DSM Total (N=96)		log(Omissions) (N=95)		log(Commisions) (N=98)		Hit Reaction Time (N=98)	
	Beta	P-value	Beta	P-value	Beta	P-value	Beta	P-value
Model		NS		NS		NS		0.042
Intercept	46.743	0.012	3.779	<<0.001	3.669	<<0.001	76.003	<0.001
centhg	-0.809		0.007		-0.011		1.167	
centpb	1.035		0.006		0.009		1.640	
Sex of Child: Female	2.808		0.004		-0.003		-2.380	
Age of Child at Visit	1.021		0.009		0.019		-1.868	
Cohort: 3	-1.126		0.121		0.112		-0.127	
SES Level	-0.137		0.001		-0.002		-0.430	
Maternal Age	-0.314		0.002		-0.003		-0.049	
Maternal Education	-0.465		0.008		-0.011		0.937	0.029
Maternal IQ	0.161	0.049	-0.001		0.002		-0.118	
Maternal Marital Status: Married	-3.538		-0.033		0.038		-3.157	
centhg*centpb	-0.228		0.060		-0.044		0.981	

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Chapter 2

Mercury Exposure-Genetic Interactions and Attention Deficits in Children from Mexico City

Abstract

Attention deficit-hyperactivity disorder (ADHD) is a common psychological disorder among school-aged children. ADHD is strongly heritable and a number of potential genes have been identified as risk factors. However, there is still relatively limited information about the interactions of these identified genes and environmental exposures. We examine the interactions of dopamine related candidate SNPs (rs6347, rs40184, rs4680, rs1800497, rs1800955, and rs27072) and blood and hair mercury and the ADHD index and DSM-IV total symptom scores of the Conners' Rating Scales-Revised (CRS-R), as measured in participants from the Early Life Exposure in Mexico to Environmental Toxicants (ELEMENT) study. Participants had a mean (SD) blood Hg concentration of 1.8 (1.3) μg/L and hair Hg concentration of 0.60 (0.47) μg/g. We found that rs1800497 and rs27072, genotypes containing the T allele were consistently associated with higher ADHD Index and DSM-IV Total scores. Mercury exposure was generally not significantly associated with the outcome measures. Two interactions between the chosen SNPs and exposure approached statistical significance. The hair Hg association with DSM-IV total symptom score differed by rs1800955 genotype ($p=0.058$) and ADHD index differed by rs4680 heterozygous genotype ($p=0.025$). Our study suggests that COMT and DRD4 genotype may modify the effect of postnatal MeHg exposure on two measures of attention in children.

Introduction

Attention deficit/hyperactivity disorder (ADHD) is characterized by persistent impulsivity, inattention, and hyperactivity that is present in multiple contexts and impairs functioning (American Psychiatric Association 2013). ADHD is one of the most common neurological disorder in school-aged children worldwide (Escobar et al. 2005; Polanczyk et al. 2015). Measures of the worldwide prevalence of ADHD vary widely, but it is estimated at 7.2% worldwide (Polanczyk et al. 2014; Thomas et al. 2015), with the CDC estimating a prevalence of 9.5% in children in the U.S. (Bloom et al. 2013).

The existing literature suggests that ADHD is highly heritable. While the heritability is estimated to be 76%, the interactions between the genes involved are complex (Faraone and Mick 2010). Candidate genes for ADHD risk are primarily dopaminergic and serotonergic. While the risk associated with these genes is generally small and cannot individually explain the entirety of a person's risk, these findings, especially related to the dopaminergic genes, have been consistent (Thapar et al. 2013; Swanson et al. 2007).

However, what is known about the genetics of ADHD cannot account fully for the heritability of the disorder (Schachar 2014). Further, there is heterogeneity in the disorder which could potentially involve environmental influences or gene-environment interactions (Thapar et al. 2013; Archer et al. 2011). These include environmental exposures such as maternal smoking during pregnancy (Thapar et al. 2013; Neuman et al. 2007), exposure to polychlorinated biphenyls (PCBs) (Boucher et al. 2009; Eubig et al. 2010; Schantz et al. 2003), and exposure to metals (Goodlad et al. 2013; Karagas et al. 2012; Sanders et al. 2015).

A number of epidemiological studies have examined the relationship between MeHg exposures and later behavioral and cognitive effects. Studies examining concurrent MeHg

exposure generally do not find an association with attention deficits (Ha et al. 2009; Nicolescu et al. 2010). More studies have found associations with prenatal exposure. A 1997 study in the Faroe Islands found attention deficits related to high cord blood levels of MeHg (Grandjean et al. 1997). A more recent study by Grandjean et al. and a study by Oken et al. found similar results (Grandjean et al. 2012; Oken et al. 2005). However, a similar cohort in the Seychelles has consistently failed to find deficits (Myers et al. 2003; Davidson et al. 2010).

A number of studies have examined changes to catecholamine signaling and processing after MeHg exposure. A 1997 study by Faro et al. found that chronic intrastriatal MeHg exposure in rats resulted in increased striatal release of DA (Faro et al. 1997). The same group later found that there was a dose-dependent relationship between MeHg administration and DA release (Faro et al. 2000). Further, a later study by Tiernan et al found that in addition to increased release of DA, MeHg administration increased DA production (Tiernan et al. 2013). Other studies found prenatally exposed rats had decreased monoamine oxidase (MAO) activity (Chakrabarti et al. 1998; Beyrouty et al. 2006). More recently, a Tiernan et al. study found that MeHg-induced DA release was associated with increases in DA synthesis, tyrosine hydroxylase activity, and intracellular levels of DA (Tiernan et al. 2013). Given that monoamine neurotransmitters have been consistently associated with ADHD, interactions between MeHg and genes related to neurotransmitter signaling and processing are a viable direction for research (Asherson and Gurling 2011).

Here, we aim to examine potential disruptions in dopaminergic pathways as a mechanism for MeHg effects on attention processes via study of genetic polymorphisms. We hypothesize that genetic variants will be associated with attention deficits and that the nature of these associations will be modified by considering MeHg exposure. There are currently few studies of

gene-environment interactions examining MeHg, genes and behavioral outcomes, and there is a need for more studies which examine gene-environment interactions (Yolton et al. 2014). Several studies examining Hg, genes related to its toxicokinetics and behavior have been done (Yolton et al. 2014; Woods et al. 2013; Basu et al. 2014a), but there are fewer studies looking at MeHg, genes related to ADHD, and behavior. For example, a study by Woods et al observed interactions between inorganic Hg and catechol-O-methyltransferase (COMT) genotype among boys (Woods et al. 2014), but did not examine MeHg.

Methods

ELEMENT Cohort

The ELEMENT study, consisting of three sequentially enrolled cohorts, was initially designed to research the influence of maternal lead exposure on offspring neurodevelopment. Pertinent details of ELEMENT, such as inclusion and exclusion criteria, collection methods, and demographics can be found elsewhere (Tellez-Rojo et al. 2006; Afeiche et al. 2011). In brief, Cohort 1 subjects were recruited 1994-1995, Cohort 2 subjects were recruited 1997-2001, Cohort 3 subjects were recruited 2001-2004 (Afeiche et al. 2011). In 2006 participants were recruited from all three cohorts for follow-up visits regarding behavioral outcomes. For analysis of concurrent exposures, children were included if their mothers were recruited into Cohorts 2 and 3, had at least one mercury exposure value and at least one attention measure from the same visit. Behavior outcomes at these visits were linked to exposure measures taken at the same time as the outcomes (i.e. concurrent exposures).

The research protocol was approved by the ethics and research committees of the partnering institutions, including the National Institute of Public Health of Mexico, the Harvard School of Public Health, the Brigham and Women's Hospital, the University of Michigan School

of Public Health, the University of Toronto, and the participating hospitals.

Human Biospecimens and Mercury Analysis

Blood, hair and urine samples were collected from the participating children. Venous whole blood samples were collected into vials certified for trace metals analysis and stored at 4^{o}C until analysis. Spot (second morning void) urine samples were collected and stored frozen until analysis. Scalp hair samples were obtained from each participant using stainless steel scissors and the proximal end was designated. Mercury was analyzed in all samples as described elsewhere (Basu et al. 2014b). Briefly, total mercury content was carried out using a Direct Mercury Analyzer 80 (DMA-80, Milestone Inc., CT). Daily instrument calibration, procedural blanks, replicates, and several certified reference materials were analyzed. Reference materials included CRM #13 for hair (National Institute for Environmental Studies, Japan), DOLT-4 (dogfish liver; National Research Council, Canada), and QMEQAS for blood (Institut National de Santé Publique du Québec). Recoveries of the reference materials ranged from 80 to 110%. The analytical detection limit was less than 1 ng mercury.

Attention Measures

Two outcome measures from the parent responses of the Conners' Rating Scales-Revised (CRS-R) were used. The CRS-R uses parent-completed questionnaires that provide information about the extent to which a child's difficulties managing attention are manifested as dysfunctions in everyday life. Each item of the questionnaire is linked to the DSM-IV criteria for ADHD-inattentive subtype, ADHD-hyperactive-impulsive subtype, and ADHD-combined subtype. The CRS-R produces index scores for hyperactivity, inattention, and ADHD overall, as well as other behaviors, such as perfectionism, opposition, and somatization. Here, the ADHD Index and the DSM-IV Total Symptoms score were used.

DNA Extraction and Genotyping

Genotyping was performed as previously described (Fortenberry et al. 2014). In brief, the University of Michigan Sequencing Core performed DNA extraction and genotyping. Genomic DNA was extracted from venous blood stored in 8.5ml Paxgene tubes, following the purification of DNA from cell lystate from compromised samples on the Qiagen Autopure LS® protocol. Genotyping was performed using the Sequenom MassARRAY iPLEX Platform (Bruker Instruments, Billerica, MA) (Gabriel et al. 2009). Population sizes for each SNP were based on the ability to make a successful call for each individual, which was done using the SpectroTYPER software supplied by Sequenom. The overall call rate was calculated as $\frac{N\ of\ individuals\ with\ genotype\ call}{N\ of\ individuals\ in\ population}$. The genes used in this study were selected for their relevance to ADHD (Li et al. 2014; Gatt et al. 2015).

Statistical Analysis

Univariate descriptive statistics and graphical displays were obtained for all variables. Data were analyzed using R x64 3.0.1. Outliers were detected using the ExtremeValues package for R, which uses a distribution based method for identifying outliers (van der Loo 2010). Spearman correlations were used to assess associations among all biomarkers. Bivariate analyses were used to relate mercury biomarker values with demographic characteristics. Data are reported as mean (standard deviation), unless otherwise indicated.

Linear models were constructed, including a number of covariates selected for potential relevance to either the exposure or the analyzed attention measures. Maternal IQ was calculated based on the mothers' scores on the Spanish Wechsler Adult Intelligence Scale (Tellez-Rojo et al. 2004; Wechsler 1968). Maternal education was the cumulative number of years that the mother attended school at time of recruitment. Information about smoking during pregnancy

(yes/no) was obtained from a questionnaire administered to the mother during pregnancy. Mothers who responded "yes" at any point during pregnancy were excluded from analyses. As previously described (Fortenberry et al. 2014), direct questions about income were deemed too intrusive within this cohort. Thus, a measure of socioeconomic status based on reported possessions and household assets was used instead. Maternal age and marital status at the time of recruitment were also included, as were child age at the follow-up visit and child sex.

Exposure measures were log-transformed prior to entering into the models. Further, model diagnostics revealed that the ADHD Index did not meet normality and was highly skewed. Thus, this outcome was log-transformed in all subsequent analyses. Given this transformation, the actual beta coefficients are presented in tables and figures. However, in the text, we also provide interpretations that consider the transformation. Specifically, we calculated the difference in the outcome for a 10% higher Hg concentration as $\Delta Outcome = \left(e^{\beta_{exposure} * \ln(1.10)} - 1\right) * 100$. For DSM-IV Total Symptoms, which did not require log-transformation, we used $\Delta Outcome = \beta_{exposure} * \ln(1.10)$.

Similarly, in the ADHD Index models, where the outcome was log-transformed, actual beta coefficients are shown in tables and figures, while in the text we provide interpretations for the genotypes that consider that transformation. Specifically, we calculated the percent difference in the outcome for a given genotype as$\%\Delta Outcome = \left(e^{\beta_{SNP}} - 1\right) * 100$. This was unneeded for DSM-IV Total Symptoms.

Possible interactions between MeHg and the DA related genes were assessed using models that included MeHg exposure, genotype as a categorical variable, and their cross-product. Exposures were log-transformed and centered. The models could then be interpreted as the change as MeHg was increased for the group with a given genotype. Once again, the actual

beta coefficients are presented in tables and figures, but interpretations that consider transformations are presented in the text.

Results

Population Characteristics

Overall, 466 Cohort 2 and 3 children with complete demographic information participated in the follow-up visit. A slight majority of child participants were male. A majority of mothers had been married at the time they were recruited. Sixteen mothers reported smoking during pregnancy. Given the small proportion of our sample and the existing literature suggesting an association between maternal smoking status in pregnancy and later attention problems (Neuman et al. 2007; Braun et al. 2006; Froehlich et al. 2011), these mothers, and their children, were omitted from subsequent analyses. (Table 2.1)

Exposure data for this cohort was previously reported for mercury (Basu et al. 2014b) and briefly summarized here. Concurrent mercury exposure data is available for 67.4-93.3% of the children, depending on the biomarker. Blood and hair mercury levels of participating children were 1.8 ± 1.3 μg/L and 0.60 ± 0.47 μg/g respectively (Table 2.1). Blood and hair mercury levels of the same individuals were correlated ($r=0.69$, $p<0.001$). Two outcome measures from the CRS-R test were available from 443 participants at the follow-up visit. For any given outcome, a maximum of eight outliers were removed. (Table 2.1)

Table 2.1 Demographic Characteristics, Exposure Assessment, Psychological Testing, & Genotypes

DEMOGRAPHIC CHARACTERISTICS			**N**	**Mean(SD)**	**Median**	**Range**
Child Age			466	9.1 (1.3)	9.3	(6.9, 12.5)
Household SES Level			466	6.7 (2.5)	6.5	(1, 14)
Maternal Age at Recruitment			466	26.0 (5.5)	26	(14, 44)
Maternal Education Level at Recruitment			466	10.9 (2.6)	11	(2, 20)
Maternal IQ			466	92.6 (18.5)	91	(60, 182)
			Total N	**N(%)**		
Sex of Child (Male)			466	237 (50.9%)		
Maternal Smoking During Pregnancy ("Ever Smoked")			466	16 (3.4%)		
Maternal Marital Status ("Married")			466	343 (73.6%)		
EXPOSURE ASSESSMENT			**N**	**Mean(SD)**	**Median**	**Range**
Blood Mercury (μg/L)			314	1.8 (1.3)	1.5	(0.23, 8.5)
Hair Mercury (μg/g)			435	0.60 (0.47)	0.46	(0.06, 3.1)
PSYCHOLOGICAL TESTING			**N**	**Mean (SD)**	**Median**	**Range**
CADS Scores			443			
ADHD Index			440	53.8 (9.8)	52.0	(40, 86)
DSM IV Total			435	54.9 (9.3)	53.0	(40, 81)
GENOTYPES				**Total N**	**N(%)**	
DAT1/ SLC6A3	rs6347	AA	103	90 (87.4)		
		AG		13 (12.6)		
DAT1/ SLC6A3	rs40184	GG	194	156 (80.4)		
		AG		38 (19.6)		
COMT	rs4680	GG	268	120 (44.8)		
		GA		114 (42.5)		
		AA		34 (12.7)		
Taq1A DRD2/ ANKK1	rs1800497	CC	239	59 (24.7)		
		TC		118 (49.4)		
		TT		62 (25.9)		
DRD4	rs1800955	TT	166	53 (31.9)		
		CT		107 (64.5)		
		CC		6 (3.6)		
DAT1/ SLC6A3	rs27072	CC	282	171 (60.6)		
		CT		97 (34.4)		
		TT		14 (5.0)		

Crude Associations

Crude associations were computed for both outcomes and the genotypes of interests (Table 2.2). Of these, several were statistically significant ($p<0.05$) or nearly so ($p<0.10$). The mean ADHD Index score for the AG genotype of rs40184 (DAT1) was lower than that of the GG genotype ($p=0.083$). The mean DSM-IV Total score for that genotype was also lower, but this was not statistically significant. Lower scores for ADHD Index and DSM-IV Total score both indicate fewer ADHD-related symptoms.

Conversely, for rs27072 (DAT1), the mean ADHD Index and DSM-IV Total score for the CT and TT genotypes was higher than that of the CC genotype ($p=0.041$, 0.034). Similarly, for rs1800497 (DRD2 Taq1A), both the TC and TT genotypes had higher average ADHD Index and DSM-IV Total scores than the CC genotype ($p_{TC}=0.005$, 0.002; $p_{TT}=0.022$, 0.045).

There were no observable or statistically significant patterns for the mean outcome scores for rs6347 (DAT1) or rs4680 (COMT). For rs1800955 (DRD4), the mean ADHD Index and DSM-IV Total scores were higher for the CT and CC genotypes than for the TT genotype, but this was not statistically significant.

Table 2.2 Crude Means of Outcomes by Genotype. Means with p<0.10 are shown in bold.

			ADHD Index		DSM Total	
	SNP[a]		Mean (SD)	P-Value	Mean (SD)	P-Value
DAT1/ SLC6A3	rs6347	AA	53.48 (9.32)		55.73 (10.23)	
		AG	54.92 (11.28)	0.644	55.08 (10.34)	0.829
DAT1/ SLC6A3	rs40184	GG	54.20 (10.01)		56.03 (10.86)	
		AG	**51.18 (9.02)**	**0.083**	53.05 (8.93)	0.119
COMT	rs4680	GG	53.85 (9.47)		55.89 (10.04)	
		GA	54.53 (10.56)	0.685	55.94 (11.04)	0.973
		AA	53.35 (10.50)	0.720	54.65 (10.13)	0.542
Taq1A DRD2/ ANKK1	rs1800497	CC	50.51 (8.39)		51.88 (8.67)	
		TC	**54.86 (10.36)**	**0.005**	**56.95 (11.12)**	**0.002**
		TT	**54.44 (9.70)**	**0.022**	**55.32 (9.30)**	**0.062**
DRD4	rs1800955	TT	53.21 (10.58)		54.81 (10.50)	
		CT/CC	54.85 (10.47)	0.314	56.16 (10.34)	0.437
DAT1/ SLC6A3	rs27072	CC	53.03 (9.29)		54.51 (9.82)	
		CT/TT	**55.65 (11.03)**	**0.041**	**57.21 (11.05)**	**0.033**

[a]Sample sizes range from 102-282. Sample sizes and genotype frequencies are shown in Supplmental Table 1.

Linear Models

Adjusted Associations

Associations that were adjusted for our selected covariates were computed for both outcomes and the genotypes of interests (Table 2.3). Statistically significant associations were only observed in the adjusted linear models for rs1800497 and rs27072 and psychometric outcomes. For rs1800497, the TC and TT genotypes were consistently associated with higher ADHD Index and DSM-IV Total scores, as they were in the crude associations. So, TC was associated with a 8.01% (95% CI: 2.32, 14.03) higher ADHD Index score than the CC reference group, while TT was associated with a 7.27% (95% CI: 0.79, 14.17) higher ADHD Index score than the CC group (calculated as $\%\Delta Outcome_{TC} = (e^{0.077} - 1) * 100$ and $\%\Delta Outcome_{TT} = (e^{0.070} - 1) * 100$). Similarly, TC was associated with a 4.89 (95% CI: 1.73, 8.06) unit increase in DSM-IV Total scores compared to the CC reference group, while TT was associated with a

3.23 (95% CI: -0.42, 6.88) unit increase in DSM-IV total scores compared to the CC group. For rs27072, the CT and TT genotypes were also consistently associated with higher ADHD Index and DSM-IV Total scores, as they were in the crude associations.

Among the other SNPs, although there are no statistically significant associations, several patterns are notable. For rs1800955 SNP, the CT/CC genotypes were associated with higher scores than the TT genotype, while for rs40184, the AG genotype was associated with lower scores than the GG genotype, although this was not statistically significant. For rs6347, the AG genotype was associated with higher ADHD Index scores than the AA genotype, but not DSM-IV Total scores. A more complicated pattern is present for rs4680: the GA genotype was associated with higher ADHD Index and DSM-IV Total scores than the GG genotype, while the AA genotype was generally associated with lower scores than the GG genotype, although this was, again, not statistically significant. Thus, GA was associated with a 1.50% (95% CI: -3.06, 6.28) higher ADHD Index score than the GG reference group, while AA was associated with a 0.514% (95% CI: -7.09, 6.52) lower ADHD Index score than the GG group. Similarly, GA was associated with a 0.378 (95% CI: -2.30, 3.06) unit increase in DSM-IV Total scores compared to the GG reference group, while AA was associated with a 0.708 (95% CI: -4.71, 3.30) unit decrease in DSM-IV Total scores compared to the GG group.

Table 2.3 Adjusted[a] associations of outcomes and genotypes. Beta coefficients with p<0.10 are shown in bold.

			ADHD Index		DSM Total	
	SNP[b]		β (Std Error)	P-Value	β (Std Error)	P-Value
DAT1/ SLC6A3	rs6347	AA	Ref (0)		Ref (0)	
		AG	0.017 (0.051)	0.734	-0.848 (3.08)	0.783
DAT1/ SLC6A3	rs40184	GG	Ref (0)		Ref (0)	
		AG	-0.043 (0.033)	0.204	-2.43 (2.00)	0.226
COMT	rs4680	GG	Ref (0)		Ref (0)	
		GA	0.015 (0.023)	0.526	0.378 (1.37)	0.782
		AA	-0.005 (0.035)	0.883	-0.708 (2.04)	0.729
Taq1A DRD2/ ANKK1	rs1800497	CC	Ref (0)		Ref (0)	
		TC	**0.077 (0.028)**	**0.006**	**4.90 (1.61)**	**0.003**
		TT	**0.070 (0.032)**	**0.028**	**3.23 (1.86)**	**0.084**
DRD4	rs1800955	TT	Ref (0)		Ref (0)	
		CT/CC	0.045 (0.032)	0.156	2.31 (1.81)	0.203
DAT1/ SLC6A3	rs27072	CC	Ref (0)		Ref (0)	
		CT/TT	**0.041 (0.022)**	**0.057**	**2.60 (1.25)**	**0.039**

[a]Adjusted for sex of child, age of child at follow-up, study cohort, SES, maternal age, maternal education, maternal IQ, and maternal marital status

[b]Sample sizes range from 102-282 participants. Sample sizes and genotype frequencies are shown in Supplmental Table 1

Gene-Environment Interactions

Models that included genotypes, exposures and the cross-product of genotype and exposure were constructed for both outcomes (Table 2.4). Only two interactions between the chosen SNPs and exposure approached statistical significance. The hair Hg association with DSM-IV total symptom score differed by rs1800955 (DRD4) genotype (p=0.058). Among the TT genotype, a 10% increase in hair Hg was associated with a 0.392 unit increase (95% CI: -3.06, 6.28) in DSM-IV total symptom score. However, among the CT/CC genotypes, a 10% increase in hair Hg exposure was associated with a 0.076 unit decrease (95% CI: -0.306, 0.154) in DSM-IV total symptom score.

Additionally, the hair Hg association with ADHD index score differed by rs4680 (COMT) genotype, but this was only statistically significant for the GA genotype (p=0.025). Among the GG genotype, a 10% increase in hair Hg was associated with a 0.182 unit decrease (95% CI: -0.554, 0.193) in ADHD index score. However, among the GA genotype, a 10% increase in hair Hg exposure was associated with a 0.484 unit increase (95% CI: 0.041, 0.930) in ADHD index score. An increase in scores was also observed among the AA genotype: there, a 10% increase in hair Hg was associated with a 0.183 unit increase (95% CI: -0.542, 0.913) in ADHD index score.

Table 2.4 Gene-environment interactions of Hg exposure and selected genotypes.[a] Beta coefficients with p<0.10 are shown in bold.

			Blood[b]				Hair			
			ADHD Index		DSM Total		ADHD Index		DSM Total	
			β (Std Error)	P-Value	β (Std Error)	P-Value	β (Std Error)	P-Value	β (Std Error)	P-Value
	log(Hg)[c]		-0.035 (0.031)	0.262	-0.383 (1.87)	0.839	-0.004 (0.025)	0.879	0.591 (1.55)	0.704
rs6347	Genotype	AA	Ref (0)		Ref (0)		Ref (0)		Ref (0)	
		AG	0.020 (0.052)	0.697	-0.447 (3.16)	0.888	0.049 (0.054)	0.368	0.877 (3.35)	0.794
	log(Hg)*AG		0.069 (0.069)	0.319	3.21 (4.14)	0.441	0.053 (0.061)	0.388	2.69 (3.76)	0.476
rs40184	log(Hg)		-0.010 (0.022)	0.665	-0.057 (1.33)	0.966	-0.008 (0.018)	0.651	-0.429 (1.11)	0.700
	Genotype	GG	Ref (0)		Ref (0)		Ref (0)		Ref (0)	
		AG	-0.053 (0.036)	0.142	-3.16 (2.16)	0.145	-0.042 (0.035)	0.241	-1.98 (2.13)	0.354
	log(Hg)*AG		-0.001 (0.052)	0.978	-1.77 (3.10)	0.570	0.014 (0.048)	0.763	0.487 (2.90)	0.867
rs4680	log(Hg)		-0.032 (0.025)	0.210	-0.919 (1.48)	0.534	-0.019 (0.020)	0.342	-0.813 (1.19)	0.493
	Genotype	GG	Ref (0)		Ref (0)		Ref (0)		Ref (0)	
		GA	0.015 (0.025)	0.550	0.481 (1.46)	0.742	0.009 (0.024)	0.708	0.021 (1.40)	0.988
		AA	0.003 (0.038)	0.944	-0.717 (2.21)	0.746	-0.009 (0.035)	0.801	-0.934 (2.08)	0.654
	log(Hg)*GA		0.052 (0.037)	0.153	1.60 (2.13)	0.455	**0.070 (0.031)**	**0.025**	2.88 (1.82)	0.116
	log(Hg)*AA		0.030 (0.050)	0.550	0.838 (2.93)	0.775	0.038 (0.043)	0.380	0.877 (2.57)	0.734
rs1800497	log(Hg)		-0.012 (0.033)	0.713	-0.387 (1.90)	0.838	-0.008 (0.029)	0.792	-0.117 (1.72)	0.946
	Genotype	CC	Ref (0)		Ref (0)		Ref (0)		Ref (0)	
		TC	**0.077 (0.030)**	**0.010**	**5.43 (1.70)**	**0.002**	**0.074 (0.028)**	**0.010**	**4.81 (1.65)**	**0.004**
		TT	**0.074 (0.034)**	**0.031**	**4.04 (1.97)**	**0.042**	**0.070 (0.033)**	**0.032**	**3.32 (1.91)**	**0.083**
	log(Hg)*TC		0.006 (0.041)	0.881	0.127 (2.35)	0.957	0.031 (0.036)	0.394	0.939 (2.12)	0.658
	log(Hg)*TT		-0.019 (0.052)	0.713	-2.47 (2.96)	0.405	-0.003 (0.041)	0.934	-2.41 (2.43)	0.321
rs1800955	log(Hg)		0.025 (0.038)	0.508	1.50 (2.15)	0.486	**0.067 (0.040)**	**0.097**	**4.11 (2.29)**	**0.075**
	Genotype	TT	Ref (0)		Ref (0)		Ref (0)		Ref (0)	
		CT/CC	0.053 (0.033)	0.116	2.63 (1.88)	0.163	0.038 (0.032)	0.237	1.99 (1.83)	0.279
	log(Hg)*CT/CC		-0.019 (0.047)	0.682	-1.22 (2.65)	0.646	-0.071 (0.045)	0.122	**-4.91 (2.57)**	**0.058**
rs27072	log(Hg)		0.001 (0.021)	0.974	0.431 (1.18)	0.717	0.013 (0.018)	0.479	1.23 (1.04)	0.235
	Genotype	CC	Ref (0)		Ref (0)		Ref (0)		Ref (0)	
		CT/TT	0.035 (0.023)	0.135	**2.43 (1.32)**	**0.067**	**0.038 (0.022)**	**0.086**	**2.55 (1.28)**	**0.047**
	log(Hg)*CT/TT		-0.023 (0.034)	0.492	-2.24 (1.95)	0.251	0.003 (0.028)	0.923	-1.87 (1.64)	0.254

[a]Adjusted for sex of child, age of child at follow-up, study cohort, SES, maternal age, maternal education, maternal IQ, and maternal marital status

[b]Sample sizes range from 89-273 participants. Sample sizes and genotype frequencies are shown in Supplmental Table 2.

[c]log(Hg) values are centered

Discussion

In our study cohort, we found associations between increased CRS-R scores and the TC and TT genotypes of rs1800497 (Taq1A DRD2) and rs27072 (DAT1/SL6A3). These remained statistically significant after adjustment for demographic covariates. Additionally, the AG genotype of rs40184 (DAT1/SL6A3) was associated with lower CRS-R scores, but this was only marginally statistically significant for ADHD index and not significant for DSM-IV total symptoms. However, neither was statistically significant after adjustment. When examining interactions between our candidate SNPs and Hg exposure, only two such interactions were statistically significant. Both only involved hair Hg exposure. For rs1800955 (DRD4), increasing Hg was associated with increasing ADHD symptoms for the TT genotype, but a decrease in those scores for all other genotypes. A similar differential association was noted for rs4680 (COMT), where increasing hair Hg exposure was associated with decreasing ADHD index scores for the GG genotype, but increasing scores about the GA and AA genotypes. This was only statistically significant for the GA genotype, however.

As with our study of MeHg at multiple time points and attention deficits, in our cohort, girls were more likely to have greater attention deficit symptoms in all measures. This was frequently significant or marginally significant. Although it was thought that ADHD predominantly affected boys, more recent research suggests that boys are more likely to be referred for treatment (Bruchmüller et al. 2012; Biederman et al. 2005). Other studies have suggested that girls with ADHD inattention symptoms have greater internalizing symptoms and perceived peer deviation (Becker et al. 2013; Cardoos et al. 2012), which could have a compounding effect on symptoms over time. Additionally, maternal age, maternal education, and marital status were consistently associated with lower attention deficit symptoms. The

association with maternal age was consistent with previous work, where the offspring of younger mothers had the greatest risk for ADHD later in life (Chang et al. 2014; Sagiv et al. 2013). Previous work has also reported that low maternal education may be a risk factor for attention deficits (Sagiv et al. 2013; Gurevitz et al. 2014).

The increased attention scores with the T risk allele of rs1800497 is consistent with previous studies (Pan et al. 2015). While rs27072 has been associated with ADHD, the C allele is generally found to be the risk allele (Feng et al. 2005; Gizer et al. 2009; Ouellet-Morin et al. 2008), although this has been inconsistent (Shang et al. 2011; Genro et al. 2008; Friedel et al. 2007). There has been some evidence that the association with the C allele of rs27072 is only seen in ADHD without the presence of comorbid disorders, such as conduct disorder (Zhou et al. 2008).

To date, there are only a few studies of ADHD-related candidate genes and concurrent MeHg exposure. (Nigg et al. 2010; Tarver et al. 2014). Existing studies generally examine other outcomes, a different series of candidate genes, different time points, or all of these. One such study examined several of our candidate genes, in addition to toxicokinetic related SNPs, in relation to prenatal MeHg exposure and cognition at age 8 years. That study presented evidence for MeHg-gene interactions with transferrin (TF), paraoxonase 1 (PON1), brain-derived neurotrophic factor (BDNF), and progesterone receptor (PGR), but not with our candidate genes (Julvez et al. 2013). Another found evidence for an interaction between prenatal MeHg exposure and apolipoprotein E (*APOE*) genotype on neurodevelopmental outcomes (Ng et al. 2013). Many existing studies of gene-Hg interactions and ADHD examined cigarette smoking, alcohol use, and other factors (Nigg et al. 2010).

However, there have been studies of some of our SNPs with other forms of Hg, such as inorganic Hg as measured in urine. A study by Woods et al examined urinary Hg exposure and several COMT SNPs, including rs4680, and found that the A allele of rs4680 was associated with impaired attention, as was exposure. They also found evidence of an interaction between exposure and the A allele of rs4680, in the direction of further attention deficits. However, this was specific to boys (Woods et al. 2014). Our findings are somewhat consistent with theirs, in that we observed some interactions in the direction of further deficits, but not entirely. Additional analyses in our cohort with sex stratification and with urinary Hg levels in addition to our current biomarkers could help clarify what is attributable to the different forms of Hg and possible sex differences.

There are several limitations to our study that must be addressed. We used a candidate gene approach for this study. Although candidate gene studies are useful, it is likely that there is variability we are unable to capture with this approach. In particular, a number of studies have suggested that the greatest risk comes from deficits in neurotransmitter systems, rather than in single genes (Hawi et al. 2015). Additionally, our procedure for outliers ensured that data was representative of the majority for the available data and was not unduly influenced by outliers, but this may have led to the exclusion of real, but extreme, values. A maximum of 8 outcome measure outliers and 4 exposure measure outliers were removed.

We observed associations with increased attention deficits for the TC and TT genotypes of rs1800497 (Taq1A DRD2) and rs27072 (DAT1/SL6A3). Our study suggests that COMT and DRD4 genotype may modify the effect of postnatal MeHg exposure on two measures of attention in children. Considering genetic factors, such as these, could help explain the discrepancies between studies of MeHg and attention deficits that are not explained by

differences in nutritional factors, such as fish consumption. Further study with additional biomarkers of exposure and examination of other time points could help us to understand these potential interactions.

Appendix 2

Supplemental Tables

Table S2.1.1 Sample sizes and genotype frequencies for crude and adjusted main effect models (found in Tables 2.2 and 2.3)

		ADHD Index		DSM Total	
SNP		**N**	**Percentage**	**N**	**Percentage**
rs6347	AA	89	87.3	90	87.4
	AG	13	12.7	13	12.6
	Total N	102		103	
rs40184	GG	154	80.2	156	80.4
	AG	38	19.8	38	19.6
	Total N	192		194	
rs4680	GG	119	44.7	120	44.8
	GA	113	42.5	114	42.5
	AA	34	12.8	34	12.7
	Total N	266		268	
rs1800497	CC	59	24.9	59	24.7
	TC	116	48.9	118	49.4
	TT	62	26.2	62	25.9
	Total N	237		239	
rs1800955	TT	53	32.1	53	31.9
	CT/CC	112	67.9	113	68.1
	Total N	165		166	
rs27072	CC	170	60.7	171	60.6
	CT/TT	110	39.3	111	39.4
	Total N	280		282	

Table S2.1.2 Sample sizes and genotype frequencies for adjusted models (found in Table 2.4)

		Blood				Hair			
		ADHD Index		DSM Total		ADHD Index		DSM Total	
SNP		N	Percentage	N	Percentage	N	Percentage	N	Percentage
rs6347	AA	77	86.5	78	86.7	86	87.8	87	87.9
	AG	12	13.5	12	13.3	12	12.2	12	12.1
	Total N	89		90		98		99	
rs40184	GG	137	80.6	139	80.8	150	81.5	152	81.7
	AG	33	19.4	33	19.2	34	18.5	34	18.3
	Total N	170		172		184		186	
rs4680	GG	108	45.0	109	45.0	114	44.0	115	44.1
	GA	103	42.9	104	43.0	111	42.9	112	42.9
	AA	29	12.1	29	12.0	34	13.1	34	13.0
	Total N	240		242		259		261	
rs1800497	CC	53	24.9	53	24.7	58	25.3	58	25.1
	TC	107	50.2	109	50.7	112	48.9	114	49.4
	TT	53	24.9	53	24.7	59	25.8	59	25.5
	Total N	213		215		229		231	
rs1800955	TT	50	33.6	50	33.3	52	32.9	52	32.7
	CT/CC	99	66.4	100	66.7	106	67.1	107	67.3
	Total N	149		150		158		159	
rs27072	CC	148	59.4	149	59.4	166	61.3	167	61.2
	CT/TT	101	40.6	102	40.6	105	38.7	106	38.8
	Total N	249		251		271		273	

Table S2.2.1 Crude and adjusted main effects models of rs6347. Darker shades of blue indicate larger positive betas, while darker shades of red indicate larger negative betas.

	rs6347 (DAT1/SLC6A3)							
	log(ADHD)				DSM Total			
	Beta	P-value	Beta	P-value	Beta	P-value	Beta	P-value
Model		NS		NS		NS		NS
Intercept	3.965	<<0.001	3.965	<<0.001	55.733	<<0.001	49.091	0.004
genotype: AG	0.024		0.017		-0.656		-0.848	
Sex of Child: Female			0.024				1.946	
Age of Child at Visit			-0.015				-0.257	
Cohort: 2a			0.042				1.245	
Cohort: 3			0.019				0.941	
SES Level			0.004				-0.141	
Maternal Age			-7.7E-04				0.107	
Maternal Education			-0.010				-0.631	
Maternal IQ			0.003	0.012			0.156	0.020
Maternal Marital Status: Married			-0.052				-2.400	

Table S2.2.2 Crude and adjusted main effects models of rs40184. Darker shades of blue indicate larger positive betas, while darker shades of red indicate larger negative betas.

	rs40184 (DAT1/SLC6A3)							
	log(ADHD)				DSM Total			
	Beta	P-value	Beta	P-value	Beta	P-value	Beta	P-value
Model		0.083		NS		NS		NS
Intercept	3.977	<<0.001	4.132	<<0.001	56.032	<<0.001	62.228	<0.001
genotype: AG	-0.055	0.083	-0.043		-2.979		-2.431	
Sex of Child: Female			0.046	0.082			3.301	0.036
Age of Child at Visit			-0.008				-0.285	
Cohort: 2a			-0.055	0.082			-2.723	
Cohort: 3			-0.011				-0.817	
SES Level			0.001				-0.116	
Maternal Age			-0.002				-0.021	
Maternal Education			-0.008				-0.590	0.081
Maternal IQ			4.7E-04				0.034	
Maternal Marital Status: Married			-0.001				0.385	

Table S2.2.3 Crude and adjusted main effects models of rs4680. Darker shades of blue indicate larger positive betas, while darker shades of red indicate larger negative betas.

	rs4680 (COMT)							
	log(ADHD)				DSM Total			
	Beta	P-value	Beta	P-value	Beta	P-value	Beta	P-value
Model		NS		NS		NS		NS
Intercept	3.972	<<0.001	4.108	<<0.001	55.892	<<0.001	56.334	<0.001
genotype: GA	0.009		0.015		0.047		0.378	
genotype: AA	-0.012		-0.005		-1.245		-0.708	
Sex of Child: Female			0.046	0.040			3.776	0.004
Age of Child at Visit			-0.007				0.249	
Cohort: 2a			-0.019				-0.809	
Cohort: 3			-0.030				-0.547	
SES Level			-0.001				-0.187	
Maternal Age			-0.003				-0.127	
Maternal Education			-0.009	0.053			-0.486	0.076
Maternal IQ			0.001				0.052	
Maternal Marital Status: Married			0.011				0.708	

Table S2.2.4 Crude and adjusted main effects models of rs1800497. Darker shades of blue indicate larger positive betas, while darker shades of red indicate larger negative betas.

	rs1800497 (Taq1A DRD2/ANKK1)							
	log(ADHD)				DSM Total			
	Beta	P-value	Beta	P-value	Beta	P-value	Beta	P-value
Model		0.014		0.007		0.008		0.006
Intercept	3.909	<<0.001	4.175	<<0.001	51.881	<<0.001	58.957	<0.001
genotype: TC	0.079	0.005	0.077	0.006	5.068	0.002	4.895	0.003
genotype: TT	0.073	0.022	0.070	0.028	3.441	0.062	3.227	0.084
Sex of Child: Female			0.044	0.056			3.266	0.014
Age of Child at Visit			-0.014				0.021	
Cohort: 2a			-0.024				-1.821	
Cohort: 3			-0.094	0.043			-3.256	
SES Level			0.002				-0.087	
Maternal Age			-0.003				-0.130	
Maternal Education			-0.009	0.047			-0.549	0.044
Maternal IQ			0.001				0.029	
Maternal Marital Status: Married			-0.011				-0.124	

Table S2.2.4 Crude and adjusted main effects models of rs1800955. Darker shades of blue indicate larger positive betas, while darker shades of red indicate larger negative betas.

	rs1800955 (DRD4)							
	log(ADHD)				DSM Total			
	Beta	P-value	Beta	P-value	Beta	P-value	Beta	P-value
Model		NS		NS		NS		NS
Intercept	3.956	<<0.001	4.003	<<0.001	54.811	<<0.001	59.251	<0.001
genotype: CT/CC	0.031		0.045		1.348		2.308	
Sex of Child: Female			0.034				1.851	
Age of Child at Visit			-0.001				-0.448	
Cohort: 2a			-0.021				-0.449	
Cohort: 3			0.021				0.405	
SES Level			-0.005				-0.476	
Maternal Age			-0.002				-0.033	
Maternal Education			-0.014	0.036			-0.604	
Maternal IQ			0.002	0.071			0.084	
Maternal Marital Status: Married			0.033				1.513	

Table S2.2.6 Crude and adjusted main effects models of rs27072. Darker shades of blue indicate larger positive betas, while darker shades of red indicate larger negative betas.

	rs27072 (DAT1/SLC6A3)							
	log(ADHD)				DSM Total			
	Beta	P-value	Beta	P-value	Beta	P-value	Beta	P-value
Model		0.041		0.025		0.033		0.011
Intercept	3.956	<<0.001	4.055	<<0.001	54.515	<<0.001	55.987	<0.001
genotype: CT/TT	0.044	0.041	0.041	0.057	2.702	0.033	2.595	0.039
Sex of Child: Female			0.054	0.014			3.494	0.006
Age of Child at Visit			2.1E-05				0.317	
Cohort: 2a			-0.026				-1.763	
Cohort: 3			-0.029				-1.161	
SES Level			-5.4E-04				-0.180	
Maternal Age			-0.003				-0.092	
Maternal Education			-0.010	0.035			-0.614	0.022
Maternal IQ			8.9E-04				0.057	
Maternal Marital Status: Married			-0.006				-0.249	

Table S2.3.1 Gene-Environment interaction model for rs6347. "centhg" refers to centered, log-transformed mercury concentration. Darker shades of blue indicate larger positive betas, while darker shades of red indicate larger negative betas.

	rs6347 (DAT1/SLC6A3)							
	Blood				Hair			
	log(ADHD)		DSM Total		log(ADHD)		DSM Total	
	Beta	P-value	Beta	P-value	Beta	P-value	Beta	P-value
Model		NS		NS		NS		NS
Intercept	3.859	<<0.001	42.917	0.016	4.057	<<0.001	56.327	0.002
centhg	-0.035		-0.383		-0.004		0.591	
genotype: AG	0.020		-0.447		0.049		0.877	
Sex of Child: Female	0.024		2.005		0.012		1.336	
Age of Child at Visit	-0.007		0.343		-0.029		-1.052	
Cohort: 2a	0.053		2.161		0.038		1.174	
Cohort: 3	0.062		3.760		-0.004		-0.852	
SES Level	0.001		-0.422		0.005		-0.027	
Maternal Age	-0.003		-0.053		2.0E-04		0.154	
Maternal Education	-0.006		-0.575		-0.009		-0.649	
Maternal IQ	0.003	0.007	0.188	0.007	0.003	0.015	0.144	0.040
Maternal Marital Status: Married	-0.038		-0.733		-0.045		-2.114	
centhg*genotype: AG	0.069		3.209		0.053		2.695	

Table S2.3.2 Gene-Environment interaction model for rs40184. "centhg" refers to centered, log-transformed mercury concentration. Darker shades of blue indicate larger positive betas, while darker shades of red indicate larger negative betas.

	rs40184 (DAT1/SLC6A3)							
	Blood				Hair			
	log(ADHD)		DSM Total		log(ADHD)		DSM Total	
	Beta	P-value	Beta	P-value	Beta	P-value	Beta	P-value
Model		NS		NS		NS		NS
Intercept	4.126	<<0.001	61.049	<0.001	4.139	<<0.001	62.440	<0.001
centhg	-0.010		-0.057		-0.008		-0.429	
genotype: AG	-0.053		-3.161		-0.042		-1.982	
Sex of Child: Female	0.044		3.339	0.046	0.040		2.954	
Age of Child at Visit	-0.008		-0.268		-0.011		-0.378	
Cohort: 2a	-0.059	0.078	-2.756		-0.058	0.076	-2.909	
Cohort: 3	0.003		-0.340		-0.018		-1.125	
SES Level	-0.001		-0.230		-1.8E-04		-0.165	
Maternal Age	-0.003		-0.112		-0.001		-0.001	
Maternal Education	-0.007		-0.480		-0.008		-0.619	0.082
Maternal IQ	0.001		0.050		0.001		0.043	
Maternal Marital Status: Married	0.009		1.766		0.006		0.747	
centhg*genotype: AG	-0.001		-1.766		0.014		0.487	

Table S2.3.3 Gene-Environment interaction model for rs4680. "centhg" refers to centered, log-transformed mercury concentration. Darker shades of blue indicate larger positive betas, while darker shades of red indicate larger negative betas.

	rs4680 (COMT)							
	Blood				Hair			
	log(ADHD)		DSM Total		log(ADHD)		DSM Total	
	Beta	P-value	Beta	P-value	Beta	P-value	Beta	P-value
Model		NS		NS		NS		NS
Intercept	4.132	<<0.001	56.379	<0.001	4.149	<<0.001	57.830	<0.001
centhg	-0.032		-0.919		-0.019		-0.813	
genotype: GA	0.015		0.481		0.009		0.021	
genotype: AA	0.003		-0.717		-0.009		-0.934	
Sex of Child: Female	0.047	0.050	3.771	0.007	0.043	0.057	3.571	0.007
Age of Child at Visit	-0.008		0.272		-0.013		0.006	
Cohort: 2a	-0.015		-0.536		-0.018		-0.790	
Cohort: 3	-0.022		0.056		-0.049		-1.341	
SES Level	-0.002		-0.292		-0.001		-0.194	
Maternal Age	-0.003		-0.213		-0.002		-0.095	
Maternal Education	-0.009	0.073	-0.477	0.098	-0.010	0.039	-0.539	0.054
Maternal IQ	0.001		0.063		0.001		0.059	
Maternal Marital Status: Married	0.025		1.991		0.027		1.332	
centhg*genotype: GA	0.052		1.596		0.070	0.025	2.877	
centhg*genotype: AA	0.030		0.838		0.038		0.877	

Table S2.3.4 Gene-Environment interaction model for rs1800497. "centhg" refers to centered, log-transformed mercury concentration. Darker shades of blue indicate larger positive betas, while darker shades of red indicate larger negative betas.

	rs1800497 (Taq1A DRD2/ANKK1)							
	Blood				Hair			
	log(ADHD)		DSM Total		log(ADHD)		DSM Total	
	Beta	P-value	Beta	P-value	Beta	P-value	Beta	P-value
Model		0.040		0.006		0.022		0.014
Intercept	4.184	<<0.001	56.804	<0.001	4.221	<<0.001	60.387	<0.001
centhg	-0.012		-0.387		-0.008		-0.117	
genotype: TC	0.077	0.010	5.426	0.002	0.074	0.010	4.807	0.004
genotype: TT	0.074	0.031	4.036	0.042	0.070	0.032	3.325	0.083
Sex of Child: Female	0.039		3.300	0.018	0.037		3.106	0.023
Age of Child at Visit	-0.014		0.243		-0.019		-0.193	
Cohort: 2a	-0.022		-1.551		-0.023		-1.587	
Cohort: 3	-0.079		-2.042		-0.111	0.020	-3.757	
SES Level	3.7E-04		-0.164		3.1E-04		-0.122	
Maternal Age	-0.004	0.063	-0.251	0.062	-0.003		-0.100	
Maternal Education	-0.009	0.089	-0.470		-0.010	0.043	-0.544	0.055
Maternal IQ	6.8E-04		0.039		0.001		0.028	
Maternal Marital Status: Married	-5.3E-05		0.892		-0.005		0.270	
centhg*genotype: TC	0.006		0.127		0.031		0.939	
centhg*genotype: TT	-0.019		-2.469		-0.003		-2.412	

Table S2.3.4 Gene-Environment interaction model for rs1800955. "centhg" refers to centered, log-transformed mercury concentration. Darker shades of blue indicate larger positive betas, while darker shades of red indicate larger negative betas.

	rs1800955 (DRD4)							
	Blood				Hair			
	log(ADHD)		DSM Total		log(ADHD)		DSM Total	
	Beta	P-value	Beta	P-value	Beta	P-value	Beta	P-value
Model		NS		NS		NS		NS
Intercept	4.019	<<0.001	60.385	<0.001	4.065	<<0.001	62.496	<0.001
centhg	0.025		1.499		0.067	0.097	4.108	0.075
genotype: CT/CC	0.053		2.635		0.038		1.987	
Sex of Child: Female	0.036		1.836		0.019		1.015	
Age of Child at Visit	-3.6E-04		-0.372		-0.007		-0.730	
Cohort: 2a	-0.015		0.080		-0.016		-0.169	
Cohort: 3	0.036		1.706		-0.004		-0.815	
SES Level	-0.010		-0.663		-0.006		-0.488	0.095
Maternal Age	-0.004		-0.171		-0.002		-0.019	
Maternal Education	-0.015	0.030	-0.668	0.082	-0.017	0.015	-0.801	0.041
Maternal IQ	0.002	0.036	0.100	0.078	0.002	0.031	0.106	0.062
Maternal Marital Status: Married	0.057		3.234		0.043		1.973	
centhg*genotype: CT/CC	-0.019		-1.219		-0.071		-4.908	0.058

Table S2.3.6 Gene-Environment interaction model for rs27072. "centhg" refers to centered, log-transformed mercury concentration. Darker shades of blue indicate larger positive betas, while darker shades of red indicate larger negative betas.

	rs27072 (DAT1/SLC6A3)							
	Blood				Hair			
	log(ADHD)		DSM Total		log(ADHD)		DSM Total	
	Beta	P-value	Beta	P-value	Beta	P-value	Beta	P-value
Model		0.081		0.021		0.069		0.023
Intercept	4.072	<<0.001	55.781	<0.001	4.083	<<0.001	56.893	<0.001
centhg	0.001		0.431		0.013		1.235	
genotype: CT/TT	0.035		2.433	0.067	0.038	0.086	2.553	0.047
Sex of Child: Female	0.055	0.020	3.863	0.005	0.050	0.026	3.358	0.009
Age of Child at Visit	-2.6E-04		0.420		-0.003		0.210	
Cohort: 2a	-0.026		-1.615		-0.025		-1.753	
Cohort: 3	-0.018		-0.355		-0.042		-1.921	
SES Level	-0.002		-0.256		-0.002		-0.195	
Maternal Age	-0.004	0.057	-0.223	0.087	-0.003		-0.084	
Maternal Education	-0.009	0.074	-0.525	0.064	-0.011	0.024	-0.660	0.017
Maternal IQ	0.001		0.066	0.124	0.001		0.066	
Maternal Marital Status: Married	-4.8E-04		0.573		0.001		-0.254	
centhg*genotype: CT/TT	-0.023		-2.244		0.003		-1.872	

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Chapter 3

Relationships between Acoustic Startle Reflex, Prepulse Inhibition, and Methylmercury in Adolescents

Abstract

Prepulse inhibition (PPI) is a sensorimotor gating process in the startle reflex that is modified under different behavioral conditions (e.g. during attention tasks). This can be impaired in a number of behavioral disorders, such as attention deficit disorder. Although extensively studied in animal models, few studies have explored the use of the PPI to assess neurological effects of toxicants in humans. We aim to describe the relationship between acoustic startle reflex (ASR), PPI and MeHg exposure in a cohort of adolescents. We report on recordings from 231 adolescents aged 8-17 years from the Early Life Exposure in Mexico to Environmental Toxicants (ELEMENT) study. We used quantile regression to examine relationships between blood and hair Hg levels and PPI. ASR without a prepulse was non-linearly associated with MeHg exposure. For both target and non-target ASR response magnitudes with prepulses, higher MeHg generally corresponded to higher ASR magnitudes, especially in the right tail of their respective distributions. There was a linear, but not necessarily significant, association with increased inhibition with MeHg in the 25^{th} percentile and median of the PPI distribution in trials with an attended tone, but a linear association with decreased inhibition in the 75^{th} percentile. These findings add to our understanding of ASR and PPI, particularly in the context of environmental exposures.

Introduction

The startle reflex is a well-characterized response to a sudden, unexpected loud sound, and involves the sudden contraction of the facial muscles, an increase in skin conductance, and an increase in heart rate (Li et al. 2009). The startle reflex is typically reduced when the startling stimulus is preceded by a weaker stimulus to which the person needs to attend, referred to as a prepulse. This is referred to as prepulse inhibition (PPI). PPI is a basic attention mechanism in the brain: a sensorimotor gating process that helps focus attention on stimuli that requires a response. The weaker stimulus causes the later startling stimulus to be "gated out," resulting in the characteristic inhibition (Alvarez-Blanco et al. 2009; Braff et al. 2001). At the neuronal level, the prepulse induces a negative feedback loop, inhibiting the startle initiating neurons. Thus, the resulting startle is not as great (Li et al. 2009).

In subjects with ADHD, the observed change in magnitude is not as great (Braff et al. 2001). This is most noticeable when subjects are asked to use selective attention, typically via a task (Filion and Poje 2003). Additionally, deficits in PPI in ADHD cases have been observed to be remediated with methylphenidate treatment (Hawk et al. 2003; Schulz-Juergensen et al. 2014). This is notable because methylphenidate, an extremely common treatment for ADHD, acts on dopamine signaling (Jenson et al. 2015).

Although PPI deficits in different psychiatric conditions and how they respond to a number of psychiatric drugs have been relatively well characterized in humans (Braff et al. 2001; Kohl et al. 2013), studies of PPI and environmental exposures in humans are comparatively few. Those that have been completed generally focus on tobacco use, alcohol, or psychosocial stressors. Some animal studies, however, suggest that one potential environmental exposure of interest is methylmercury.

Methylmercury (MeHg) exposure has been linked with attention and cognition deficits (Karagas et al. 2012), which is not surprising as this organic form of mercury (Hg) is an established neurodevelopmental toxicant (Clarkson and Magos 2006). Exposure to MeHg is primarily via fish and seafood consumption and is thus ubiquitous amongst populations that consume fish (Driscoll et al. 2013). Mercury exposure has been suggested to modify the processing and release of several neurotransmitters, particularly dopamine, which have been implicated in the etiology of deficits in attention and cognition (Faraone and Mick 2010). It has previously been suggested that PPI might be useful as a measure of dopaminergic function (Swerdlow et al. 2003). The remediation of PPI deficits in ADHD with methylphenidate treatment, which acts on dopamine levels (Hawk et al. 2003; Schulz-Juergensen et al. 2014), also suggests MeHg may have effects on PPI deficits.

To our knowledge, no studies have examined the relationship between Hg exposure and PPI in humans. To date, only a few animal studies have been conducted examining MeHg and PPI. Wu et al found that rats with MeHg poisoning had deficits in PPI, though there was not a clear dose-response relationship (Wu et al. 1985). Vezer at al found similar results in sub-chronically dosed rats (Vezer et al. 2005). Beyrouty et al saw disrupted unmodified startle responses after prenatal MeHg exposure in rats, with few significant difference in behavioral tests, including motor activity, swimming performance, and a functional observation battery (Beyrouty et al. 2006).

Here, we aim to explore the relationship between concurrent MeHg exposure and both the basic acoustic startle reflex (ASR) and prepulse inhibition (PPI). We hypothesize that increased ASR and deficient PPI will be associated with high concurrent MeHg exposure, as suggested by the existing animal studies.

Methods

ELEMENT Cohort

The ELEMENT study, consisting of three sequentially enrolled cohorts, was initially designed to research the influence of maternal lead exposure on offspring neurodevelopment. Pertinent details of ELEMENT, such as inclusion and exclusion criteria, collection methods, and demographics can be found elsewhere (Tellez-Rojo et al. 2006; Afeiche et al. 2011). In brief, Cohort 1 subjects were recruited 1994-1995, Cohort 2 subjects were recruited 1997-2001, Cohort 3 subjects were recruited 2001-2004 (Afeiche et al. 2011). In 2006 participants were recruited from all three cohorts for follow-up visits regarding behavioral outcomes. For analysis of concurrent exposures, children were included if they had at least one mercury exposure value and also had electromyography response data.

The research protocol was approved by the ethics and research committees of the partnering institutions, including the National Institute of Public Health of Mexico, the Harvard School of Public Health, the Brigham and Women's Hospital, the University of Michigan School of Public Health, the University of Toronto, and the participating hospitals.

Human Biospecimens and Mercury Analysis

Blood and hair samples were collected from the participating children at the follow-up visit. Venous whole blood samples were collected into vials certified for trace metals analysis and stored at 4^{o}C until analysis. Scalp hair samples were obtained from each participant using stainless steel scissors and the proximal end was designated. Mercury was analyzed in all samples as described elsewhere (Basu et al. 2014). Briefly, total mercury content was carried out using a Direct Mercury Analyzer 80 (DMA-80, Milestone Inc., CT). Daily instrument calibration, procedural blanks, replicates, and several certified reference materials were analyzed.

Reference materials included CRM #13 for hair (National Institute for Environmental Studies, Japan), DOLT-4 (dogfish liver; National Research Council, Canada), and QMEQAS for blood and urine (Institut National de Santé Publique du Québec). Recoveries of the reference materials ranged from 80 to 110%. The analytical detection limit was less than 1.0 ng mercury.

PPI Recording and Analysis

Participants were placed in an isolated setting during the clinic visit. Surface electrodes (Ambu, Balerup, Denmark) were affixed to participants over their orbicularis oculi muscle (for eyeblink), wrist, and chest to measure electromyography (EMG), skin conductance, and heart rate, respectively. Using integrated stimulus and presentation software (BIOPAC, Goleta, CA) participants were then presented with a series of tones at 75 dB over headphones until they could accurately distinguish between high (1200 Hz) and low (400 Hz) pitched tones and short (5 second) and long (8 second) duration tones. Following this, during the tone discrimination task, three randomly presented blocks of tones in a pseudorandom order were presented, including a total of 36 startle probes (a 50 ms burst of white at 102dB). Each block included 6 presentations each of the low and high frequency tones, and 3 during the intertrial interval (ITI; i.e. those with no tone). Startle probes that were presented with tones came either 120 ms or 240 ms after the onset of the tone (referred to as a stimulus onset asynchrony, SOA). Prior to starting the discrimination task, participants were instructed to press the space bar in response to the long tones. Thus, this selected pitch was considered the "target tone" as it was the one the participant was expected to attend to. The other tone was considered the "non-target tone" as the participant did not need to respond (and therefore attend) to this tone. A cartoon diagram of example responses is shown in Figure 3.1.I. After the participant completed the task, the electrodes were

removed. This methodology follows a similar design to that used in other studies of prepulse inhibition (Hawk et al. 2003).

PPI was calculated for each block as $\frac{(Average\ Magnitude\ X - Average\ Magnitude\ ITI)}{Average\ Magnitude\ ITI}$, where average magnitude *X* is the average magnitude of responses in the presence of a tone within the given block and average magnitude ITI is the average magnitude of responses during the ITI of the same block. We considered PPI for target tones and non-target tones separately, and responses within a block were averaged. Because there may be differences in the extent of PPI when the SOA is 120 ms or 240 ms, we considered PPI separately for these SOA as well as PPI when considering all target or non-target responses irrespective of the SOA.

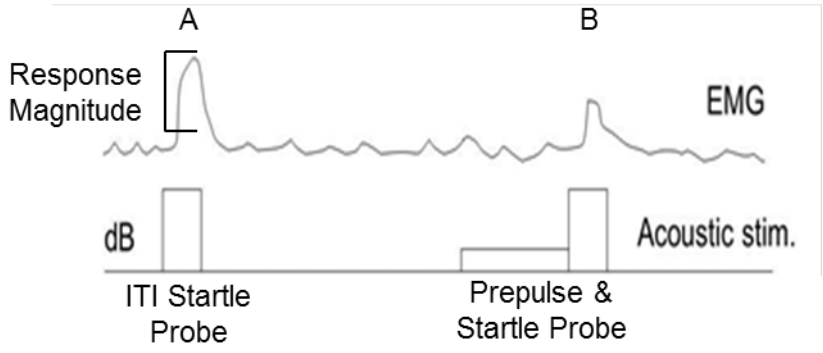

Figure 3.1.I Cartoon of acoustic stimuli vs expected EMG results. This shows the startle probes for ITI trials and those with prepulses. Response magnitudes (shown for response A) are calculated as the peak EMG value less the baseline EMG value. PPI is then calculated as $\frac{(Average\ Magnitude\ B - Average\ Magnitude\ A)}{Average\ Magnitude\ A}$.

Statistical Analysis

Data were analyzed using R x64 3.0.1. Univariate descriptive statistics and graphical displays were obtained for all variables. Outliers were detected using the ExtremeValues package for R, which uses a distribution based method for identifying outliers (van der Loo 2010). Because most values of PPI were negative, this package was not suitable for outlier

detection. Thus, outliers were identified and removed by identifying the values of the 1st and 99th percentiles. Spearman correlations were used to assess association among all biomarkers. Bivariate analyses (correlations with t-test) were used to relate mercury biomarker values with demographic characteristics. Descriptive statistics are reported as mean (standard deviation), unless otherwise indicated.

The crude and adjusted associations between the neurological outcomes and mercury were estimated using quantile regression. A distribution of residuals from linear modeling deviated from normality. Because a large portion of the distribution for PPI included negative values, log-transformation to correct this was not possible. Thus, we sought alternative methods that would yield valid inferences. Quantile regression enables us to study shifts at any quantile of the distribution, rather than at the mean as done in ordinary linear regression. We examined the 25th, 50th, and 75th percentiles, since these are representative of the overall distribution. This enables us to look at the tails of the distribution in addition to the center. Additionally, regressions were run with exposure as a continuous variable and as quartiles of exposure (referred to as "Low", "Medium-Low", "Medium-High", and "High").

Models included a number of covariates selected for potential relevance to either the exposure or the analyzed attention measures. Maternal IQ was calculated based on the mothers' scores on the Spanish Wechsler Adult Intelligence Scale (Tellez-Rojo et al. 2004; Wechsler 1968). Maternal education was the cumulative number of years that the mother attended school at time of recruitment. Smoking during pregnancy (yes/no) was obtained from a questionnaire administered to the mother at or before 1-month post-partum. A "yes" response at any point in pregnancy was coded as a yes for this covariate. Because only a small number of mothers reported smoking during pregnancy, data from children whose mothers' smoked were excluded

from analyses. As previously described (Fortenberry et al. 2014), direct questions about income were deemed too intrusive within this cohort. Thus, a measure of socioeconomic status based on reported possessions and household assets was used instead. Maternal age and marital status at recruitment were also included, as were child age at the follow-up visit and child sex. Protocol block sequence was also included.

Because we were using the quantile regression method, outcome measures were not transformed. However, exposure measures were log-transformed prior to entering into the models, given their high skewness. The actual beta coefficients are presented in tables and figures. However, in the text, we also provide interpretations that consider the transformation. Specifically, we calculated the difference in the outcome for a 10% higher Hg concentration as $\Delta Outcome = \beta_{exposure} * \ln(1.10)$. These differences in the outcomes are given for the 25^{th}, 50^{th}, and 75^{th} percentiles of the outcome distribution.

Results

Population Characteristics

Overall, 450 children participated in the visit where startle response was tested. Of these, 231 had complete covariates and at least one of the startle response measures. A slight majority of participants were male (Table 3.1). A majority of mothers had been married at the time they were recruited. Six mothers reported smoking during pregnancy. Given this small proportion of smokers in our sample and the existing literature suggesting an association between maternal smoking status in pregnancy and later attention problems (Duncan et al. 2001a; Popke et al. 1997), these mothers, and their children, were omitted from subsequent analyses.

Exposure data for this cohort was previously reported for mercury (Basu et al. 2014) and briefly summarized here. Mercury exposure data is available for 88.8-91.0% of the children,

depending on the biomarker. Blood and hair mercury levels of participating children were 2.07 ± 2.03 µg/L and 0.53 ± 0.44 µg/g respectively (Table 3.1). Blood and hair mercury levels of the same individuals were correlated ($r=0.75$, $p<0.001$).

The EMG data, including outcomes related to EMG response amplitude and calculated PPI, were available at least in part from 231 participants.

Table 3.1

DEMOGRAPHIC CHARACTERISTICS	**N**	**Mean(SD)**	**Median**	**Range**
Child Age	231	13.13 (2.53)	12.3	(8.1, 17.4)
Household SES Level	231	6.63 (2.63)	6.0	(1.0, 13.5)
Maternal Age at Recruitment	231	26.11 (5.22)	26.0	(16.0, 42.0)
Maternal Education Level at Recruitment	231	10.73 (2.98)	11.0	(1.0, 20.0)
Maternal IQ	233	92.32 (17.46)	91.0	(60.0, 138.0)
	Total N	**N(%)**		
Sex of Child (Male)	231	125 (54.11)		
Maternal Smoking During Pregnancy ("Ever Smoked")	231	6 (2.60)		
Maternal Marital Status ("Married")	231	168 (72.73)		
EXPOSURE ASSESSMENT	**N**	**Mean(SD)**	**Median**	**Range**
Mercury (µg/L or µg/g)				
Blood (µg/L)	212	2.07 (2.03)	1.37	(0.18, 11.6)
Hair (µg/g)	207	0.53 (0.44)	0.38	(0.07, 2.5)
TRIAL RESPONSES	**N**	**Mean (SD)**	**Median**	**Range**
EMG MAGNITUDE				
ITI	180	2.95 (2.56)	2.10	(0.51, 16.13)
Target (All)	188	2.21 (1.96)	1.57	(0.51, 10.62)
Target (120 SOA)	138	2.13 (1.87)	1.41	(0.52, 9.44)
Target (240 SOA)	148	2.28 (2.04)	1.47	(0.50, 12.02)
Non-Target (All)	169	1.94 (1.51)	1.37	(0.51, 7.39)
Non-Target (120 SOA)	128	2.06 (1.79)	1.51	(0.51, 8.94)
Non-Target (240 SOA)	139	2.04 (1.65)	1.37	(0.52, 8.16)
PRE-PULSE INHIBITION				
Target (All)	160	0.002 (0.909)	-0.175	(-0.827, 5.20)
Target (120 SOA)	115	-0.152 (0.781)	-0.398	(-0.872, 4.86)
Target (240 SOA)	133	-0.046 (0.752)	-0.226	(-0.843, 4.83)
Non-Target (All)	146	-0.151 (0.602)	-0.331	(-0.808, 2.85)
Non-Target (120 SOA)	115	-0.162 (0.699)	-0.415	(-0.857, 2.52)
Non-Target (240 SOA)	124	-0.177 (0.573)	-0.369	(-0.863, 2.36)

Quantile Regression for Response Magnitude

Quantile regression models were constructed that considered exposure in two different ways. First, exposure was considered as a continuous log-transformed variable to look for linear associations. Then, quartiles of exposure were considered to examine possible non-linearity. In all models using quartiles of exposure used the "Low" group as the reference group.

Table 3.2 Upper ends of exposure quartiles

	Blood (μg/L)	**Hair (μg/g)**
Low	0.815	0.240
Medium-Low	1.37	0.381
Medium-High	2.49	0.528
High	11.56	0.657

Baseline Acoustic Startle Response

The baseline ASR response, i.e. that evoked during the ITI, showed non-linear associations with blood Hg at all quantiles of the response distribution (Figure 3.1). The pattern of association appeared U-shaped, with greater responses in the medium-low and medium-high exposure groups compared with the low group (some even reaching statistical significance), but not in the highest exposure group. This pattern was stronger for the higher quartiles of the distribution. A similar pattern was seen with hair Hg.

For instance, the medium-low exposure group of blood Hg was associated with a 0.376 unit increase (95% CI: 0.039, 0.713) in the 25^{th} percentile, a 0.214 unit increase (95% CI: -0.658, 1.09) in the median, but a 0.640 unit increase (95% CI: -0.379, 1.66) in the 75^{th} percentile, as compared to the low exposure group. The medium-high exposure group of blood Hg was associated with a 0.510 unit increase (95% CI: -0.079, 1.10) in the 25^{th} percentile, a 0.685 unit increase (95% CI: -0.455, 1.83) in the median, but a 1.69 unit increase (95% CI: 0.198, 3.19) in the 75^{th} percentile, as compared the low exposure group. In the high exposure group of blood Hg

was associated with a 0.260 unit increase (95% CI: -0.155, 0.675) in the 25th percentile, a 0.107 unit increase (95% CI: -0.866, 1.08) in the median, but a 0.124 unit increase (95% CI: -1.14, 1.39) in the 75th percentile, as compared to the low exposure group.

A similar pattern was observed with hair Hg exposure. In the 25th and 75th percentile, the same U-shaped pattern where the largest magnitude of association was seen in the medium-low and medium-high exposure groups. Again, the largest magnitudes of association overall were observed in the upper-tail of the outcome distribution. There was a deviation from the pattern in the median of the distribution. There, the medium-low exposure group of hair Hg was associated with a 0.484 unit increase (95% CI: -0.130, 1.10) and the medium-high exposure group was associated with a 0.307 unit increase (95% CI: -0.440, 1.05) as compared to the low exposure group. However, in the high exposure group, a 0.563 unit increase (95% CI: -0.262, 1.39) as compared the low exposure group.

Quantile Regression of Prepulse Inhibition

TARGET

There were several linear associations between exposure and prepulse inhibition for some startle probe types (Figures 3.2A and 3.2B). In the 25th percentile, there was a linear association between exposure and both broad responses and 240 ms tone responses. A 10% increase in blood Hg was associated with a 0.001 unit decrease (95% CI: -0.011, 0.009; p=0.063) in the 25th percentile of all target response magnitudes, while a 10% increase in hair Hg was associated with a 0.001 unit increase (95% CI: -0.011, 0.012) in the same (calculated as $\Delta Outcome_{Blood} = -0.001 = -0.011 * \ln(1.10)$ and $\Delta Outcome_{Hair} = 0.001 = 0.006 * \ln(1.10)$ based on the log-transformation used in the models). For 240 ms tone target responses, a 10% increase in blood Hg was associated with a 0.004 unit increase (95% CI: -0.011, 0.019; p=0.069) in the 25th

percentile of response magnitude, while a 10% increase in hair Hg was associated with a 0.002 unit decrease (95% CI: -0.016, 0.012). There were also linear associations between exposure and the 75th percentile of the broad responses. There, a 10% increase in blood Hg levels was associated with a 0.045 unit increase (95% CI: 0.006, 0.085; p=0.058) in the 75th percentile, while a 10% increase in hair Hg levels was associated with a 0.062 unit increase (95% CI: 0.028, 0.097; p=0.035) in the same. Additionally, a 10% increase in hair Hg levels was associated with a 0.029 unit increase (95% CI: -0.002, 0.059) in the median distribution of 120 ms tone. However, this was not observed for blood Hg exposure (Figures 3.2A-B).

A U-shaped pattern of response was noted for the median of the distribution of all target responses and both biomarkers (Figure 3.2C). However, while both have the greatest magnitude of difference, as compared to the low reference group, in the medium-low or medium-high exposure groups, for hair Hg exposure these were increases compared to the reference, while for blood this was a decrease compared to the reference group. A 10% increase in blood Hg exposure was associated with a 0.003 unit increase (95% CI: -0.020, 0.025) in the median of all target responses when examined linearly. When examining the exposure as quartiles, the medium-low group was associated with a 0.120 unit decrease (95% CI: -0.360, 0.119) in the median as compared to the low exposure group, the medium-high group was associated with a 0.064 unit decrease (95% CI: -0.433, 0.306), and the high exposure group was associated with a 0.058 unit decrease (95% CI: -0.433, 0.306). A similar pattern was observed for the 25th percentile of the 120 ms tone distribution and blood Hg exposure. However, none of these associations was significant.

NON-TARGET

There were several linear associations between non-target PPI responses and blood Hg exposure, but not hair Hg exposure (Figures 3.3A and 3.3B). Generally, there was much less of a consistent pattern between exposure and PPI for non-target tones than for the target tones. Most notably, a 10% increase in blood Hg exposure was associated with a 0.010 (95% CI: 0.0003, 0.020; p=0.045) unit increase in the 25th percentile and a 0.008 (95% CI: -0.005, 0.021) unit increase in the median, but a 0.014 (95% CI: -0.046, 0.018) unit decrease in the 75th percentile of the 120 ms tone non-target responses. Additionally, a 10% increase in blood Hg exposure was associated with a 0.003 unit increase (95% CI: -0.003, 0.009) in the 25th percentile of the distribution of all non-target responses and a 0.005 unit decrease (95% CI: -0.015, 0.005) in the median of the 240 ms tone non-target response distribution.

A 10% increase in hair Hg was associated with a 0.006 unit increase (95% CI: -0.002, 0.014) in the 25th percentile of the broad response distribution. However, when examining exposure by quartile, a U-shaped pattern of responses was observed (Figure 3.3C). The medium-low exposure group was associated with a 0.083 unit decrease (95% CI: -0.270, 0.104) in the 25th percentile of the broad non-target responses, as compared to the low exposure group, and the medium-high exposure group was associated with a 0.156 unit decrease (95% CI: -0.350, 0.038) as compared to the reference. The high exposure group was then associated with a 0.066 unit decrease (95% CI: -0.240, 0.108) in the 25th percentile of the broad non-target responses as compared to the low exposure group. Thus, the higher exposure groups had decreasing PPI as compared to the low exposure group, but the magnitude difference of this peaked in the medium-high group. A similar pattern was observed for the median of the 120 ms tone and the 75th

percentile of the 240 ms tone non-target response distribution. None of these were statistically significant.

Discussion

Our objective for this work was to examine the relationships between MeHg exposure and acoustic startle reflex (ASR) and prepulse inhibition (PPI). We found that ITI responses corresponded non-linearly to increasing MeHg, where the greatest magnitude of difference was in the medium-low and medium-high exposure groups. All three of the higher quartiles of exposure were associated with higher response magnitudes than that of the reference group. This was strongest in the 75th percentile of the distribution, suggesting that percentile of the distribution was most sensitive to any changes. These results suggest that in general, individuals with higher MeHg have a greater startle response.

For target PPI responses, higher MeHg generally corresponded to less PPI in the upper tail of the distribution. However, it was notable that in the 25th percentile, higher Hg generally corresponded to more negative values of PPI (more inhibition), while in the 75th percentile, higher Hg generally corresponded to more positive values of PPI (less inhibition). Similarly, among non-target responses, there was little pattern to linear or non-linear relationships and only one linear association was statistically significant (120 ms tone responses vs blood Hg in the 25th percentile). However, a pattern opposite to that seen in the target responses was observed. There, for broad and 120 ms tone responses, in the 25th percentile and median, higher Hg generally corresponds to more positive values of PPI (less inhibition), and in the 75th percentile, higher Hg generally corresponded to more negative values of PPI (more inhibition). For 240 ms tone responses, higher Hg generally corresponded to more inhibition at all points in the distribution.

This study represents, to our knowledge, the first study of ASR and PPI in relation to MeHg exposure in human subjects. Existing studies of PPI in humans are generally meant to characterize deficits in the context of psychological disorders (Braff et al. 2001). There are studies of PPI in relation to smoking (Duncan et al. 2001a; Popke et al. 1997; Kumari et al. 2001; Della Casa et al. 1998), psychosocial stressors (Rahman et al. 2003), and drug use (Abel et al. 2003; Duncan et al. 2001b), but there are few published studies examining relationships with pollutant exposures.

Existing studies of PPI and MeHg are primarily in animal models. An early study looking at this relationship, looked at the startle reflex via cutaneous stimuli. That study found that animals that had been sub-chronically dosed with MeHg led to less PPI and exaggerated reaction to startling stimuli (Wu et al. 1985). However, a later study by Vezer et al. found that both the ASR responses were lower in the rats that were dosed with MeHg. They also found that PPI was reduced in the dose groups, which was consistent with the previous study by Wu et al (Vezer et al. 2005).

Other animal studies of PPI look specifically at prenatal exposure or exposure with other neurotoxic metals. While Carratu et al. found no relationship between prenatal MeHg exposure and later ASR and PPI measurements (Carratu et al. 2006), Beyrouty et al. found that prenatally dosed rats had higher mean startle responsiveness than controls (Beyrouty et al. 2006). A study by Geyer et al. also reported impaired ASR performance (Geyer et al. 1985). Commissaris et al. examined chronic low-level lead exposure in rats and found no effect on ASR, but that lead dosed rats had some facilitation instead of inhibition in response to prepulses (Commissaris et al. 2000).

PPI deficits are observed in in multiple neurological disorders (Braff et al. 2001). These deficits are best characterized in schizophrenia (Braff et al. 1999; Geyer et al. 2001), but have also been observed in obsessive compulsive disorder, Tourette's syndrome, and bipolar disorder (Kohl et al. 2013). There may be deficits in other disorders, such as post-traumatic stress disorder, but these are still being characterized (Kohl et al. 2013). Many in this diverse group of disorders share deficits in gating processes and possible abnormalities in the cortico-striato-pallido-pontine domain (Swerdlow et al. 2001). Deficits are also seen in individuals with risk factors for psychotic disorders (Ziermans et al. 2011) and in unaffected siblings of patients with bipolar disorder (Giakoumaki et al. 2007), suggesting PPI deficits may be observable for disorders in a sub-clinical state.

There are several limitations that could have affected our results. First, although we controlled for age, our cohort did cover a wide age range. Differences in processing at different ages and stages of neurodevelopment have been documented (Kofler et al. 2013). Second, PPI deficits in ADHD tends to be most prominent during tasks which require selective attention, rather than passive attention (Hawk et al. 2003). Further study of their omission and commission errors during the startle testing could help to address this limitation. Additionally, our procedure for outliers made sure that data was representative of the majority for the available data and was not unduly influenced by outliers, but this may have led to the exclusion of real, but extreme, values.

Our study suggests that acoustic startle responses in children are non-linearly associated with MeHg exposure. Additionally, there was a linear, but not necessarily significant, association with increased inhibition with MeHg in the 25^{th} percentile and median of the PPI distribution in trials with an attended tone, but a linear association with decreased inhibition in the 75^{th}

percentile. These findings add to our understanding of ASR and PPI, particularly in the context of environmental exposures rather than clinical symptoms of psychological disorders. Additional studies repeating this methodology can help to confirm these relationships, as would expanding the study to biomarkers of other forms of Hg or additional time points.

Figures

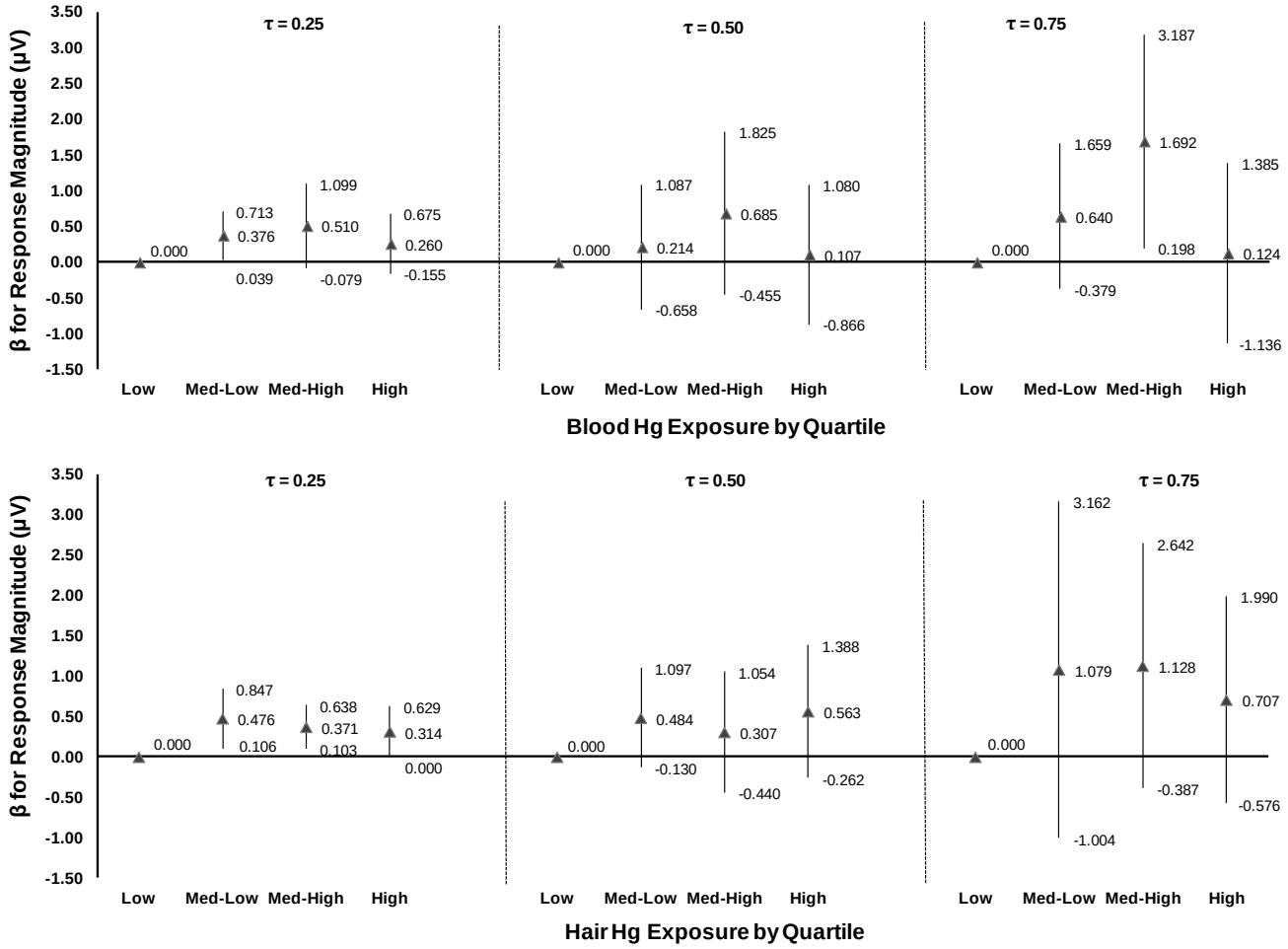

Figure 3.1 Results from Quantile Regression by Quartile of Exposure for Response Magnitude ITIs; point and 95% confidence intervals represent the association between the quartile of exposure for each biomarker (blood or hair) and the $\tau = 25^{th}$, 50^{th}, or 75^{th} percentile of the distribution of ITI response magnitude as compared to the reference group. Displayed values are the coefficients and the 95% confidence intervals.

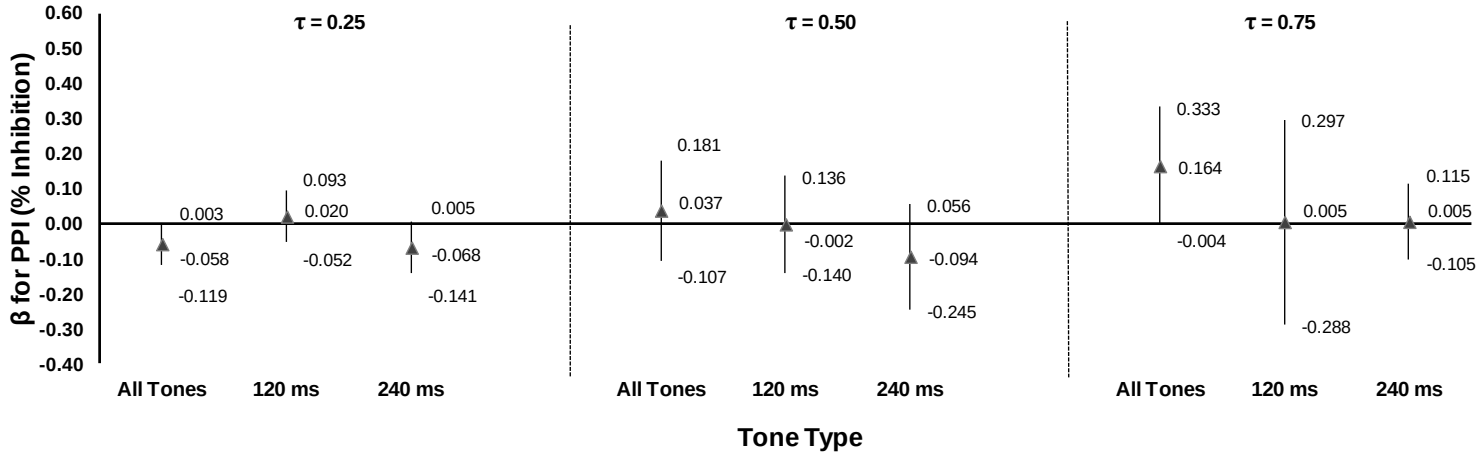

Figure 3.2A Results from Quantile Regressions for Prepulse Inhibition and Blood Exposure in Target Trials; point and 95% confidence intervals represent the association between blood exposure (μg/L) and the $\tau = 25^{th}$, 50^{th}, or 75^{th} percentile of the distribution of the target response magnitudes.

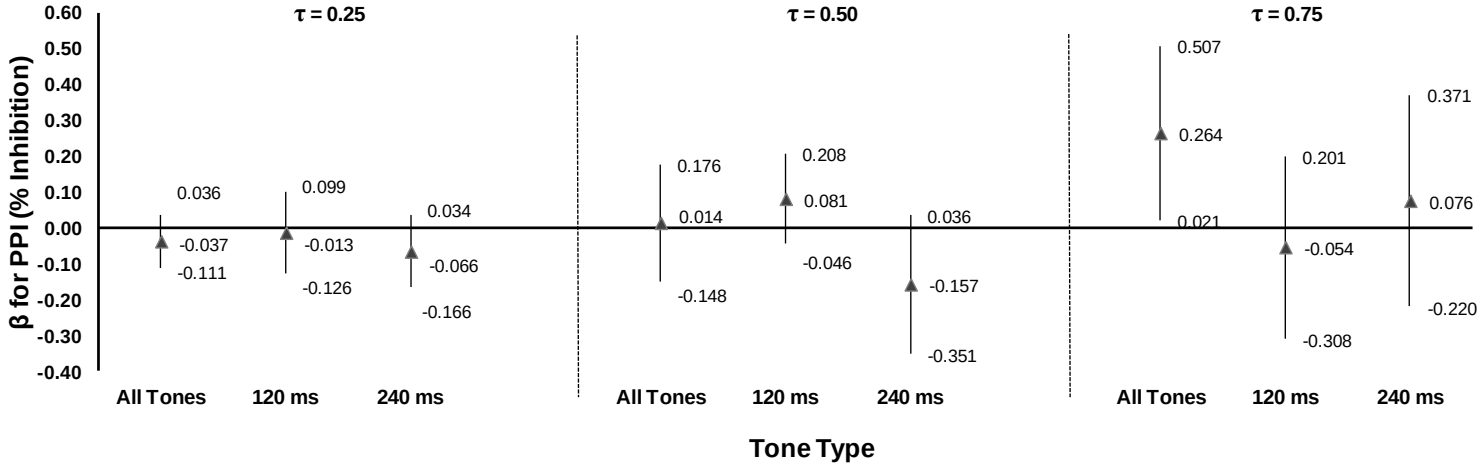

Figure 3.2B Results from Quantile Regressions for Prepulse Inhibition and Hair Exposure in Target Trials; point and 95% confidence intervals represent the association between hair exposure (μg/g) and the $\tau = 25^{th}$, 50^{th}, or 75^{th} percentile of the distribution of the target response magnitudes.

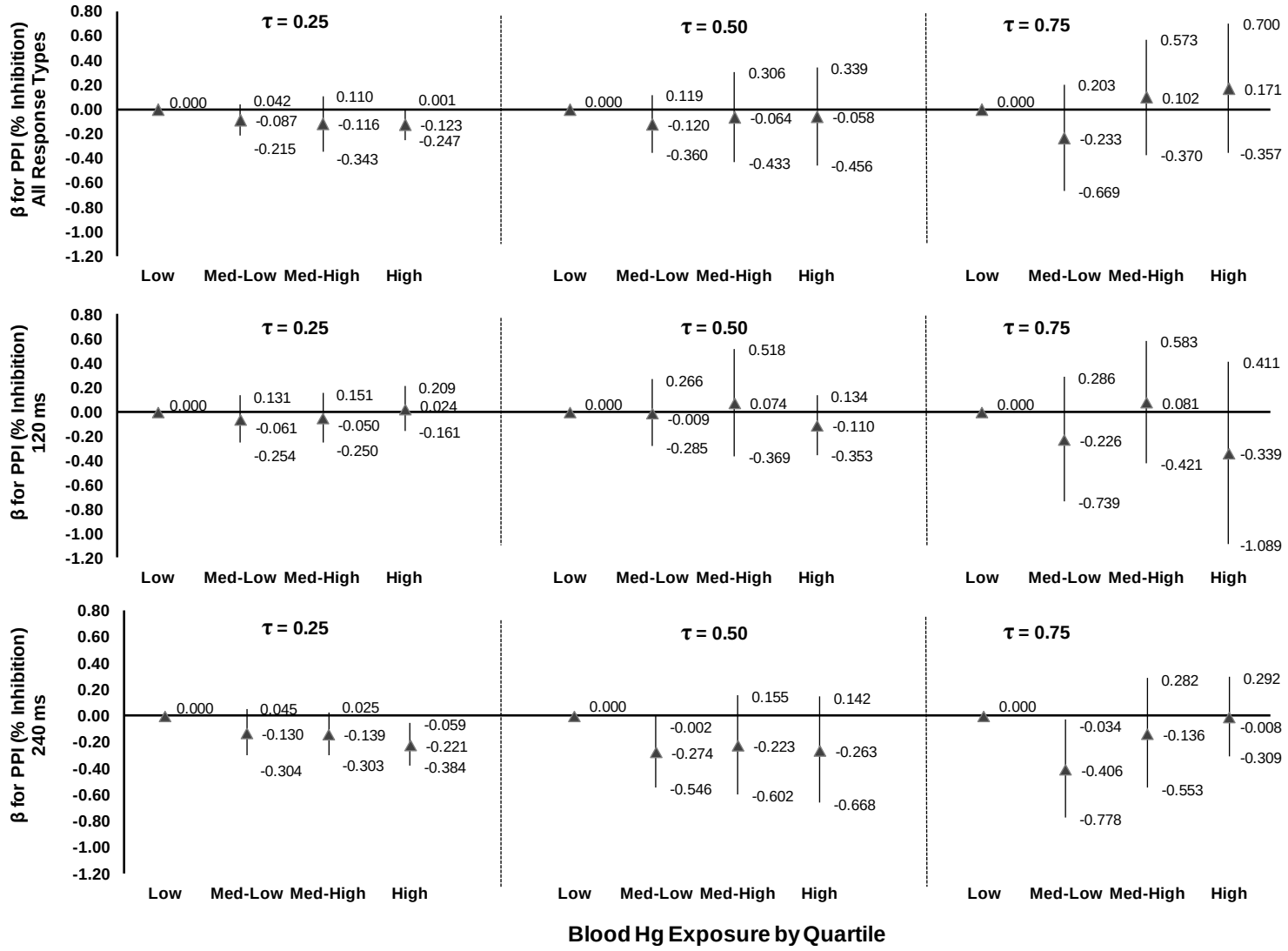

Figure 3.2C Results from Quantile Regression by Quartile of Exposure for Prepulse Inhibition in Target trials; point and 95% confidence intervals represent the association between the quartile of exposure for the biomarker (blood) and the $\tau = 25^{th}$, 50^{th}, or 75^{th} percentile of the distribution of target response magnitude.

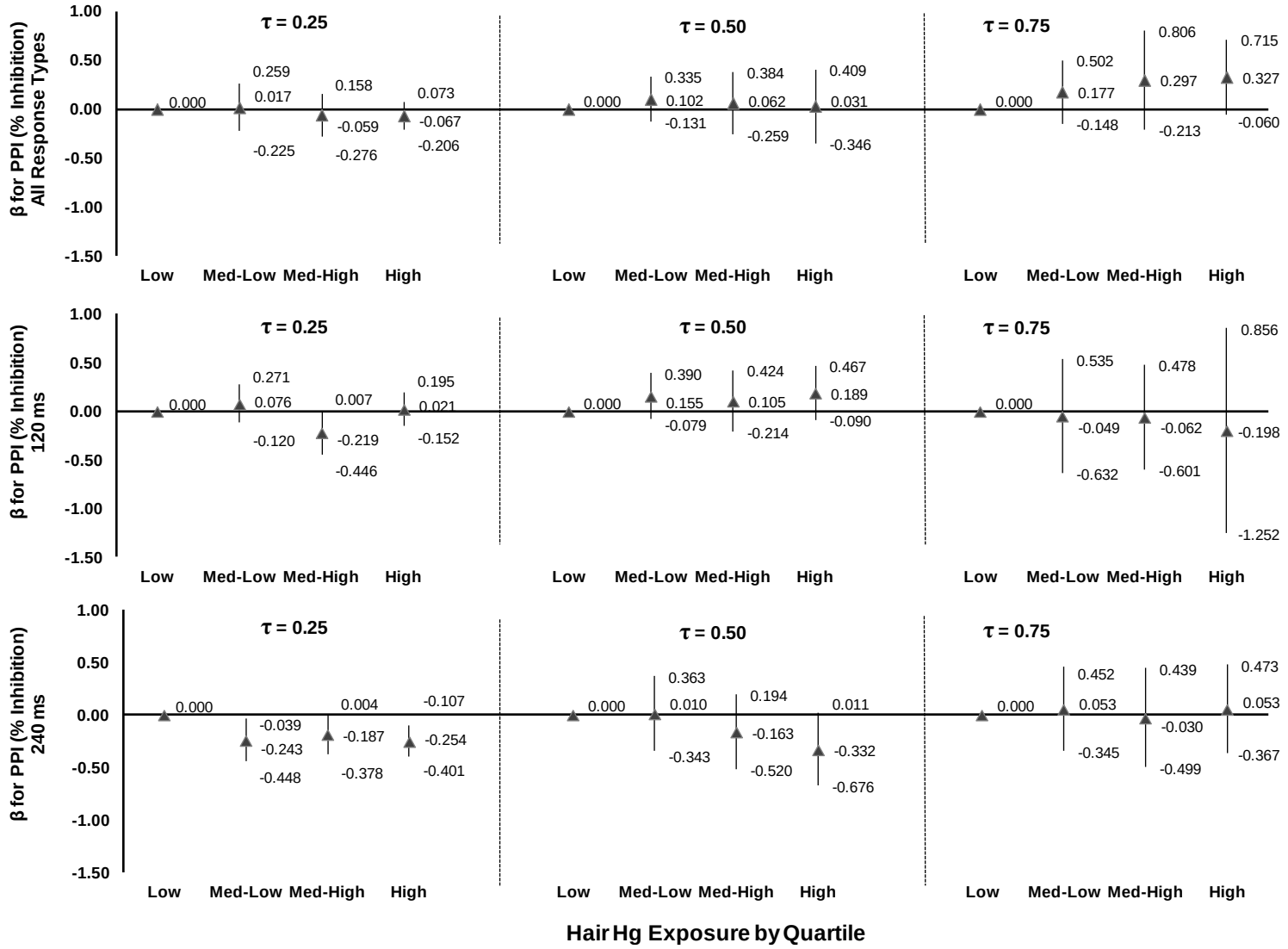

Figure 3.2D Results from Quantile Regression by Quartile of Exposure for Prepulse Inhibition in Target trials; point and 95% confidence intervals represent the association between the quartile of exposure for the biomarker (hair) and the $\tau = 25^{th}$, 50^{th}, or 75^{th} percentile of the distribution of target response magnitude.

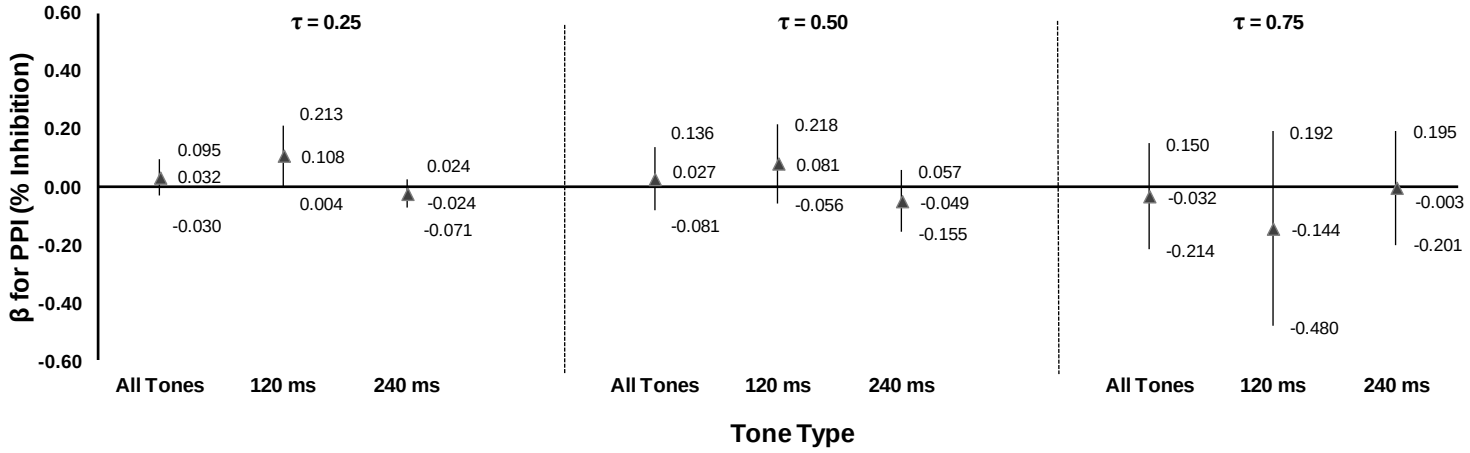

Figure 3.3A Results from Quantile Regressions for Prepulse Inhibition and Blood Exposure in Non-Target Trials; point and 95% confidence intervals represent the association between blood exposure (μg/L) and the $\tau = 25^{th}$, 50^{th}, or 75^{th} percentile of the distribution of the non-target response magnitudes.

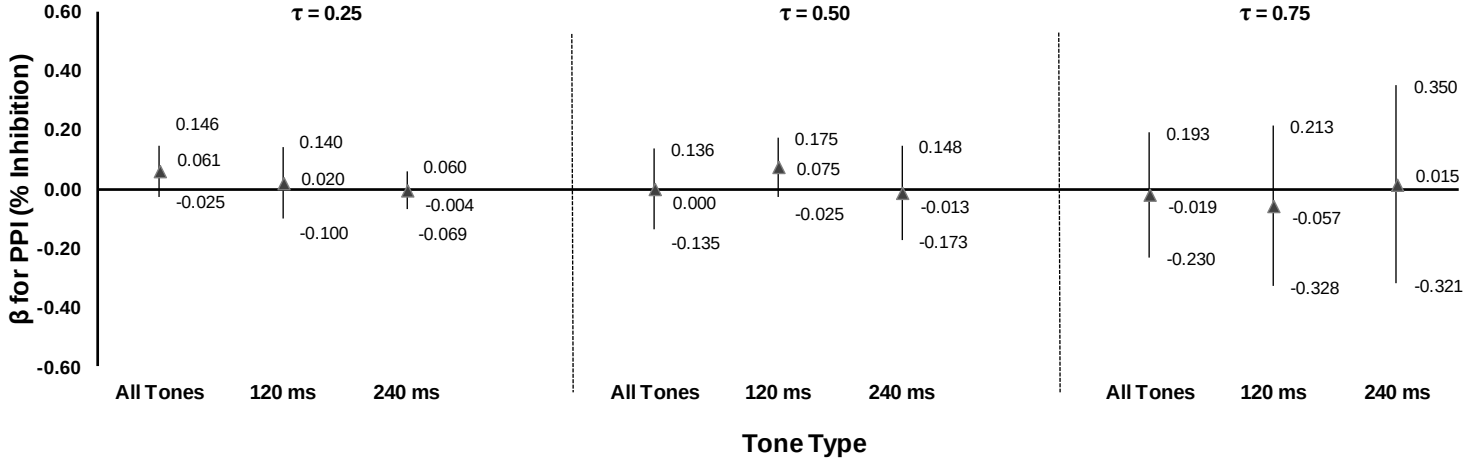

Figure 3.3B Results from Quantile Regressions for Prepulse Inhibition and Hair Exposure in Non-Target Trials; point and 95% confidence intervals represent the association between hair expsoure (μg/g) and the $\tau = 25^{th}$, 50^{th}, or 75^{th} percentile of the distribution of the non-target response magnitudes.

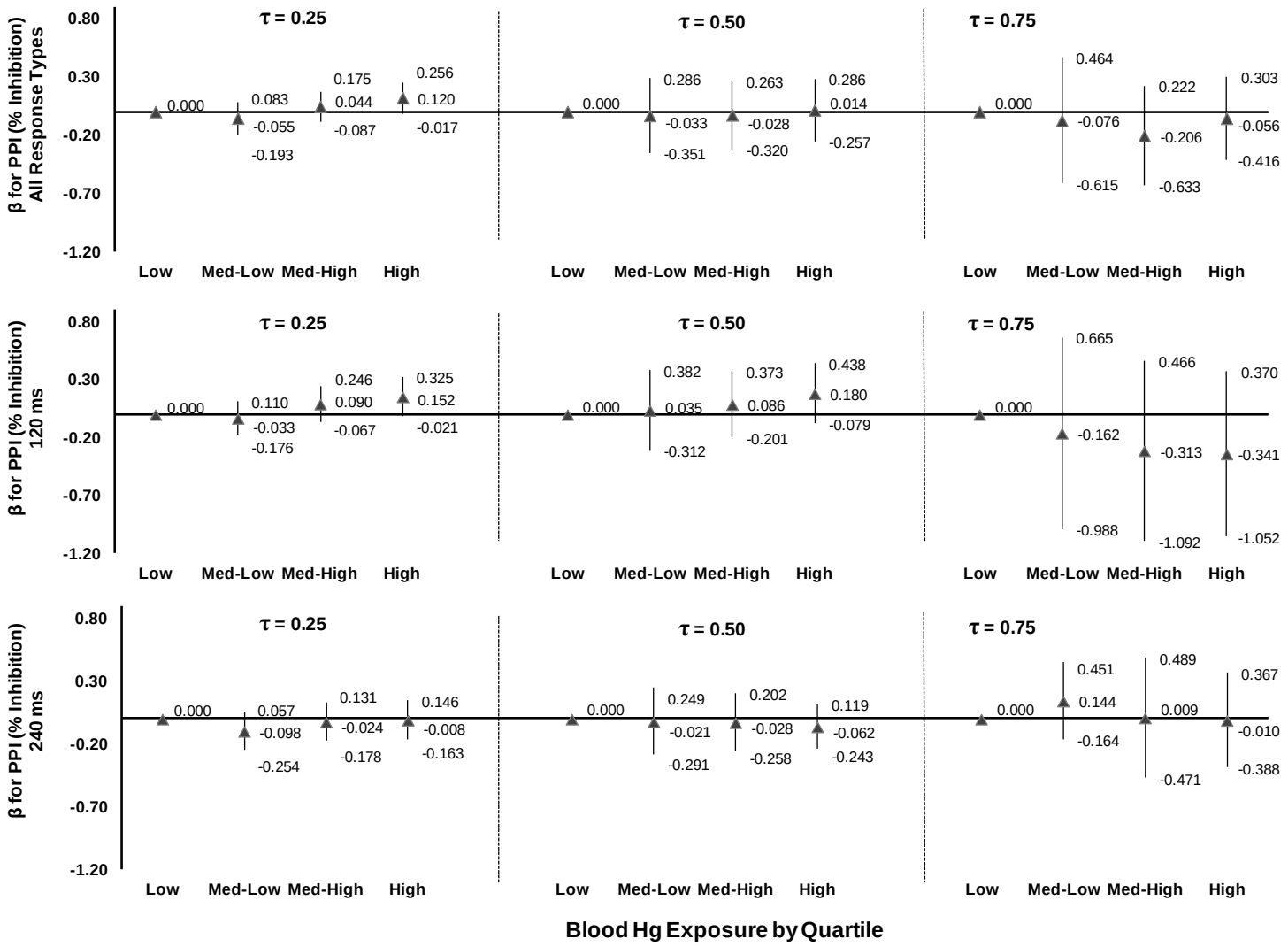

Figure 3.3C Results from Quantile Regression by Quartile of Exposure for Prepulse Inhibition in Non-target trials; point and 95% confidence intervals represent the association between the quartile of exposure for the biomarker (blood) and the $\tau = 25^{th}$, 50^{th}, or 75^{th} percentile of the distribution of non-target response magnitude.

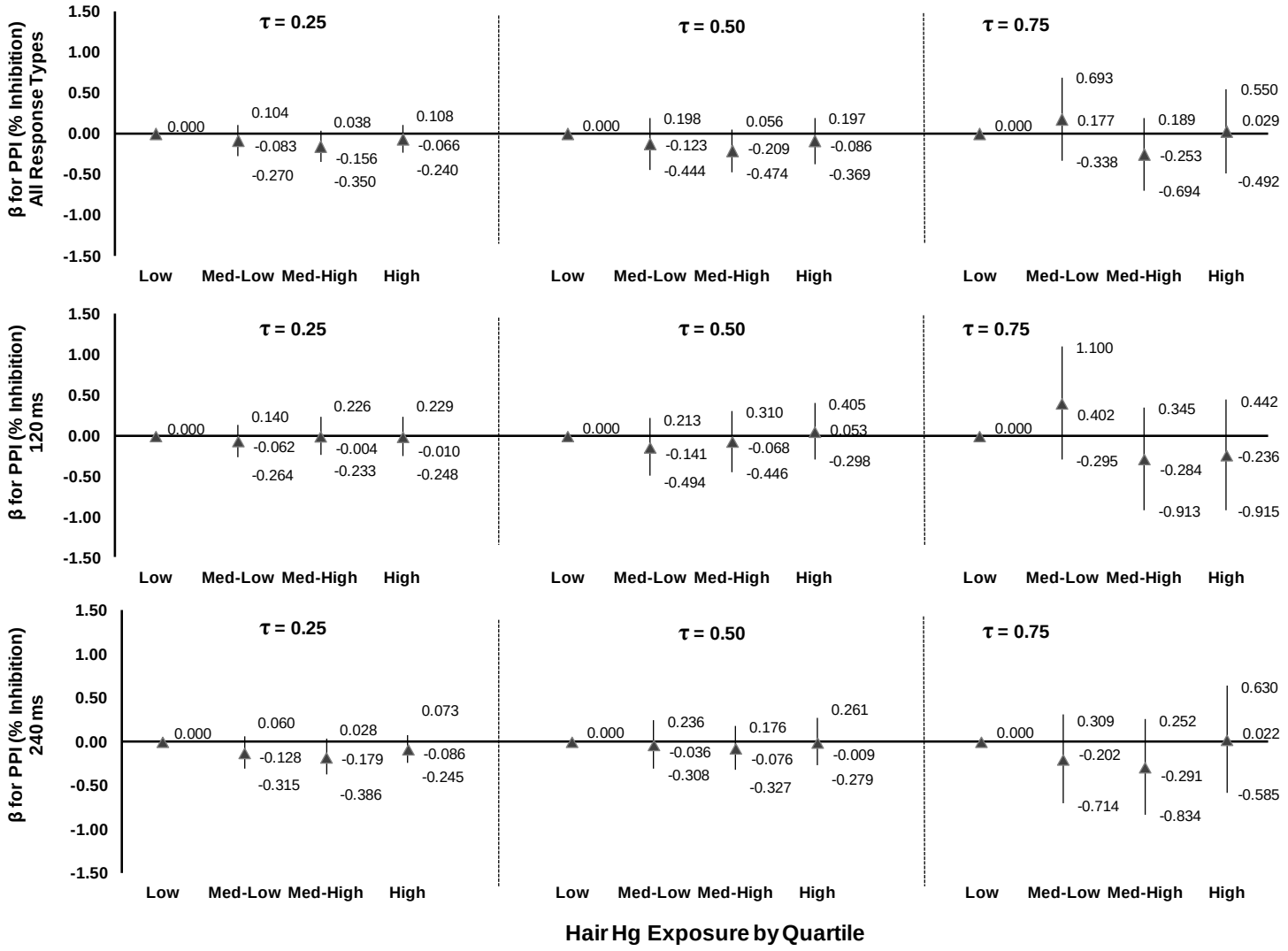

Figure 3.3D Results from Quantile Regression by Quartile of Exposure for Prepulse Inhibition in Non-target trials; point and 95% confidence intervals represent the association between the quartile of exposure for the biomarker (hair) and the $\tau = 25^{th}$, 50^{th}, or 75^{th} percentile of the distribution of non-target response magnitude.

Appendix 3

Supplemental Tables

Table S3.1.1 Sample Sizes Used in Modeling of ASR and PPI

		Blood	Hair
Response Magnitudes			
ITI		179	177
Prepulse Inhibition			
All Responses	Target	159	156
	Non-Target	145	145
120 SOA	Target	114	109
	Non-Target	114	113
240 SOA	Target	132	129
	Non-Target	124	124

Table S3.2.1 Models for Response Magnitude of Intertrial Intervals

	Intertrial Interval (ITI)											
	Blood						Hair					
	τ=0.25		τ=0.50		τ=0.75		τ=0.25		τ=0.50		τ=0.75	
	Beta	P-value	Beta	P-value	Beta	P-value	Beta	P-value	Beta	P-value	Beta	P-value
Model												
Intercept	1.822		-1.482		-4.270		2.719	0.016	0.488		-6.850	
log (Exposure)	0.104		0.099		0.269		0.095		0.215		0.276	
Sex of Child: Female	0.240		0.568	0.081	1.002		0.094		0.709	0.003	0.916	0.066
Age of Child at Visit	-0.086		0.251		0.677		-0.148		0.193		0.933	0.087
Cohort: 1	0.261		-1.433		-4.068	0.083	0.502		-1.374		-5.273	0.034
Cohort: 2a	0.229		0.556		-0.305		0.292		0.227		-0.269	
Cohort: 3	-0.358		1.151		0.814		-0.646	0.028	0.277		1.384	
SES Level	0.018		0.044		0.178		-0.011		0.052		0.174	0.020
Maternal Age	0.024		0.035		-0.048		0.009		0.011		-0.079	
Maternal Education	0.013		-0.039		-0.102		0.027		-0.064	0.061	-0.148	0.088
Maternal IQ	0.000		0.011		0.024		0.002		0.011		0.037	0.024
Maternal Marital Status: Married	-0.719	0.002	-1.527	0.002	-1.895	0.005	-0.481	0.003	-1.524	0.001	-1.558	0.005
Presentation Order: B 1st	-0.065		-0.031		1.394	0.073	-0.027		-0.364		1.201	0.074
Presentation Order: C 1st	-0.224		-0.226		0.486		-0.168		-0.409		0.507	

Table S3.3.1 Models for PPI of All Target Responses

	All Target											
	Blood						Hair					
	τ=0.25		τ=0.50		τ=0.75		τ=0.25		τ=0.50		τ=0.75	
	Beta	P-value	Beta	P-value	Beta	P-value	Beta	P-value	Beta	P-value	Beta	P-value
Model												
Intercept	-0.678		0.363		0.132		-1.705	0.002	-2.005	0.065	-1.139	
log (Exposure)	-0.058	0.063	0.037		0.164	0.058	-0.037		0.014		0.264	0.035
Sex of Child: Female	-0.067		0.005		0.121		-0.072		0.079		0.038	
Age of Child at Visit	0.059		-0.003		0.047		0.108	0.007	0.172	0.045	0.165	
Cohort: 1	-0.312		-0.161		-0.384		-0.504	0.012	-0.870	0.036	-0.828	
Cohort: 2a	-0.076		-0.008		-0.096		-0.093		-0.068		-0.157	
Cohort: 3	0.241	0.013	0.004		0.795		0.427	0.001	0.661	0.090	1.032	0.096
SES Level	-0.004		0.011		0.022		-0.007		0.006		0.017	
Maternal Age	-0.008		-0.010		-0.011		-0.005		-0.006		-0.003	
Maternal Education	0.018	0.093	0.015		-0.004		0.004		0.024		-0.036	
Maternal IQ	-0.006	0.002	-0.007	0.030	-0.006		0.000		-0.005		-0.001	
Maternal Marital Status: Married	0.034		0.071		-0.079		0.015		-0.045		0.024	
Presentation Order: B 1st	0.045		0.216	0.085	0.256		0.050		0.174		0.182	
Presentation Order: C 1st	0.133	0.061	0.234	0.046	0.529	0.026	0.168	0.045	0.297	0.039	0.630	0.027

Table S3.3.2 Models for PPI of All Non-Target Responses

	All Non-Target											
	Blood						Hair					
	τ=0.25		τ=0.50		τ=0.75		τ=0.25		τ=0.50		τ=0.75	
	Beta	P-value	Beta	P-value	Beta	P-value	Beta	P-value	Beta	P-value	Beta	P-value
Model												
Intercept	0.429		0.465		1.222		-0.248		-0.384		0.222	
log (Exposure)	0.032		0.027		-0.032		0.061		0.000		-0.019	
Sex of Child: Female	0.021		0.040		0.229		0.012		0.110		0.071	
Age of Child at Visit	-0.069		-0.087		-0.104		-0.007		-0.018		-0.023	
Cohort: 1	0.190		0.358		0.356		-0.150		0.010		-0.032	
Cohort: 2a	-0.049		0.039		0.031		-0.140		-0.083		0.116	
Cohort: 3	0.072		0.119		-0.142		0.164		0.396		0.470	
SES Level	0.028	0.014	0.025		-0.001		0.026	0.033	0.018		0.013	
Maternal Age	-0.009	0.063	-0.003		0.007		-0.006		-0.001		0.000	
Maternal Education	-0.006		0.013		-0.025		-0.001		0.029		-0.035	
Maternal IQ	-0.002		-0.004		-0.003		-0.002		-0.003		0.002	
Maternal Marital Status: Married	0.083	0.073	0.149		0.322	0.046	0.037		0.064		0.324	0.040
Presentation Order: B 1st	0.078		0.126		0.004		0.033		0.022		-0.270	
Presentation Order: C 1st	0.111		0.453	0.001	0.408		0.123		0.312	0.065	0.284	

Table S3.3.3 Models for PPI of 120 ms Tone Target Responses

	120 SOA Target											
	Blood						Hair					
	τ=0.25		τ=0.50		τ=0.75		τ=0.25		τ=0.50		τ=0.75	
	Beta	P-value	Beta	P-value	Beta	P-value	Beta	P-value	Beta	P-value	Beta	P-value
Model												
Intercept	-0.997		-1.605		-2.167		-1.119	0.059	-2.913	0.025	-4.253	0.062
log (Exposure)	0.020		-0.002		0.005		-0.013		0.081		-0.054	
Sex of Child: Female	-0.044		-0.092		0.014		-0.056		0.002		0.059	
Age of Child at Visit	0.086	0.056	0.165	0.073	0.259		0.069		0.275	0.004	0.411	0.017
Cohort: 1	-0.460	0.035	-0.710		-1.479	0.057	-0.349		-1.287	0.008	-2.113	0.008
Cohort: 2a	-0.071		0.052		-0.030		0.001		-0.104		-0.089	
Cohort: 3	0.067		0.272		0.239		0.232	0.056	0.649	0.073	1.168	0.006
SES Level	-0.018		-0.010		-0.019		-0.006		-0.004		0.024	
Maternal Age	-0.011	0.046	-0.016		-0.019		-0.003		-0.021	0.053	-0.024	
Maternal Education	-0.007		-0.014		0.071	0.093	0.008		0.002		0.063	0.094
Maternal IQ	-0.001		-0.002		-0.011		-0.003		0.001		-0.008	
Maternal Marital Status: Married	-0.031		0.119		0.060		0.063		-0.017		-0.046	
Presentation Order: B 1st	-0.086		-0.037		-0.098		-0.107		-0.209	0.060	-0.180	
Presentation Order: C 1st	0.159	0.053	0.230		0.742	0.018	0.049		0.044		0.755	0.002

Table S3.3.4 Models for PPI of 120 ms Tone Non-Target Responses

	120 SOA Non-Target											
	Blood						Hair					
	τ=0.25		τ=0.50		τ=0.75		τ=0.25		τ=0.50		τ=0.75	
	Beta	P-value	Beta	P-value	Beta	P-value	Beta	P-value	Beta	P-value	Beta	P-value
Model												
Intercept	-0.810		0.215		1.606		-0.869		0.290		0.531	
log (Exposure)	0.108	0.045	0.081		-0.144		0.020		0.075		-0.057	
Sex of Child: Female	-0.110	0.076	-0.045		0.015		0.032		-0.099		-0.091	
Age of Child at Visit	0.031		-0.034		-0.075		0.041		-0.025		-0.024	
Cohort: 1	-0.265		0.133		0.126		-0.282		0.098		-0.071	
Cohort: 2a	-0.168	0.022	-0.026		-0.237		-0.117		-0.024		-0.102	
Cohort: 3	0.519	0.044	0.254		-0.192		0.336	0.055	0.288		0.512	
SES Level	0.007		0.009		-0.021		0.010		0.002		-0.020	
Maternal Age	-0.015	0.023	-0.014	0.058	-0.019		-0.006		-0.010		-0.013	
Maternal Education	0.003		-0.011		-0.008		-0.007		-0.010		0.001	
Maternal IQ	0.001		-0.001		0.000		-0.001		-0.002		0.002	
Maternal Marital Status: Married	0.137	0.031	0.251	0.013	0.169		0.032		0.276	0.003	0.326	
Presentation Order: B 1st	-0.007		-0.013		-0.190		-0.056		-0.088		-0.441	
Presentation Order: C 1st	0.173	0.056	0.302	0.062	0.415		0.103		0.206		0.245	

Table S3.3.5 Models for PPI of 240 ms Tone Target Responses

	240 SOA Target											
	Blood						Hair					
	τ=0.25		τ=0.50		τ=0.75		τ=0.25		τ=0.50		τ=0.75	
	Beta	P-value	Beta	P-value	Beta	P-value	Beta	P-value	Beta	P-value	Beta	P-value
Model												
Intercept	-0.991	0.053	-0.034		1.084		-2.410	0.000	-2.722	0.065	0.334	
log (Exposure)	-0.068	0.069	-0.094		0.005		-0.066		-0.157		0.076	
Sex of Child: Female	0.018		0.073		0.156		-0.058		0.104		0.316	0.041
Age of Child at Visit	0.062		-0.039		-0.050		0.136	0.003	0.146		-0.018	
Cohort: 1	-0.263		-0.095		0.207		-0.648	0.005	-0.826		0.045	
Cohort: 2a	-0.043		0.001		-0.008		-0.059		-0.070		-0.044	
Cohort: 3	0.333	0.029	0.051		0.494		0.496	0.012	0.804	0.056	0.817	0.100
SES Level	0.019	0.076	0.025		0.034		0.015		0.039		0.037	
Maternal Age	-0.001		-0.003		-0.015		0.005		0.005		0.005	
Maternal Education	0.007		0.017		0.019		0.019		0.024		-0.001	
Maternal IQ	-0.005	0.008	-0.001		-0.006	0.027	-0.002		-0.001		-0.005	
Maternal Marital Status: Married	0.019		0.128		-0.020		0.041		-0.029		0.002	
Presentation Order: B 1st	0.086		0.061		0.259	0.069	-0.011		0.100		0.134	
Presentation Order: C 1st	0.041		0.194		0.368		0.088		0.143		0.426	

Table S3.3.6 Models for PPI of 240 ms Tone Non-Target Responses

	240 SOA Non-Target											
	Blood						Hair					
	τ=0.25		τ=0.50		τ=0.75		τ=0.25		τ=0.50		τ=0.75	
	Beta	P-value	Beta	P-value	Beta	P-value	Beta	P-value	Beta	P-value	Beta	P-value
Model												
Intercept	-0.716		-0.670		0.699		-1.120	0.029	-0.775		0.500	
log (Exposure)	-0.024		-0.049		-0.003		-0.004		-0.013		0.015	
Sex of Child: Female	0.076		0.105		0.188		0.089	0.077	0.107		0.170	
Age of Child at Visit	-0.044		-0.055		-0.101		-0.007		-0.025		-0.029	
Cohort: 1	0.150		0.272		0.671		-0.106		0.028		0.241	
Cohort: 2a	-0.036		0.012		0.306		-0.139		-0.084		0.373	
Cohort: 3	0.054		0.182		-0.080		0.050		0.365		0.284	
SES Level	0.035	0.028	0.037	0.034	0.007		0.012		0.026		0.010	
Maternal Age	0.007		0.011		0.032		0.014	0.004	0.010		0.022	
Maternal Education	0.022	0.077	0.050	0.011	-0.034		0.036	0.000	0.048	0.016	-0.018	
Maternal IQ	-0.001		-0.004		-0.003		-0.002		-0.004		-0.008	
Maternal Marital Status: Married	0.030		0.063		0.287		0.010		-0.035		0.240	
Presentation Order: B 1st	0.096		0.144		-0.180		-0.006		0.063		-0.113	
Presentation Order: C 1st	0.097		0.179		0.167		-0.032		0.214		0.178	

Table S3.4.1 Models for Response Magnitude of Intertrial Intervals with Quartiles of Exposure

	Intertrial Interval (ITI)											
	Blood						Hair					
	τ=0.25		τ=0.50		τ=0.75		τ=0.25		τ=0.50		τ=0.75	
	Beta	P-value	Beta	P-value	Beta	P-value	Beta	P-value	Beta	P-value	Beta	P-value
Model												
Intercept	2.019		-1.724		-3.762		3.200	0.012	0.511		-2.791	
Exposure Med-Low	0.376	0.030	0.214		0.640		0.476	0.013	0.484		1.079	
Exposure Med-High	0.510	0.092	0.685		1.692	0.028	0.371	0.007	0.307		1.128	
Exposure High	0.260		0.107		0.124		0.314	0.052	0.563		0.707	
Sex of Child: Female	0.133		0.621	0.081	0.686		0.093		0.624	0.061	0.899	
Age of Child at Visit	-0.090		0.247		0.634		-0.180	0.084	0.137		0.574	
Cohort: 1	0.051		-1.405		-4.370	0.034	0.605		-1.252		-3.684	
Cohort: 2a	0.165		0.738		-0.354		0.407	0.072	0.233		0.291	
Cohort: 3	-0.486		1.278		1.031		-0.739	0.016	0.149		0.617	
SES Level	0.020		0.055		0.132		-0.005		0.023		0.186	
Maternal Age	0.017		0.024		0.010		0.000		0.019		-0.076	
Maternal Education	-0.015		-0.043		-0.079		-0.018		-0.034		-0.086	
Maternal IQ	0.004		0.014		0.010		0.007		0.008		0.017	
Maternal Marital Status: Married	-0.748	0.001	-1.471	0.005	-1.922	<0.001	-0.460	0.001	-1.552	0.003	-1.433	0.009
Presentation Order: B 1st	-0.197		-0.160		1.023		-0.280	0.088	-0.207		1.066	
Presentation Order: C 1st	-0.426		-0.437		-0.043		-0.355	0.044	-0.265		0.051	

Table S3.5.1 Models for PPI of All Target Responses with Quartiles of Exposure

	All Target											
	Blood						Hair					
	τ=0.25		τ=0.50		τ=0.75		τ=0.25		τ=0.50		τ=0.75	
	Beta	P-value	Beta	P-value	Beta	P-value	Beta	P-value	Beta	P-value	Beta	P-value
Model												
Intercept	-1.009	0.049	-0.330		0.292		-1.661	0.003	-1.500		-1.363	
Exposure Med-Low	-0.087		-0.120		-0.233		0.017		0.102		0.177	
Exposure Med-High	-0.116		-0.064		0.102		-0.059		0.062		0.297	
Exposure High	-0.123	0.053	-0.058		0.171		-0.067		0.031		0.327	
Sex of Child: Female	-0.054		0.017		0.101		-0.092		0.065		0.098	
Age of Child at Visit	0.074	0.064	0.049		0.044		0.114	0.002	0.148		0.132	
Cohort: 1	-0.328	0.099	-0.403		-0.451		-0.511	0.007	-0.781	0.077	-0.802	
Cohort: 2a	-0.018		-0.038		-0.090		-0.078		-0.080		-0.213	
Cohort: 3	0.349	0.003	0.053		0.547		0.407	0.005	0.347		0.963	
SES Level	-0.001		0.004		-0.044		-0.006		0.001		0.043	
Maternal Age	-0.006		-0.008		-0.008		-0.004		-0.008		-0.002	
Maternal Education	0.016		0.007		0.014		0.006		0.021		-0.040	
Maternal IQ	-0.004	0.035	-0.004		-0.004		-0.001		-0.006	0.090	0.000	
Maternal Marital Status: Married	0.026		0.070		-0.016		0.043		-0.024		0.038	
Presentation Order: B 1st	0.025		0.200		0.211		0.051		0.168		0.127	
Presentation Order: C 1st	0.136	0.074	0.285	0.025	0.482	0.082	0.137		0.332	0.019	0.521	0.051

Table S3.5.2 Models for PPI of All Non-Target Responses with Quartiles of Exposure

	All Non-Target											
	Blood						Hair					
	τ=0.25		τ=0.50		τ=0.75		τ=0.25		τ=0.50		τ=0.75	
	Beta	P-value	Beta	P-value	Beta	P-value	Beta	P-value	Beta	P-value	Beta	P-value
Model												
Intercept	0.588		0.165		0.295		-0.755		-1.016		-0.081	
Exposure Med-Low	-0.055		-0.033		-0.076		-0.083		-0.123		0.177	
Exposure Med-High	0.044		-0.028		-0.206		-0.156		-0.209		-0.253	
Exposure High	0.120	0.088	0.014		-0.056		-0.066		-0.086		0.029	
Sex of Child: Female	-0.027		0.123		0.211		0.054		0.082		0.053	
Age of Child at Visit	-0.087	0.072	-0.064		-0.033		0.011		0.019		-0.005	
Cohort: 1	0.270		0.253		0.077		-0.225		-0.002		0.010	
Cohort: 2a	-0.012		-0.018		-0.052		-0.129	0.094	-0.048		-0.068	
Cohort: 3	0.095		0.186		0.225		0.163		0.628	0.030	0.532	
SES Level	0.022	0.052	0.032	0.089	-0.002		0.026	0.015	0.018		0.041	
Maternal Age	-0.010	0.028	-0.001		0.006		-0.005		0.001		0.001	
Maternal Education	-0.006		0.013		-0.037		0.010		0.031		-0.029	
Maternal IQ	-0.001		-0.004		0.001		0.000		-0.002		-0.001	
Maternal Marital Status: Married	0.091	0.073	0.129		0.288	0.069	0.085	0.067	0.112		0.298	
Presentation Order: B 1st	0.069		0.093		-0.051		0.024		0.010		-0.064	
Presentation Order: C 1st	0.102		0.472	0.002	0.468	0.074	0.047		0.345	0.040	0.417	

Table S3.5.3 Models for PPI of 120 ms Tone Target Responses with Quartiles of Exposure

	120 SOA Target											
	Blood						Hair					
	τ=0.25		τ=0.50		τ=0.75		τ=0.25		τ=0.50		τ=0.75	
	Beta	P-value	Beta	P-value	Beta	P-value	Beta	P-value	Beta	P-value	Beta	P-value
Model												
Intercept	-0.628		-0.955		-1.689		-1.647	0.042	-2.977	0.020	-4.652	0.072
Exposure Med-Low	-0.061		-0.009		-0.226		0.076		0.155		-0.049	
Exposure Med-High	-0.050		0.074		0.081		-0.219	0.061	0.105		-0.062	
Exposure High	0.024		-0.110		-0.339		0.021		0.189		-0.198	
Sex of Child: Female	-0.044		-0.153		-0.050		-0.049		-0.039		0.054	
Age of Child at Visit	0.052		0.111		0.197		0.123	0.049	0.272	0.008	0.455	0.034
Cohort: 1	-0.287		-0.415		-1.133		-0.557	0.054	-1.302	0.011	-2.319	0.014
Cohort: 2a	-0.084		0.093		0.189		-0.054		-0.087		-0.055	
Cohort: 3	0.062		0.129		-0.051		0.378	0.052	0.724	0.031	1.247	0.050
SES Level	-0.013		0.002		-0.008		-0.024		-0.010		0.023	
Maternal Age	-0.011	0.042	-0.016		-0.011		-0.009		-0.020	0.080	-0.021	
Maternal Education	-0.007		0.002		0.036		0.001		0.000		0.062	
Maternal IQ	-0.001		-0.005		-0.005		0.001		0.001		-0.009	
Maternal Marital Status: Married	-0.022		0.124		0.056		0.018		0.007		-0.054	
Presentation Order: B 1st	-0.035		0.006		-0.291		-0.169	0.081	-0.178		-0.206	
Presentation Order: C 1st	0.163	0.040	0.308	0.094	0.475		0.068		0.117		0.713	0.031

Table S3.5.4 Models for PPI of 120 ms Tone Non-Target Responses with Quartiles of Exposure

	120 SOA Non-Target											
	Blood						Hair					
	τ=0.25		τ=0.50		τ=0.75		τ=0.25		τ=0.50		τ=0.75	
	Beta	P-value	Beta	P-value	Beta	P-value	Beta	P-value	Beta	P-value	Beta	P-value
Model												
Intercept	-0.721		0.409		1.831		-1.076		0.232		0.600	
Exposure Med-Low	-0.033		0.035		-0.162		-0.062		-0.141		0.402	
Exposure Med-High	0.090		0.086		-0.313		-0.004		-0.068		-0.284	
Exposure High	0.152	0.088	0.180		-0.341		-0.010		0.053		-0.236	
Sex of Child: Female	-0.031		-0.083		0.027		0.018		-0.119		-0.057	
Age of Child at Visit	0.002		-0.049		-0.085		0.047		-0.036		-0.028	
Cohort: 1	-0.131		0.182		0.298		-0.310		0.161		-0.072	
Cohort: 2a	-0.161	0.044	-0.007		-0.138		-0.162	0.052	0.002		-0.194	
Cohort: 3	0.365		0.210		-0.212		0.352	0.070	0.220		0.586	
SES Level	0.011		0.004		-0.024		0.022		-0.005		-0.006	
Maternal Age	-0.006		-0.014	0.025	-0.018		-0.004		-0.008		-0.004	
Maternal Education	-0.007		-0.010		-0.015		-0.008		-0.010		-0.002	
Maternal IQ	0.002		-0.001		0.002		-0.001		0.000		0.001	
Maternal Marital Status: Married	0.084		0.253	0.004	0.074		0.008		0.276	0.002	0.168	
Presentation Order: B 1st	0.007		0.009		-0.163		-0.009		-0.097		-0.295	
Presentation Order: C 1st	0.119		0.295	0.049	0.452		0.165		0.235	0.088	0.080	

Table S3.5.5 Models for PPI of 240 ms Tone Target Responses with Quartiles of Exposure

	240 SOA Target											
	Blood						Hair					
	τ=0.25		τ=0.50		τ=0.75		τ=0.25		τ=0.50		τ=0.75	
	Beta	P-value	Beta	P-value	Beta	P-value	Beta	P-value	Beta	P-value	Beta	P-value
Model												
Intercept	-1.081	0.007	-1.565		0.648		-1.803	0.002	-1.902		-0.383	
Exposure Med-Low	-0.130		-0.274	0.051	-0.406	0.035	-0.243	0.022	0.010		0.053	
Exposure Med-High	-0.139	0.099	-0.223		-0.136		-0.187	0.057	-0.163		-0.030	
Exposure High	-0.221	0.009	-0.263		-0.008		-0.254	0.001	-0.332	0.061	0.053	
Sex of Child: Female	0.027		0.045		0.154		0.068		0.087		0.214	
Age of Child at Visit	0.076	0.008	0.085		0.022		0.101	0.014	0.132		0.034	
Cohort: 1	-0.335	0.021	-0.639		-0.227		-0.488	0.022	-0.790		-0.140	
Cohort: 2a	-0.033		-0.060		-0.191		0.016		-0.130		-0.090	
Cohort: 3	0.390	0.019	0.340		0.444		0.558	0.002	0.831	0.036	0.951	0.062
SES Level	0.020	0.031	0.037		0.014		0.028	0.009	0.034		0.025	
Maternal Age	0.001		-0.004		-0.029	0.004	0.007		0.004		-0.005	
Maternal Education	0.012		0.003		0.013		0.009		0.016		0.014	
Maternal IQ	-0.006	0.000	0.004		-0.002		-0.003		-0.002		-0.004	
Maternal Marital Status: Married	0.023		0.092		-0.101		0.056		-0.033		0.058	
Presentation Order: B 1st	0.052		-0.012		0.323	0.006	-0.069		0.063		0.180	
Presentation Order: C 1st	0.047		0.131		0.358		-0.034		0.033		0.476	0.055

Table S3.5.6 Models for PPI of 240 ms Tone Non-Target Responses with Quartiles of Exposure

	240 SOA Non-Target											
	Blood						Hair					
	τ=0.25		τ=0.50		τ=0.75		τ=0.25		τ=0.50		τ=0.75	
	Beta	P-value	Beta	P-value	Beta	P-value	Beta	P-value	Beta	P-value	Beta	P-value
Model												
Intercept	-0.463		-0.259		0.189		-0.474		-0.515		0.223	
Exposure Med-Low	-0.098		-0.021		0.144		-0.128		-0.036		-0.202	
Exposure Med-High	-0.024		-0.028		0.009		-0.179	0.093	-0.076		-0.291	
Exposure High	-0.008		-0.062		-0.010		-0.086		-0.009		0.022	
Sex of Child: Female	0.016		0.080		0.070		0.071		0.080		0.169	
Age of Child at Visit	-0.058		-0.085		-0.077		-0.050		-0.024		-0.026	
Cohort: 1	0.198		0.360		0.603		0.128		0.052		0.221	
Cohort: 2a	0.017		0.025		0.295	0.068	-0.039		-0.018		0.393	
Cohort: 3	-0.001		0.115		-0.031		-0.004		0.354	0.061	0.469	
SES Level	0.018		0.041	0.020	0.008		0.019	0.050	0.021		-0.002	
Maternal Age	0.011	0.042	0.010		0.036	0.034	0.011	0.071	0.008		0.027	
Maternal Education	0.022	0.086	0.047	0.010	-0.027		0.029	0.048	0.048	0.032	-0.021	
Maternal IQ	-0.002		-0.004		-0.002		-0.002		-0.006		-0.005	
Maternal Marital Status: Married	0.083		0.077		0.281		0.072		-0.012		0.138	
Presentation Order: B 1st	0.054		0.141	0.057	-0.183		-0.005		0.022		-0.106	
Presentation Order: C 1st	-3.0E-05		0.170		0.153		-0.048		0.156		0.073	

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Conclusion

Objectives and significance

Our long term goal for this research was to better understand the relationship between mercury and attention deficits, via the central hypothesis that MeHg might contribute to ADHD through the disruption of dopaminergic pathways.

This work aimed to answer several questions that were unanswered by the existing literature. Within the first aim, looking at prenatal and postnatal Hg exposure and ADHD screening scores, our objective was to add to the understanding of which windows of exposure might be of most concern for ADHD. In particular, prenatal versus postnatal exposure and several time points during gestation, were the focus. Additionally, we sought to examine how MeHg and Pb, another common neurotoxic metal, could potentially interact. Previous work in this area has found inconsistent results for postnatal Hg exposure (MeHg or IHg) (Bellinger et al. 2007; Debes et al. 2006; DeRouen et al. 2006). Studies of prenatal exposure primarily used biomarkers of exposure related to late pregnancy, such as cord blood (Grandjean et al. 1999; Oken et al. 2005; Sagiv et al. 2012; van Wijngaarden et al. 2013). Here, we also include measures from earlier in pregnancy, potentially exposing vulnerabilities earlier in gestation. Further, examining possible interactions between more than one neurotoxic metal reflects real world exposures, where an individual is unlikely to be exposed to only one potentially harmful substance.

Second, we examined gene-environment interactions between dopamine (DA) related SNPs and MeHg. All of the SNPs we examined have been previously related to ADHD or

correspond to a gene which has been related to ADHD (Gatt et al. 2015; Li et al. 2014). Changes to DA synthesis, signaling, and processing have been implicated in ADHD etiology (Swanson et al. 2007; Thapar et al. 2013). This is interesting in relation to MeHg, as a number of animal studies have found MeHg induced changes to DA signaling, most notably changes to the release of DA into the synapse (Asherson and Gurling 2011; Faro et al. 2000; Faro et al. 1997; Tiernan et al. 2013). A relationship between MeHg and these SNPs could support the idea that MeHg could impact ADHD symptoms.

Finally, we examined the potential relationships between concurrent MeHg exposure and acoustic startle reflex (ASR) and prepulse inhibition (PPI). There are limitations to studying ADHD in epidemiology (Faraone et al. 2014). Specifically, screening instrument scores are generally used rather than clinically diagnosed disorders. While this allows for study of sub-clinical symptoms, it also assumes that all factors are relevant across the continuum. Further, depending on the screening instrument, there is a possibility for reporter based differences between studies (e.g. teacher versus parent responses) (Lavigne et al. 2012). PPI potentially allows for a way to look at attention related processes from a physiological standpoint (Swerdlow et al. 2003). Further, the relationship between PPI and DA signaling makes it a compelling choice for studies related to MeHg.

Major results

Aim 1: Explore the relationship between Hg exposure in multiple windows of exposure and screening instrument scores

In general we found no significant relationships were found between CPT-II and CRS-R scores and prenatal (Trimesters 1, 2, 3 and delivery) and postnatal (ages 6-12 years) mercury exposures. While we did find that concurrent exposure to Hg, as measured in blood, hair, and urine, generally corresponded to higher CRS-R and CPT-II scores, this relationship was not

significant, except for one marginally significant association between urinary Hg and omission errors (p=0.076). Similarly, prenatal Hg exposure typically corresponded to higher CRS-R and CPT-II scores, although these relationships were again not statistically significant.

We found no statistically significant interactions between concurrent MeHg and Pb exposure. Existing studies generally find that Pb is associated with ADHD, but MeHg is not (Ha et al. 2009; Kim et al. 2013; Nicolescu et al. 2010). Previous studies in our group found non-linear associations with Pb exposure and attention (Huang et al. 2015), so additional analyses with an altered methodology may further clarify this relationship. Prenatal exposure to both metals presented a somewhat more complicated picture. Exposures to both metals in the first trimester showed a synergistic interaction that was significant or marginally significant in many cases. However, in the second trimester, interactions were observed in the opposite direction. No statistically significant interactions were observed between concurrent Pb and prenatal MeHg exposure.

While our results were still unclear in many places, there does seem to be evidence that prenatal exposures are more likely to be relevant to ADHD etiology. Further, we observed the most compelling results when examining interactions between first trimester Pb and MeHg.

Aim 2: Examine potential disruptions in dopaminergic pathways as a mechanism for MeHg effects on attention processes, via study of genetic polymorphisms

In our study cohort, we found associations between increased CRS-R ADHD index and DSM-IV total symptom scores and the genotypes of rs1800497 (Taq1A DRD2) and rs27072 (DAT1/SL6A3) containing "T" alleles. These crude associations then remained statistically significant after adjustment for demographic covariates. One other association was seen between the AG genotype of rs40184 (DAT1/SL6A3) and lower CRS-R scores, but this was only

marginally statistically significant for ADHD index and was not significant after demographic covariate adjustment.

Only two interactions between SNPs and MeHg were statistically significant and both involved hair Hg exposure, but not blood Hg exposure. For rs1800955 (DRD4), increasing Hg was associated with increasing ADHD symptoms for the TT genotype, but a decrease in those scores for all other genotypes. A similar differential association was observed for rs4680 (COMT), where increasing hair Hg exposure was associated with decreasing ADHD index scores for the GG genotype, but increasing scores about the GA and AA genotypes. This was only statistically significant for the GA genotype, however.

While further work, especially with a larger sample size or other SNPs related to these genes and pathway, will continue to clarify these potential relationships, these results suggest that an interaction between MeHg exposure and dopamine related SNPs might exist.

Aim 3: Explore the relationship between concurrent MeHg exposure and ASR and PPI

We found that ASR responses without a prepulse (intertrial intervals, ITIs) were non-linearly associated with MeHg exposure. The greatest magnitude of association was seen in the middle two quartiles of exposure, and all three of the upper quartiles of exposure corresponded to higher magnitudes of response. That is, individuals with higher MeHg levels than the reference group tended to startle more in response to a stimulus. Similarly, among both target and non-target ASR responses, higher MeHg generally corresponded to higher response magnitudes. These results all suggest that individuals with higher MeHg are generally having greater startle responses to a startling stimulus.

There was no clear relationship between MeHg and PPI. Although for target responses, in the lower tail of the distribution, higher MeHg corresponded to more inhibition, while in the upper tail, higher MeHg corresponded to less inhibition. An opposite pattern was seen in the

non-target responses. However, none of these were statistically significant. Because relationships were observed between MeHg and ASR, it is possible that that relationship is making any association between PPI and MeHg harder to detect.

Although our results are not conclusive, they represent one of the first studies of MeHg and ASR and PPI in human participants, rather than in animal models. Additional work with a larger sample size could potentially make any relationships more clear. Further, similarly to our work with CRS-R and CPT-II, ASR and PPI is an outcome which could be used to study interactions between MeHg and Pb. Animal studies have also focused on prenatal exposure, so further study of this outcome with exposures from pregnancy could be informative.

Public Policy Concerns and Potential Interventions

There are three core functions of public health: assessment, policy development, and assurance (Schneider 2014). Assessment includes monitoring the health status and identifying health problems in a given population. This would also incorporate investigating these problems, their risk factors, and other observed health hazards. Policy development includes the creation of laws meant to educate the public on health issues and initiate community-based efforts to solve health problems. Assurance consists of enforcement of existing regulations, efforts to connect individuals to clinical care and health services, and evaluation of population-based health service effectiveness (Schneider 2014).

These assertions provide a framework for how researchers might consider public health policies relevant to ADHD. The previous sections of this paper covered the assessment function of public health and the ongoing research into potential environmental risk factors. The following details several areas where research may assist in the development of health and environmental policy, using MeHg as the focus. While many of the results in this work were

inconclusive, they represent an attempt to better understand the health effects of MeHg. Refining that knowledge will eventually impact policymaking decisions by furthering the knowledge base available to policymakers. Moreover, exposures to toxic substances like MeHg can be limited or removed with effective policies and education, which could help to reduce the burden of and excess cost due to health effects of this pollutant.

Health Behavior and Education

Two of the major cohort studies examining the cognitive and behavioral effects of MeHg consistently find contrasting results. The studies in the Faroe Islands find deficits, while those in the Seychelles do not. A major difference in these two cohorts, which may partially explain these differing results, is source of exposure. In the Faroe Islands, exposure is primarily via consumption of pilot whales, while in the Seychelles exposure is via consumption of ocean fish (Davidson et al. 2010; Grandjean et al. 2012; Grandjean et al. 1997; Myers et al. 2003). Fish contain a number of compounds, such as ω-3 fatty acids (Mahaffey 2004; Ponce et al. 2000) that may have beneficial effects during neurodevelopment (Karr et al. 2011). Additionally, fish consumption is nutritionally beneficial for other conditions (Ginsberg and Toal 2009). Our study does not neatly align with the exposures seen in the Faroe Islands or the Seychelles. In our population, exposure is most frequently through consumption of canned tuna and it is not as large a staple of the diet as in either of those populations (Basu et al. 2014). Thus, the question is how to best balance the potential protective benefits of fish, as possibly seen in the Seychelles, with the deficits observed in the Faroe Islands.

In 2004, the FDA and EPA released a joint statement attempting to communicate guidelines for fish consumption to women of child bearing age (U.S. EPA and U.S. CDC 2004). These include:

1. Not eating species of fish known to have very high levels of MeHg. These include shark and swordfish.
2. Eating up to 12 oz. of low MeHg fish up to twice per week. Information is also provided about what constitutes a low MeHg species of fish.
3. Seeking information about local fish advisories which may affect the above guidelines.

This is a good start to help limit pre-natal exposure, while maximizing the nutritional benefits of fish consumption. However, there are still flaws. A recent study by Oken et al. found that participants had concerns over the ecological and economic impacts of their consumption habits. There is also a need for the guidelines to be clear and comprehensive, while not overwhelming the audience with technical information (Oken et al. 2012).

Environmental Policy

As mentioned in previous sections, a major source of Hg in the environment is coal-fired utilities (Clarkson and Magos 2006) , though never research suggests that this may now be derived from artisanal and small-scale gold mining operations. A major way to reduce exposure without reducing fish consumption, is reducing anthropogenic Hg emissions. As of 2010, it appeared that Europe and North America were, overall, reducing their Hg emissions. However, emissions from developing nations are still rising (Rallo et al. 2012), creating concerns as developing nations often have disparate health risks compared to other nations (Chongsuvivatwong et al. 2011; Doherty and Clayton 2011; Hanna and Kangolle 2010). In 2013, the United Nations Environment Programme (UNEP) agreed to the text of the Minamata

Convention on Mercury, which has the objective of protecting health and the environment from mercury pollution originating from anthropogenic emissions. It provides goals related to reducing emissions, dealing with existing contamination and mercury- containing waste, as well as measures to support the efforts of developing nations to address those goals (UNEP 2013). There is continued need for Hg specific policy to reduce emissions and potential exposure.

In Conclusion

The high prevalence and associated costs of ADHD make it a pressing public health concern. There is consistent evidence that a diagnosis of ADHD is related to reduced quality of life, poor academic performance, and difficulties in later employment (Birnbaum et al. 2005; DeShazo Barry et al. 2002; Escobar et al. 2005; Secnik et al. 2005). A thorough understanding of the etiology of ADHD could help us identify possible cases early, refine treatment, and, in the case of environmental risk factors, reduce incidence rates.

The chemical exposures of most interest for ADHD risk are those that have been previously observed to cause dysfunction in catecholamine signaling or lesions in areas of the brain suspected to be responsible for functional deficits. There is existing evidence that MeHg can disrupt normal DA signaling (Faro et al. 1997; Tiernan et al. 2013; Beyrouty et al. 2006; Chakrabarti et al. 1998). At high levels, it can cause lesions in some of the same brain regions which are affected in cases (Bertossi et al. 2004; Ceccatelli et al. 2010; Cherkasova and Hechtman 2013; Eto 2000), suggesting there is biological plausibility for it to add to ADHD risk.

Additionally, risk is likely to be associated with a particular window of development. In the case of MeHg, exposure current evidence points towards pre-natal exposure as the most susceptible period (Oken et al. 2005; Grandjean et al. 2012; Grandjean et al. 1997), although the exact window is not yet known. Further research to determine this vulnerable period could allow

existing interventions, such as the EPA and FDA fish consumption guidelines, to be more effectively targeted.

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