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The undersigned, appointed by the Committee on Higher Degrees in Population Health Sciences

Department of Population Health Sciences

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presented by **Anna Oppenheimer**

candidate for the degree of Doctor of Philosophy and hereby certify that it is worthy of acceptance.

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Date: May 10, 2021

Prenatal Exposure to Chemical Mixtures and Executive Function Among Adolescents in the New Bedford Cohort

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Prenatal Exposure to Chemical Mixtures and Executive Function Among Adolescents in the New Bedford Cohort

Abstract

Background: The three core executive functions – inhibition, working memory, and cognitive flexibility – are employed in higher-level cognitive processes such as problem-solving. Executive functions undergo substantial development during adolescence and decrements play a key role in many psychiatric and behavioral disorders. There is some evidence that prenatal exposure to individual chemicals may adversely impact executive functions among children, but few studies have explored the association of co-exposure to multiple chemicals with executive function in adolescence, a time when decrements may become discernable. In addition, few studies have investigated the association of prenatal chemical exposures with higher-level executive functions or potential mediators of this association. Determining the neuropsychological mechanisms that are most sensitive to chemical-related impacts and characterizing the relationships between core and higher-level executive functions are both essential to understanding the relation of chemical exposures with executive function. Therefore, the purpose of this dissertation was to identify modifiable environmental risk factors that may impact executive functions and to determine how the relationships between the core and higher-level executive functions may play a role in these relationships. We did this by first investigating the association of prenatal exposure to chemical mixtures composed of prevalent and neurotoxic organochlorines and metals with the core executive functions. We then investigated the association of prenatal

exposure to manganese (Mn) with problem-solving among adolescents and assessed the extent to which the core executive functions mediate this association.

Methods: Among a diverse group of 373 adolescents living near the New Bedford Harbor Superfund site in Massachusetts, we investigated the association of dichlorodiphenyldichloroethylene (DDE), hexachlorobenzene (HCB), polychlorinated biphenyls (PCBs), lead (Pb), and Mn with inhibition, working memory, and cognitive flexibility measured with subtests of the Delis-Kaplan Executive Function System (D-KEFS) and Wide Range Assessment of Memory and Learning, 2nd Edition (WRAML2). We did this by using Bayesian kernel machine regression (BKMR) as an exploratory tool to inform a traditional multivariable regression approach. We also assessed effect modification by sex and social disadvantage as well as the association of a secondary chemical mixture that included methylmercury (MeHg) and arsenic (As) in addition to the aforementioned chemicals with executive function. As we observed Mn to be consistently adversely associated with the core executive functions, we focused on Mn in our subsequent analyses. We used multivariable linear regression to assess the relation of prenatal Mn exposure with problem-solving skills followed by causal mediation methods to investigate whether the core executive functions may be potential mediators of this association.

Results: In BKMR models, we observed adverse joint associations of the chemical mixture with primarily verbal, but not non-verbal, measures of inhibition, working memory, and cognitive flexibility. In co-exposure and covariate-adjusted regression models, a twofold increase in cord blood Mn was associated with worse performance on a verbal inhibition task and multiple working memory and cognitive flexibility tasks. There were no other consistently adverse associations between biomarkers of prenatal exposure to the other targeted chemicals and executive functions. There was also little evidence of effect modification by sex but some evidence of effect

modification by social disadvantage in associations of organochlorine chemicals with working memory and cognitive flexibility. Cord blood Mn was also associated with lower problem-solving scores. In a subsequent mediation analysis, the combination of inhibition, working memory, and cognitive flexibility mediated 44% of the total effect of prenatal Mn on problem-solving. When each mediator was analyzed individually, working memory mediated a larger proportion of the effect than inhibition or cognitive flexibility.

Conclusions: This dissertation provided evidence of an adverse joint association of a chemical mixture with core executive functions, particularly when assessed with verbal tasks, and implicated Mn as more adverse than other chemicals in the mixture. It was also among the first to find evidence of an adverse association of prenatal exposure to Mn with a higher-level executive function and to identify certain neuropsychological mechanisms as mediators on the pathway of this association.

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CHAPTER 1:

Introduction

Executive functions are cognitive processes involved in higher-level, goal-oriented thinking and behavior such as planning, reasoning, and problem-solving.[1] The foundational components or building blocks of executive function are inhibition, working memory, and cognitive flexibility.[2] Inhibition is the ability to control one's attention or behavior by suppressing an automatic response, working memory is the ability to hold and manipulate information in one's mind, and cognitive flexibility is the ability to adapt and switch between tasks.[1,3] Development of these three core executive functions begins in childhood and continues through adolescence and into young adulthood, however their developmental trajectories differ.[3] Specifically, inhibition skills undergo substantial development in early childhood followed by more gradual improvements in mid-childhood, adolescence, and young adulthood.[3] Meanwhile, working memory and cognitive flexibility steadily improve from childhood though adolescence and early adulthood.[3] As the three core executive functions develop, they are employed to solve complex problems, however the way in which they are utilized varies by age and stage of development.[3]

The prefrontal cortex mediates executive function tasks, though other brain regions such as the anterior cingulate cortex, parietal cortex, and hippocampus are also involved.[4] Adolescence is a critical time for brain development including structural changes in the prefrontal cortex.[4] Specifically, magnetic resonance imaging (MRI) studies have found that in adolescents, synaptogenesis and synaptic pruning occur in the prefrontal cortex, while the axonal myelination process continues throughout numerous brain regions.[4,5] Because executive functions and the brain region most directly associated with these skills undergo substantial development in adolescence, this is an optimal time to not only distinguish between the different components of executive

function and to measure them, but also to discern decrements that may not become apparent until this time. Executive functions are essential for most daily activities and play a role in social development, school performance, and the ability to find and maintain employment.[3,6,7] Poor executive function skills are associated with numerous psychiatric and behavioral disorders such as substance use disorder, attention-deficit/hyperactivity disorder (ADHD), conduct disorder, depression, obsessive compulsive disorder (OCD), and schizophrenia.[8–13] Specific decrements, such as those found in working memory, are important features of schizophrenia, ADHD, and autism.[14–17]

The purpose of this dissertation was to identify modifiable environmental risk factors that may impact the different core executive functions that play an important role in many disorders of public health importance; to investigate which types of executive function skills may be especially susceptible to chemical impacts; and to determine how the relationships between the core and higher-level executive functions may play a role in these associations. We focused on a vulnerable exposure window, the prenatal time period, and assessed executive function during adolescence when decrements are likely to manifest. Specifically, among adolescents, we first investigated the potential associations of prenatal chemical exposures with the three core executive functions. These analyses focused on exposure mixtures composed of prevalent and potentially neurotoxic contaminants and considered specific populations that may be at the highest risk of chemical-related impacts. We also investigated the association of a chemical exposure with problem-solving, a higher-order executive function that employs the core executive functions. By using an innovative consideration of the potential for the complex inter-relationships among executive function measures to play a role in observed associations, we were able to identify

neuropsychological mechanisms that may mediate the association of certain chemicals with problem-solving.

In the prenatal time period, the brain undergoes rapid development.[18] However, the fetus is not-well protected from neurotoxic injury at this time as many environmental toxicants are not blocked by the placenta.[19] Toxic exposures in the prenatal period may result in adverse cognitive impacts throughout the life course.[20] The exposures assessed in this study included organochlorine chemicals: dichlorodiphenyldichloroethylene (DDE), hexachlorobenzene (HCB), polychlorinated biphenyls (PCBs) and metals: lead (Pb), manganese (Mn), methylmercury (MeHg), and arsenic (As). These were chosen based on epidemiologic evidence of an association of prenatal exposure to each chemical of interest with executive function measures in childhood or adolescence, as well prevalence of exposure and availability of biomarker data within our population of interest. Although the organochlorine chemicals were banned and use discontinued in the United States, they are still found ubiquitously in human serum due to their persistence in the environment and bioaccumulation in animals and humans.[21] The metals analyzed in this study are also ubiquitous in the environment and may be neurotoxic even at low levels.[18,22] Mn differs from the remaining metals as it is necessary for proper brain development and functioning, however it may be neurotoxic at deficient or excess levels.[23,24] We assessed the impact of co-exposure to multiple pollutants as it has been well-established that humans are exposed to multiple pollutants simultaneously and these exposures may often interact resulting in synergistic or antagonistic effects on health.[25,26]

Previous studies have observed associations of prenatal exposure to each of the chemicals of interest with executive functions in childhood and, to a much lesser extent, in adolescence. Specifically, there is evidence of adverse associations of prenatal exposure to DDE, HCB, PCBs,

Pb, and Mn with inhibition and working memory measured with both psychometric testing and behavioral checklist assessments in early to mid-childhood, though these findings have not been consistent across studies.[27–38] There are fewer studies of cognitive flexibility or higher-level executive functions, however there is some evidence of associations of prenatal exposures to PCBs and Pb with poor performance on cognitive flexibility tasks in mid-childhood.[27,37] Less is known about the association of prenatal MeHg or As exposure with executive function. However, one prospective study did not find an association of prenatal MeHg with working memory in adolescence.[39] Meanwhile, cross-sectional studies have found adverse correlations of exposure to As with working memory, switching attention, and problem-solving among children ranging from pre-school through adolescence.[40–43]

Few studies have investigated the association of prenatal co-exposure to multiple chemicals and executive function in childhood or adolescence. One study found that an adverse association between current blood Pb and an inhibition task was stronger in children with lower current MeHg and PCB exposures.[44] In another, researchers found that among adolescent participants with low cord blood levels of MeHg, high cord blood Pb concentrations were adversely associated with a measure of working memory.[45] Other studies of chemical mixtures and executive function have been cross-sectional, [46,47] resulting in the inability to establish the direction of the association thereby limiting the interpretability of findings .

Although these studies have been vital in establishing the evidence of adverse associations of prenatal exposures to organochlorines and metals with executive function outcomes, they have some important limitations. First, the vast majority measured executive functions in childhood. Although executive function skills begin to develop in childhood, they mature and become more distinct in adolescence, therefore decrements may not become apparent until this developmental

time period. In addition, few studies have explored the association of prenatal exposures with higher level executive functions such a problem-solving or planning among adolescents. There has also been no exploration into the different neuropsychological pathways through which such an association may occur. Finally, few studies have assessed co-exposure to multiple chemicals simultaneously despite mounting evidence that the impact of exposure to multiple chemicals may be different than exposure to each chemical individually.[26] Those studies that have assessed chemical mixtures have either been cross-sectional resulting in the inability to establish the direction of the association, or used traditional statistical methods. Using traditional statistical methods such as multivariable parametric regression to analyze chemical mixtures may be limited for several reasons: there may be non-linear or non-additive relationships between the exposures and executive functions; exposures may be highly correlated resulting in multicollinearity; and including multiple chemical exposures, covariates, and interactions in the model may lead to model overspecification and unstable estimates.[48]

In this dissertation, we attempted to fill these gaps in the literature by investigating the association of prenatal exposure to chemical mixtures composed of organochlorines and metals with different levels of executive function among a group of diverse adolescents living near a superfund site in Massachusetts. First, we investigated the association of prenatal exposure to chemical mixtures with each of the core executive functions: inhibition (Chapter 2), working memory (Chapter 3), and cognitive flexibility (Chapter 4) using both multivariable parametric regression as well as Bayesian kernel machine regression (BKMR), a method that accounts for the limitations of a more traditional approach. To better capture the various components of each executive function, we used multiple subtests of standardized psychometric instruments (Table 1.1) and where applicable, combined scores on different tasks (e.g., speed and accuracy).

Table 1.1 Components of Delis-Kaplan Executive Function System (D-KEFS)[1] and Wide Range Assessment of Memory and Learning, 2nd Edition (WRAML2)[2] used to measure executive functions.

Executive Function	Test	Subtest	Condition	Description
Inhibition				
	D-KEFS[1]	Design Fluency	Empty Dots Only	Inhibit connecting filled dots and only connect empty dots to create as many different designs as possible.
	D-KEFS[1]	Color-Word Interference	Inhibition	Name font color of words denoting colors, rather than the words that are printed.
Working Memory				
	WRAML2[2]	Verbal Working Memory		After hearing list of words, recall animal words in size order followed by non-animal words in any order. Next, recall both animal words and non-animal words in size order.
	WRAML2[2]	Symbolic Working Memory		After hearing series of numbers, identify listed numbers in correct order on Number Stimulus Card. Next, after hearing number-letter series, identify listed numbers and letters in correct order on Number-Alphabet Stimulus Card.
Cognitive Flexibility				
	D-KEFS[1]	Trail-making	Number-Letter Switching	Alternate connecting numbers then letters in numeric and alphabetic order.
	D-KEFS[1]	Verbal Fluency	Category Switching	Alternate saying words from two different categories as quickly as possible.
	D-KEFS[1]	Design Fluency	Filled Dots + Empty Dots Switching	Draw designs alternating connections between filled & empty dots.
	D-KEFS[1]	Color-Word Interference	Inhibition/ Switching	Switch between naming font colors of color words and reading words denoting colors.
Problem-Solving				
	D-KEFS[1]	Sorting	Free Sorting	Sort mixed-up cards into two groups according to as many rules as possible.
	D-KEFS[1]	Sorting	Sort Recognition	Identify the correct rules used by the examiner to sort two sets of cards.
	D-KEFS[1]	Tower	Total Achievement	Move different sized disks, one at a time, across three pegs to build pictured towers in fewest number of moves possible.

1. D-KEFS: Delis Kaplan Executive Function System.[49]
2. WRAML2: Wide Range Assessment of Memory and Learning, 2nd Edition.[50]

As previous studies of prenatal chemical exposures and cognition have found evidence of effect modification by sex and social disadvantage,[29,33,34,46,51,52] we accounted for these potential interactions with sex- and social disadvantage-stratified models where possible. Building on the first three chapters of this dissertation in which Mn was found to the be strongest predictor of the building blocks of executive function, we focused on prenatal exposure to Mn and assessed its association with problem-solving skills in the fifth chapter. In an attempt to elucidate the neuropsychological mechanisms through which Mn may impact problem-solving, we then used causal mediation methods to identify the extent to which the core executive functions mediated the association of Mn with problem-solving skills. In doing so, we hope this work contributes to identifying modifiable environmental risk factors that may impact executive functions and the neuropsychological mechanisms through which these associations may be mediated with a focus on vulnerable populations.

Chapter 2:

Prenatal exposure to chemical mixtures and inhibition among adolescents

Anna Oppenheimer, David Bellinger, Brent Coull, Marc Weisskopf, Michele Zemplenyi, and Susan Korrick

Abstract

Background: Inhibition, one of the building blocks of executive function, is the ability to focus one's attention despite interference from external stimuli. It undergoes substantial development during adolescence and may be susceptible to adverse impacts of prenatal exposure to chemical mixtures, yet few studies have explored this association.

Methods: The New Bedford Cohort (NBC) is a birth cohort of residents living near the New Bedford Harbor Superfund site in Massachusetts. Among adolescents from the NBC, we investigated the association of biomarkers of prenatal exposure to organochlorines (DDE, HCB, PCBs) and metals (Pb, Mn) with inhibition, assessed with the Delis-Kaplan Executive Function System Design Fluency (non-verbal task) and Color-Word Interference (verbal task) subtests. The outcomes were dichotomized into the best (reference) and the poor performance group based on the medians of two sets of scores most reflective of inhibition skills. An exploratory mixtures analysis using Bayesian Kernel Machine Regression (BKMR) informed a traditional multivariable regression approach assessing all chemical exposures in a single model.

Results: NBC adolescents are diverse with 29% non-white and 31% in a low-income household at birth. Cord serum organochlorine concentrations and cord blood metals concentrations were generally similar to other general population-based birth cohorts. In BKMR models, we observed a suggestive adverse association of the chemical mixture with Color-Word Interference but not

Design Fluency. In covariate-adjusted logistic regression models, a doubling of cord blood Mn was associated with 1.72 increased odds (95% CI: 1.05, 2.86) of being in the poor compared to the best Color-Word Interference performance group. Prenatal exposure to organochlorines or other metals were either null (ΣPCB_4, HCB) or unexpectedly associated with a decreased odds of being in the poor performance group (DDE, Pb).

Conclusion: This study provided evidence of an adverse joint association between a chemical mixture and a verbal inhibition task and implicated Mn as more adverse than other chemicals in the mixture.

Introduction

Executive functions are mental processes that form the basis of higher-level cognition, including problem-solving, planning, and reasoning.[1] The three core executive functions are inhibition, working memory, and cognitive flexibility.[2] Inhibition, the focus of the present study, is the ability to resist impulse and to focus one's attention, behavior, and thoughts, despite external stimuli.[1] Although some executive function development begins at a young age, inhibition undergoes substantial evolution during adolescence. This evolution parallels structural and functional changes in the pre-frontal cortex, a part of the brain critical for most executive functions, that occur in this age group.[53] Executive function skills predict school achievement and poor executive functions can lead to difficulty finding and maintaining employment.[6,7] In addition, altered executive function is associated with psychiatric and behavioral disorders including substance use disorder, attention-deficit/hyperactivity disorder (ADHD), conduct disorder, depression, obsessive compulsive disorder (OCD), and schizophrenia.[8–13] Therefore, identifying

modifiable risk factors associated with executive function decrements may diminish the impact of such disorders.

Epidemiologic studies have provided evidence that prenatal exposures to environmental contaminants may be associated with cognitive impacts throughout the life course. The fetus is not well-protected from some environmental exposures. The placenta does not block the maternal transmission of pregnancy exposure to many environmental toxicants including organochlorines and some metals, which are ubiquitous in the environment.[22,54,55] In utero, the developing brain undergoes rapid neurological growth and is therefore highly sensitive to potential injury from toxic chemicals that may result in long-term neurotoxic impacts.

Prenatal exposures to organochlorines such as dichlorodiphenyldichloroethylene (DDE), hexachlorobenzene (HCB), and polychlorinated biphenyls (PCBs) have not been studied in relation to inhibition among adolescents specifically. However, among younger children in the Great Lakes region of the United States, two prospective cohort studies found evidence of an association between cord serum PCB levels and poor inhibition measured by psychometric tests of impulse control such as errors of commission on a Continuous Performance Test (CPT) and perseverative errors on the Wisconsin Card Sorting Test (WCST).[27,28,31] In contrast, in the New Bedford Cohort (NBC), researchers did not find errors of commission on the Neurobehavioral Examination System 2 (NES2)-CPT to be adversely associated with cord serum DDE or PCB concentrations among 8-year-olds who had lower or similar exposure levels, respectively, to the two Great Lakes cohorts.[32,56] Of note, the NES2-CPT is less sensitive to errors of commission than some CPT instruments.[57] Prenatal exposure to metals may also adversely impact childhood inhibition skills. Manganese (Mn) is an essential trace element necessary for proper brain functioning, though it has been found to induce neurotoxicity at high levels.[24] The impact of

prenatal exposure to Mn on inhibition has not been well-studied among adolescents, but in an exploratory study of younger children located in ten locations across the United States, deciduous tooth Mn levels corresponding to 20 weeks' gestation were associated with multiple measures of behavioral disinhibition assessed with a Forbidden Toy task, a CPT, and a children's Stroop Test at ages 36 and 54 months.[38] Finally, in a study of a high fish-eating population in the Seychelles, pre- and post-natal exposure to methylmercury (MeHg) measured in maternal and participant hair, respectively, was not found to be associated with inhibition, as measured by the Stroop Color Word Test among 24-year-olds.[58]

Exposure to chemical contaminants rarely occurs independently[25] and co-exposure to chemical mixtures may result in different, often worse, health effects.[26] Only one study has assessed the association of a chemical mixture with inhibition. In a prospective cohort of children from Arctic Quebec, researchers found that the adverse association between current blood Pb and a child's ability to inhibit a response in a Go/No-Go task was stronger in children with lower current MeHg and PCB exposures.[44] Other studies have assessed the relation of metal mixtures or metal-PCB mixtures with general executive function or other specific components of executive function, such as working memory. In a prospective cohort study based in Spain, prenatal co-exposure to a metal mixture (composed of cobalt, copper, As, cadmium, antimony, thallium, and Pb) measured in maternal urine during pregnancy was not associated with McCarthy Scales of Children's Abilities (MSCA) executive function scores among 4-year-olds.[59] In a cross-sectional study of 8 to 11-year-old children from Bangladesh, researchers found that blood Mn was associated with lower Wechsler Intelligence Scale for Children (WISC-IV) working memory scores, but they did not observe a significant interaction between Mn and As.[47] Finally, in a birth cohort study based in the Faroe Islands, among participants with low cord blood levels of MeHg,

higher cord blood Pb concentrations were associated with lower Digit Span Backward scores, a measure of working memory, at age 14.[45]

In summary, many studies have linked prenatal exposures to organochlorines and metals with decrements in executive function among children. Few have focused on inhibition or on adolescence, when the impact of earlier exposures on executive function may become most readily apparent due to it being a time of substantial executive function development. In addition, even though it has been well-established that the developing brain may be exposed to multiple pollutants simultaneously in utero, few studies have assessed the impact of prenatal exposure to mixtures of prevalent neurotoxic chemicals on executive function and only one has focused on inhibition. Therefore, the purpose of this study was to address these key gaps in the literature by investigating the association of prenatal exposure to a previously unstudied chemical mixture of organochlorines (DDE, HCB, PCBs) and metals (Pb, Mn, MeHg, As) with detailed measures of inhibition among adolescents.

Methods

Study population. The New Bedford Cohort (NBC) is an ongoing, prospective birth cohort study originally designed to assess the effects of prenatal exposures to common chemical pollutants on child development. Between 1993 and 1998, 788 mother-infant pairs were recruited and enrolled in the study shortly after birth at St. Luke's Hospital, New Bedford, Massachusetts. Mothers were eligible to participate if they were at least 18 years old, spoke English or Portuguese, and were living in one of the four towns surrounding the New Bedford Harbor for at least the duration of their pregnancy. This region of southeastern Massachusetts was chosen for study because the New Bedford Harbor, an EPA Superfund site, was highly contaminated with PCBs and metals from

local industrial emissions and there was concern about potential chemical exposure risk to surrounding communities.[60,61] Participation exclusion criteria included birth by cesarean section and infants requiring high-grade neonatal care or who were too ill to undergo neonatal examination. Biomarkers of prenatal chemical exposure were collected at birth or approximately ten days later at a postpartum home visit. The NBC study participants have undergone neuropsychological testing periodically since birth. This analysis focuses on the 528 adolescents who participated in 15-year follow-up exams (median age 15.5, range 13-17 years) between 2008 and 2014, which included psychometric tests of executive function. Of the 528 participants, 373 had complete data on all executive function outcomes and covariates of interest as well as biomarkers of prenatal exposure to DDE, HCB, PCBs, Pb, and Mn. This group will be referred to as *Set 1.* A subset of 235 participants had complete data on the *Set 1* measures , as well as biomarkers of prenatal exposure to MeHg and As, and will be referred to as *Set 2.*

Chemical exposure assessment. Cord blood samples were collected at birth, centrifuged, and the serum fraction removed and stored at -20 degrees Celsius prior to analyses at the Harvard T.H. Chan School of Public Health Organic Chemistry Laboratory (Boston, Massachusetts). After liquid-liquid extraction, cord serum was analyzed for DDE, HCB, and 51 individual PCB congeners using gas chromatography with electron capture detection.[60,62,63] For this analysis, we used the sum of the four most prevalent PCB congeners - 118, 138, 153, 180 (ΣPCB_4) - as they were measured with the least measurement error and are most frequently used to assess congener-specific health effects in other population-based studies.[63] For the organochlorines, the limits of detection (LODs) ranged from 0.001 ng/g to 0.07 ng/g serum. Organochlorine chemical analyses

were highly reproducible with within-batch coefficients of variation ranging from 5% to 7.5% and the between-batch coefficients of variation ranging from 20% to 39% over 5 years of analysis.[62]

Cord whole blood samples were also collected at birth and refrigerated prior to metals analyses at the Harvard T.H. Chan School of Public Health Trace Metals Laboratory (Boston, MA). Blood Pb and Mn were measured using isotope dilution (ID) inductively coupled plasma mass spectrometry (ICP-MS, Sciex Elan 5000, Perkin Elmer, Norwalk, CT) and external calibration on a dynamic reaction cell-inductively coupled plasma-mass spectrometer (DRC-ICP-MS, Elan 6100, Perkin Elmer, Norwalk, CT), respectively. Concentrations were reported as the mean of five replicate measurements. Procedural blanks, duplicates, spiked samples, standard reference material (NIST SRM 955b Pb in blood; NIST SRM 1643d trace elements in water); biological reference material (ICP03B-05 and ICP03B-02 multi-elements in human blood from INSPQ/ Laboratoire de Toxicologie, Quebec); and certified reference material (GBW 09101 human hair, Shanghai Institute of Nuclear Research, Academia Sinica, China) were used for quality control (QC) monitoring. Recovery rates for QC and spiked samples were 90-110% and precision >95%. The LOD was 0.02 µg/dL.

Maternal hair samples were cut from the occiput, on average, 10 days postpartum and analyzed for mercury (Hg) at the Harvard T.H. Chan School of Public Health Trace Metals Analysis Laboratory. Prior to analysis, hair samples were cleaned using sonication, rinsed with distilled deionized water, and dried for 24 hours.[64] Where the proximal end was identified, the three centimeters closest to the scalp, which approximates Hg exposure in the third trimester of pregnancy, were analyzed for total Hg by atomic absorption spectroscopy using a DM-80 Direct Mercury analyzer. Hair total Hg concentrations are a reasonable proxy for hair MeHg levels.[65] Quality control procedures included daily calibration verification, procedural blanks and certified

reference material (CRM-397).[66] Recovery rates for quality control standards were 90-110%, precision >95% and the average LOD was 50 ng/g of hair.[64]

Arsenic (As) was measured in maternal toenails collected, on average, 10 days after birth. Analyses were done at the Trace Element Analysis Laboratory at Dartmouth College (Hanover, New Hampshire). The toenail samples were cleaned by sonication, rinsed with distilled deionized water, and dried prior to analysis. The samples were then weighed and digested with 1 ml of HNO_3 acid for 24 hours at room temperature. Analyses were performed using an external calibration method on a dynamic reaction cell-inductively coupled plasma-mass spectrometer (Agilent 7700x ICP-MS), which used 5 standards at concentrations ranging from 0 to 50 ng/ml. Quality control procedures included analyses of daily calibration verification, a procedural blank, and certified reference material. Coefficients of variation for reference standards were less than 15% for toenail As.[67] The average LOD for As in toenails was 0.03 ng/g.

Inhibition assessment. At the NBC 15-year follow-up, a trained study examiner administered six subtests of the Delis-Kaplan Executive Function System (D-KEFS).[49] Inhibition was assessed using two of these subtests: Design Fluency: Empty Dots Only condition (a non-verbal task) and Color-Word Interference: Inhibition condition (a verbal task). In Design Fluency: Empty Dots Only, the examinee is presented with response boxes that contain 5 filled dots and 5 empty dots and the examinee must inhibit connecting filled dots and only connect those dots that are empty to create as many different designs as possible within 60 seconds. Performance was measured by combining the two scores most reflective of inhibitory skills: total correct and total set loss designs raw scores. Set loss designs are those designs that violate the principle rule for the condition, such as connecting a filled dot to create a design. Performance was dichotomized: the best performance

group included those who performed better than the population median score for both dimensions (total correct raw score > 10 and total set loss designs raw score = 0) and the poor performance group included the remaining participants. In Color-Word Interference: Inhibition, the examinee must inhibit reading words denoting colors to name dissonant font colors in which those words are printed. For example, if the word '*red'* is printed in green font, the examinee must inhibit saying the word *'red'* and, instead, say the word '*green'.* Performance was measured by combining the two main Color-Word Interference: Inhibition scores reflecting inhibition – total completion time and total errors raw scores – and dichotomized. The best performance group included those who had performed better than the population median score for both dimensions (total completion time raw score < 52.0 seconds and total errors raw score < 2), while the poor performance group included the remaining participants. Measuring performance on each of the inhibition subtests by integrating two different scoring criteria for each task allowed us to create a more comprehensive representation of overall performance by, for example, simultaneously considering both speed and accuracy in assessing performance on the Color-Word Interference task rather than considering each separately, as is commonly done. This approach also created comparable outcome measures for both D-KEFS subtests.

Covariate assessment. Periodic medical record review as well as parental and child self-reported questionnaire data were used to obtain and update demographic, health, lifestyle, and exposure information for the study participants. At birth, a trained study nurse reviewed medical records to obtain infant race/ethnicity, birth weight, gestational age, information about the mother's pregnancy and delivery, and the baby's initial pediatric examination and any laboratory test results after delivery.[64] Approximately ten days later, participating mothers were interviewed at a home

visit to gather information about maternal pregnancy diet, smoking, alcohol, and drug use, medical and reproductive histories, infant feeding, demographic information, income, and occupational and educational histories for both parents. Medical record reviews and questionnaire data were updated at 8-year and 15-year follow-up assessments. These follow-up assessments also included a home visit to assess the quality of the child's home environment and parent-child relationship using the Home Observation for Measurement of the Environment (HOME) questionnaire.[68] Maternal IQ was assessed using the Kaufman Brief Intelligence Test (KBIT)[69] either at the 8-year or 15-year follow-up.

Statistical analysis. The main exposure of interest was a chemical mixture composed of biomarkers of prenatal exposure to DDE, HCB, ΣPCB_4, Pb, and Mn. In secondary analyses, we added biomarkers of prenatal exposure to MeHg and As to the mixture. MeHg and As were not included in the primary analyses in order to improve power, as MeHg and As concentrations were measured in maternal hair and nails collected 10 days postpartum rather than at delivery, which resulted in significant missingness.

Regression diagnostics supported log-transforming chemical exposures to reduce the influence of extreme values. Log_2-transformation was used so all effect estimates represent a two-fold increase in exposure levels. As an exploratory tool, we first used Bayesian Kernel Machine Regression (BKMR) to assess potential non-linear dose-response relationships and interactions among exposures in determining inhibition skills. BKMR is an exposure-response surface estimation technique for mixtures that models the relationship between a high-dimensional set of predictors and an outcome using a flexible exposure-response function.[70] Using Markov Chain Monte Carlo (MCMC) for Bayesian inference, this method is able to overcome issues such as collinearity and overfitting that can be problematic with other approaches to exposure mixture

assessments.[70] BKMR accommodates binary outcomes using generalized linear modeling, specifically with the probit link function expressing a binary outcome as a dichotomization of a continuous latent variable.[70] Due to the high dimensionality of the exposure mixture, it is not possible to visualize the entire exposure-response function resulting from a BKMR analysis. However, it is possible to visualize the relationship between each individual exposure and an outcome or the joint effect of two exposures on an outcome, while fixing the other exposures to pre-specified values, such as the median of each distribution. The resulting visualizations of the exposure-response relationship facilitate identification of non-linear exposure-outcome associations and potential interactions among exposures.

Specifically, we visually inspected plots of the estimated exposure-response functions and 95% credible intervals of the five main exposures (DDE, HCB, ΣPCB_4, Pb, and Mn) and the seven secondary exposures (5 main exposures plus MeHg and As) with inhibition performance while assigning the remaining exposures to their median value. Where the exposure-response functions appeared non-linear, we formally tested whether a quadratic term for the chemical should be included in the model with a Wald test and by fitting covariate-adjusted logistic regression models with and without a quadratic term and comparing model fit using a likelihood ratio test for nested models. Next, we visually inspected plots of the estimated exposure-response functions between one of the five main exposures or seven secondary exposures and inhibition performance, where a second exposure was fixed at varying levels of exposure while the remaining exposures were assigned to their median value. We assumed no interaction where the slope of each chemical was similar at varying levels of the second exposure. BKMR analyses were also used to assess the joint association of the chemical mixture with each of the inhibition subtests. All analyses were conducted using R version 3.6.0[71] with BKMR analyses conducted using the *bkmr* package.[72]

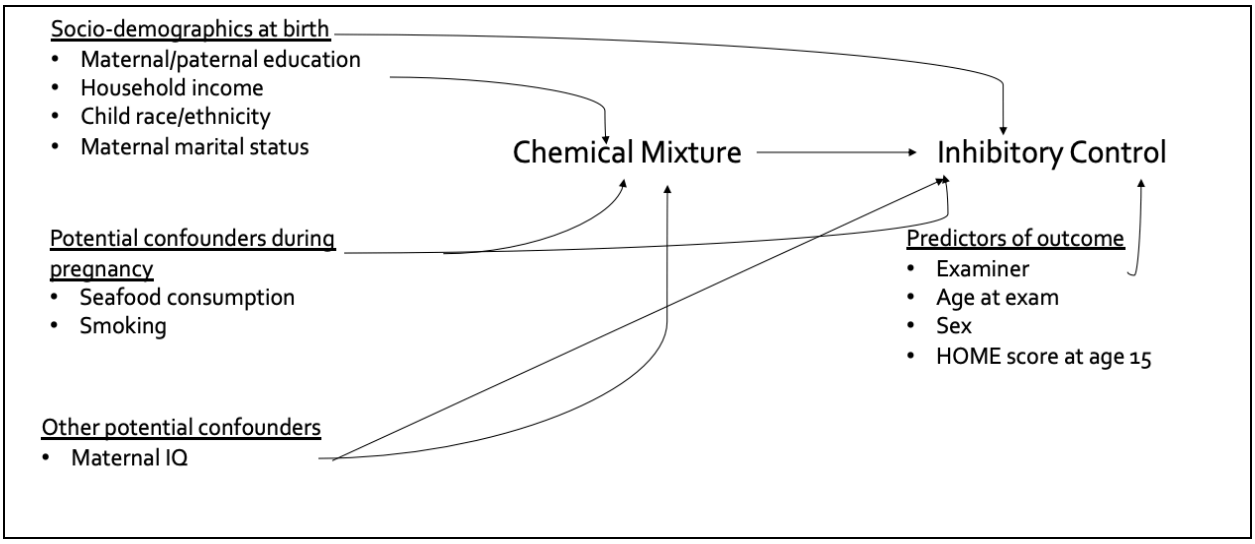
We used the results of our BKMR analyses to inform parametric logistic regression models estimating the odds of being in the poor compared to the best performance (reference) group using odds ratios (ORs), while adjusting for covariates. All five (*Set 1*) or seven (*Set 2*) exposures were included in the models simultaneously. In the main (*Set 1*) analyses, we also assessed potential effect modification of exposure associations by sex in sex-stratified logistic regression models. To assess the potential for each component of our outcome measures to be differentially impacted by exposures, we also analyzed the association between the five-chemical mixture and each dimension of each binary outcome, one-at-a-time: Design Fluency total correct, Design Fluency set loss designs, Color-Word Interference completion time, and Color-Word Interference total errors raw scores. Specifically, as Design Fluency number of total correct and Color-Word Interference completion time (measured in seconds) were normally distributed, we used linear regression to estimate the association between the chemical mixture and these outcomes using betas or differences. However, as the distributions of Design Fluency set loss designs and Color-Word Interference total errors were consistent with over-dispersed count data, negative binomial regression was used to estimate the relationship of these outcomes with chemical mixtures using rate ratios (RRs). Once again, all five (*Set 1*) exposures were included in the models simultaneously.

To account for potential selection bias due to loss to follow-up, we used logistic regression with inverse probability weights (IPW) for censoring.[73] IPW is a technique in which individuals in the analytic group are weighted based on the inverse of the probability of being included in the analysis, given their particular exposure and covariate values. The following exposures and covariates were chosen for the IPW missingness model based on their prediction of loss to follow-up for this analysis as well as for other longitudinal cohort studies reported in the literature:

biomarker levels of DDE, HCB, ΣPCB_4, and Pb and socio-demographic characteristics of the mother at birth such as education and household income and child characteristics such as race/ethnicity and sex. This weighting procedure created a pseudo-population that represented the original source population that was recruited to the NBC at birth but, by definition, did not include those who were missing covariates used to create the weights. The distributions of non-missing covariates were comparable between the source population (n=622) and the original cohort (n=788) supporting the representativeness of our population weights. We used stabilized IPW,[73] trimmed at the 2.5th and 97.5th percentile.

Potential covariates were selected using a Directed Acyclic Graph (DAG) (Figure 2.1) that was developed based on a review of the literature regarding potential confounders of the relationship of prenatal organochlorine and metal exposures with cognition. We also considered covariates that had been previously found to predict cognitive outcomes in the NBC. Based on DAGs and priors, the following covariates were included in the final models: adolescent race/ethnicity, sex, age at exam, and HOME score; maternal marital status at birth, IQ, seafood consumption and smoking during pregnancy; maternal and paternal education and household income at child's birth; and examiner. Characteristics of participants who were included in the main and secondary analyses were compared to those not included using t-tests, Wilcoxon Rank Sum tests, and chi-square tests where appropriate.

Figure 2.1 Directed acyclic graph (DAG) describing potential confounders and predictors of inhibition that were included in adjusted models.



Results

Study population: Table 2.1 describes the characteristics of adolescents in the main analytic group who had complete executive function outcome measures, covariates, and biomarkers of exposure (*Set 1*: DDE, HCB, ΣPCB_4, Pb, Mn) and those who were excluded from the main analysis. Supplemental Table 2.1 describes the characteristics of adolescents in the secondary analytic group who had complete executive function outcome measures, covariates, and biomarkers of exposure (*Set 2*: DDE, HCB, ΣPCB_4, Pb, Mn, MeHg, As) and those who were excluded from the secondary analysis. The NBC population included in the main analysis was socio-demographically diverse with 29% of participants being non-white, 50% having mothers with less than or equal to a high school education at the time of their birth, and 31% having an annual household income of less than $20,000 per year at the time of their birth (Table 2.1). Those included in both the main and secondary analyses had, on average, characteristics consistent with greater sociodemographic advantage compared to those excluded. Specifically, compared to those excluded, participating adolescents were more likely to be white and to live in households with higher incomes and HOME scores. They also tended to be younger at the time of their exam. Their mothers had higher IQs and were more likely to be married at birth; both parents had higher educational attainment. In addition, 15-year follow-up participants in this analysis had higher serum levels of DDE and lower cord blood Pb levels than those excluded from the study. Lastly, in *Set 1*, adolescents committed fewer Design Fluency set loss design errors than those who were excluded (Table 2.1), while in *Set 2*, adolescents performed better on all tests of inhibition than those who were excluded (Supplemental Table 2.1).

Table 2.1 Characteristics of New Bedford Cohort participants who were evaluated as adolescents and included in the main analysis group[1] and those who were excluded from the main analysis group.

Descriptive Characteristic	**Main analysis group, n=373**			**Excluded group, n=415**			
Inhibition Measures[3]	**n (%)**	**Mean (SD)**	**Range**	**n (%)**	**Mean (SD)**	**Range**	**p-value[2]**
Design Fluency raw scores							
Total number correct	373	9.9 (3.1)	1-19	155	9.6 (3.5)	0-23	0.3
Total number set loss designs	373	0.3 (0.8)	0-9	155	0.8 (2.3)	0-22	0.01*
Overall Design Fluency performance							
Best performance	129 (34.6)			40 (25.8)			0.06
Poor performance	244 (65.4)			115 (74.2)			
Color-Word Interference raw scores							
Completion time (seconds)	373	53.9 (13.3)	29-132	154	53.9 (12.4)	35-108	1
Total number errors	373	2.3 (2.5)	0-19	154	2.3 (2.3)	0-12	0.7
Overall Color-Word Interference performance							
Best performance	117 (31.4)			37 (23.9)			0.1
Poor performance	256 (68.6)			117 (75.5)			
Missing	0			1 (0.6)			
Exposure Measures[4]							
Cord serum DDE (ng/g)	373	0.6 (1.2)	0.02-14.9	378	0.4 (0.5)	0-4.2	0.003*
Cord serum HCB (ng/g)	373	0.03 (0.02)	0-0.1	378	0.03 (0.05)	0-0.7	0.2
Cord serum ΣPCB_4 (ng/g)	373	0.3 (0.3)	0.01-4.4	378	0.2 (0.2)	0.01-1.9	0.05
Cord blood Pb (µg/dL)	373	1.4 (0.9)	0-9.4	375	1.7 (1.7)	0-17.4	<0.001*
Cord blood Mn (µg/dL)	373	4.2 (1.6)	0.7-14.6	335	4.3 (2.0)	0.2-22.1	0.6
Covariate Measures[5]							
Child Characteristics							
Race/Ethnicity							0.09
Non-Hispanic White	263 (70.5)			268 (64.6)			
Hispanic	33 (8.8)			56 (13.5)			
Other	77 (20.6)			89 (21.4)			
Missing	0			2 (0.5)			
Sex							0.05
Male	179 (48.0)			229 (55.2)			
Female	194 (52.0)			186 (44.8)			
Age at Exam	373	15.5 (0.6)	14.4-17.8	155	15.7 (0.7)	14.0-17.9	<0.001*

Table 2.1 (Continued)

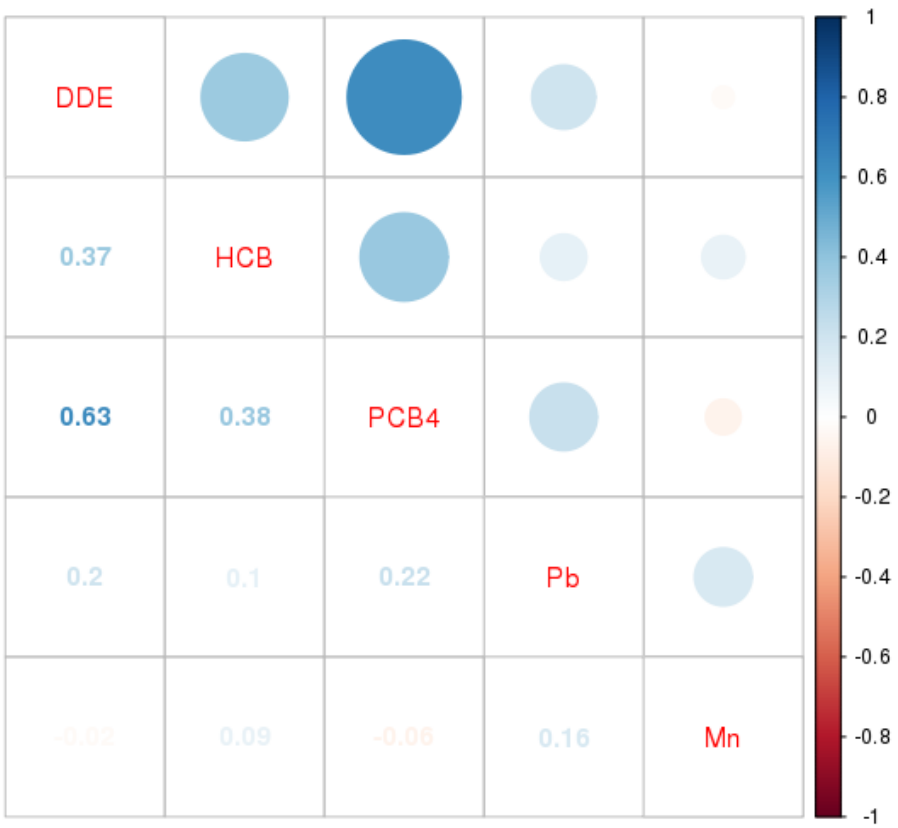
Home Score	373	43.9 (6.3)	21-56	118	42.7 (6.0)	27-53	0.07
Maternal Characteristics							
Marital status at birth							<0.001*
Not married	136 (36.5)			195 (47.0)			
Married	237 (63.5)			165 (39.8)			
Missing	0			55 (13.3)			
Maternal IQ	373	99.4 (10.4)	57-124	262	95.8 (10.2)	72-126	<0.001*
Seafood during pregnancy (serv/day)	373	0.5 (0.6)	0-5.3	260	0.6 (0.7)	0-6	0.6
Smoking during pregnancy							0.1
No	272 (72.9)			210 (50.6)			
Yes	101 (27.1)			103 (24.8)			
Missing	0			102 (24.6)			
Household Characteristics at Birth							
Maternal education							<0.001*
≤ High School	190 (50.9)			231 (55.7)			
> High School	183 (49.1)			127 (30.6)			
Missing	0			57 (13.7)			
Paternal Education							0.002*
≤ High School	246 (66.0)			266 (64.1)			
> High School	127 (34.0)			81 (19.5)			
Missing	0			68 (16.4)			
Annual Household Income							0.001*
< $20,000	115 (30.8)			150 (36.1)			
≥ $20,000	258 (69.2)			201 (48.4)			
Missing	0			64 (15.4)			
Examination Characteristics							
Examiner							0.4
1	277 (74.3)			121 (78.1)			
2	96 (25.7)			34 (21.9)			

[1]Main analytic group (*Set 1*): complete outcome, covariate, and exposure data for, DDE, HCB, ΣPCB_4, Pb, and Mn, n=373. [2]P-values represent results comparing characteristics between participants included in *Set 1* and those excluded from *Set 1* using t-tests, Wilcoxon rank sum tests, and chi-square tests. P-values reflect comparisons based on non-missing data. [3]NBC participants with missing inhibition measures: Design Fluency total correct n=260, total set loss designs n=260; Color-Word Interference completion time n=261, total errors n=261. [4]NBC participants with missing exposure measures: DDE n=37; HCB n=37; ΣPCB_4 n=37; Pb n= 40; Mn n=80. [5]NBC participants with missing covariate measures: age at exam n=260; HOME score n= 297; maternal IQ n=153; seafood during pregnancy n= 155. *p<0.05

Abbreviations: DDE: dichlorodiphenyldichloroethylene; HCB: hexachlorobenzene; ΣPCB_4: Sum of 4 PCB congeners (118, 138, 153, 180); Pb: lead; Mn: manganese.

Chemical exposure measures: Biomarker concentrations of organochlorines and metals in the NBC study participants were similar to the general population of the United States and Canada, with the exception of total hair Hg concentrations which were similar to those observed in high fish-eating populations. [56,74–77] In *Set 1*, the organochlorines were moderately correlated with each other (Spearman r: 0.4-0.6), Pb was weakly correlated with the organochlorines and Mn (Spearman r: 0.1-0.2), and Mn was not correlated with the organochlorines (Figure 2.2). In *Set 2*, MeHg was moderately correlated with the organochlorines (Spearman: 0.2-0.5) and weakly correlated with Pb (Spearman r = 0.1) but not with the other metals (Supplemental Figure 2.1). As was not correlated with the organochlorines or other metals (Supplemental Figure 2.1).

Figure 2.2 Spearman correlations between exposures in *Set 1*[1].

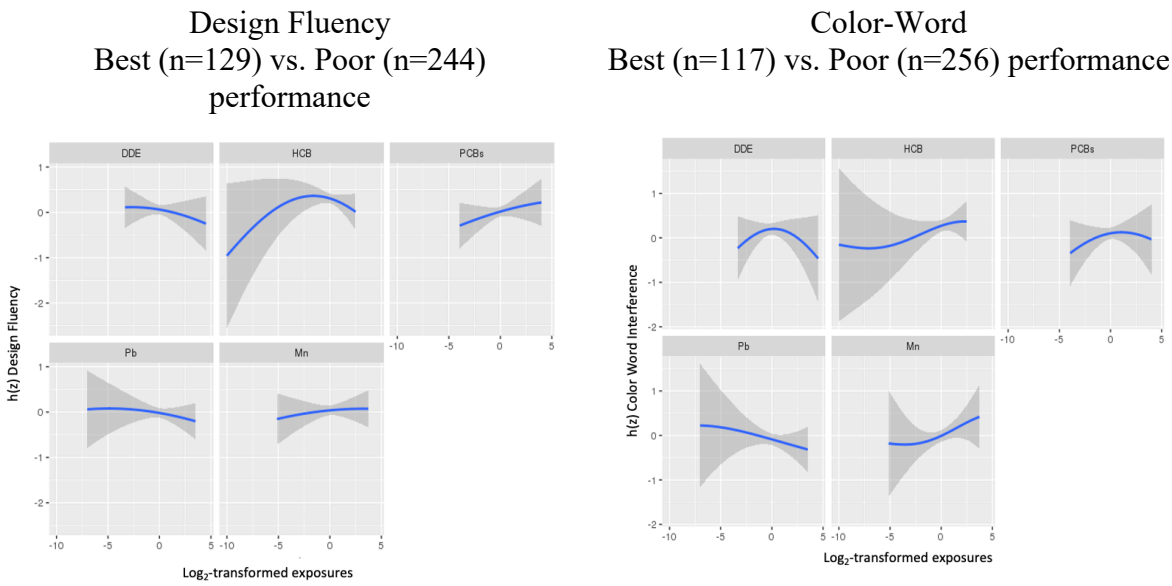


[1]*Set 1*: complete outcome, covariate and exposure data for PCBs, DDE, HCB, Pb and Mn, n=373
Abbreviations: DDE: dichlorodiphenyldichloroethylene; HCB: hexachlorobenzene; ΣPCB_4: sum of 4 PCB congeners (118, 138, 153, 180); Pb: lead; Mn: manganese.

Executive Function Measures: Design Fluency total correct and total set loss design raw scores were weakly negatively correlated (Spearman r = -0.1), whereas Color-Word Interference total completion time and total errors (raw scores) were moderately positively correlated (Spearman r = 0.4). Across the two inhibition subtests, Design Fluency total correct and Color-Word Interference completion time raw scores were modestly and negatively correlated (Spearman r = -0.2), as expected given that a higher number of correct designs and shorter completion times reflect better performance.

BKMR analysis of inhibition and prenatal chemical mixture exposures: Visual inspection of BKMR results suggested potential non-linear associations of HCB with Design Fluency and of DDE and ΣPCB_4 with Color-Word Interference (Figure 2.3, Supplemental Figure 2.2). The Wald and likelihood ratio tests only supported including a quadratic term for DDE in the Color-Word Interference model, therefore in the subsequent main analyses, a quadratic term for DDE was included in the model for Color-Word Interference. The BKMR results did not provide any evidence of interactions among exposures (Figure 2.4, Supplemental Figure 2.3), therefore, no interactions were included in subsequent analyses. We also used BKMR to assess the joint association of the chemicals with the odds of being in the poor performance group compared to the best performance group of the inhibition subtests (Figure 2.5, Supplemental Figure 2.4) by visually comparing the effect of the chemical mixture at various percentiles to their median levels. In *Set 1*, there appeared to be an adverse overall association of the chemical mixture with Color-Word Interference but not Design Fluency. There was no evidence of an adverse joint association of the chemicals with inhibition in *Set 2*.

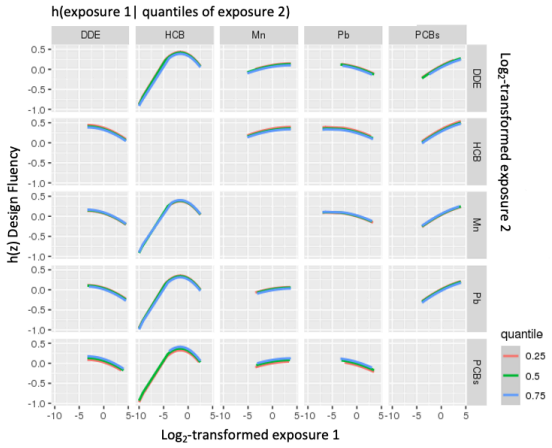
Figure 2.3 Estimated univariate exposure-response functions and 95% credible intervals[1] between each of the 5 main exposures in *Set 1*[2] and the latent continuous outcome representing the binary inhibition outcomes[3] - Design Fluency[4] and Color-Word Interference[5] - where all remaining exposures are assigned to their median value among adolescents in the main analysis group.



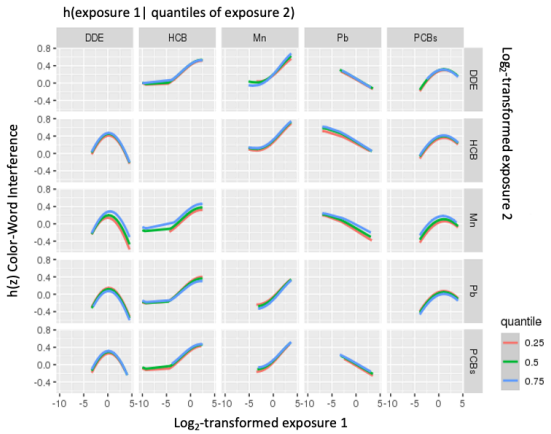
[1]Exposures have been log2-transformed and models have been adjusted for child race, sex, age at exam, and HOME score; maternal marital status at child's birth, IQ, seafood consumption during pregnancy, and smoking during pregnancy; maternal and paternal education and annual household income at child's birth; and study examiner.
[2]*Set 1*: complete outcome, covariate and exposure data for PCBs, DDE, HCB, Pb and Mn, n=373
[3]h(z) can be interpreted as the association between the chemical mixture and a latent continuous variable which represents being in the poor Design Fluency or Color-Word Interference performance group. When h(z) > 0, the probability of being in the poor performance group is equal to 1, and 0 otherwise.
[4]Reference is best performance group (total correct raw scores > median and total errors raw score < median) compared to remaining participants (poor performance group).
[5]Reference is best performance group (total completion time raw score < median and total errors raw score < median) compared to remaining participants (poor performance group).
Abbreviations: DDE: dichlorodiphenyldichloroethylene; HCB: hexachlorobenzene; PCBs: Sum of 4 PCB congeners (118, 138, 153, 180); Pb: lead; Mn: manganese.

Figure 2.4 Bivariate exposure-response functions[1] between one of the five prenatal chemical exposures (*Set 1*[2]) combined with a second exposure fixed at various quantiles, and Design Fluency[3] and Color-Word Interference[4] measures of inhibition[5], while the remaining exposures are assigned to their median value among adolescents in the main analysis group.

Design Fluency
Best (n=129) vs. Poor (n=244) performance

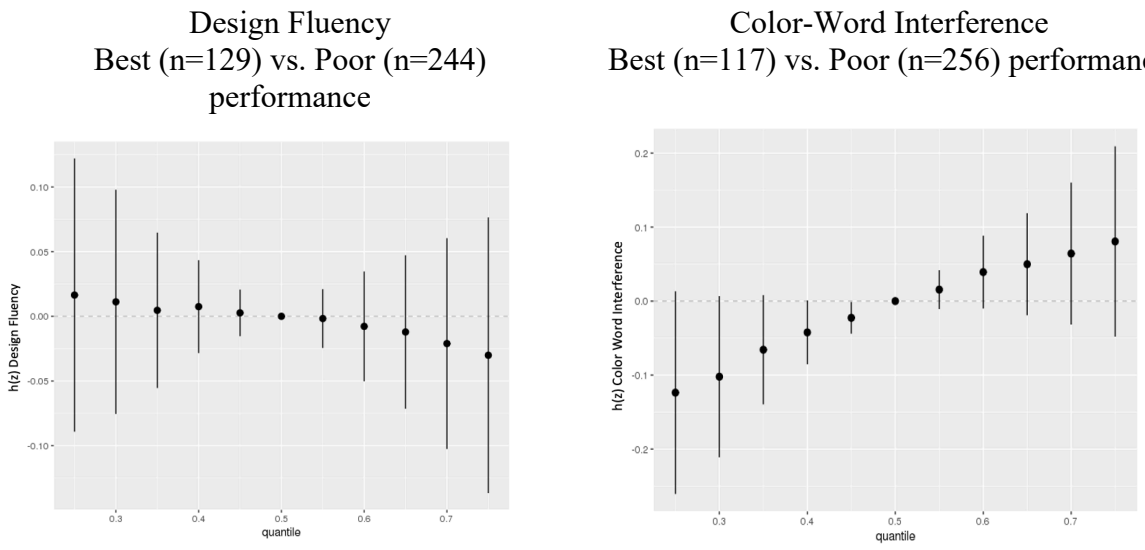


Color-Word
Best (n=117) vs. Poor (n=256) performance



[1]Exposures have been log2-transformed and models have been adjusted for child race, sex, age at exam, and HOME score; maternal marital status at child's birth, IQ, seafood consumption during pregnancy, and smoking during pregnancy; maternal and paternal education and annual household income at child's birth; and study examiner.
[2]*Set 1*: complete outcome, covariate and exposure data for PCBs, DDE, HCB, Pb and Mn, n=373
[3]Reference is best performance group (total correct raw scores > median and total errors raw score < median) compared to remaining participants (poor performance group).
[4]Reference is best performance group (total completion time raw score < median and total errors raw score < median) compared to remaining participants (poor performance group).
[5]h(z) can be interpreted as the association between the chemical mixture and a latent continuous variable which represents being in the poor Design Fluency or Color-Word Interference performance group. When h(z) > 0, the probability of being in the poor performance group is equal to 1, and 0 otherwise.
Abbreviations: DDE: dichlorodiphenyldichloroethylene; HCB: hexachlorobenzene; ΣPCB$_4$: Sum of 4 PCB congeners (118, 138, 153, 180); Pb: lead; Mn: manganese.

Figure 2.5 Joint association (estimates and 95% credible intervals)[1] of the five-chemical mixture[2] (DDE, HCB, ΣPCB$_4$, Pb and Mn) with Design Fluency[3] and Color-Word Interference[4] measures of inhibition[5] among adolescents in the main analysis group. Chemical mixture levels at each percentile are compared to the same mixture with each component at its median level, where higher outcome values reflect greater odds of poor performance.



[1]Exposures have been log2-transformed and models have been adjusted for child race, sex, age at exam, and HOME score; maternal marital status at child's birth, IQ, seafood consumption during pregnancy, and smoking during pregnancy; maternal and paternal education and annual household income at child's birth; and study examiner.
[2]*Set 1*: complete outcome, covariate and exposure data for PCBs, DDE, HCB, Pb and Mn, n=373
[3]Reference is best performance group (total correct raw scores > median and total errors raw score < median) compared to remaining participants (poor performance group).
[4]Reference is best performance group (total completion time raw score < median and total errors raw score < median) compared to remaining participants (poor performance group).
[5]h(z) can be interpreted as the association between the chemical mixture and a latent continuous variable which represents being in the poor Design Fluency or Color-Word Interference performance group. When h(z) > 0, the probability of being in the poor performance group is equal to 1, and 0 otherwise
Abbreviations: DDE: dichlorodiphenyldichloroethylene; HCB: hexachlorobenzene; ΣPCB$_4$: Sum of 4 PCB congeners (118, 138, 153, 180); Pb: lead; Mn: manganese.

Logistic regression analyses of inhibition and prenatal five chemical mixture exposure: In the main logistic regression analyses (*Set 1*), we observed an adverse association between Mn and Color-Word Interference. Specifically, a doubling of cord blood Mn was associated with 1.72 increased odds (95% CI: 1.05, 2.86) of being in the poor compared to the best Color-Word Interference performance group (Table 2.2). We also observed decreased odds of being in the poor Color-Word performance group per doubling of Pb and DDE (Table 2.2). Among the remaining chemical exposures, estimates of potential increased odds (ΣPCB_4, HCB) of being in the poor inhibition performance groups had wide confidence intervals and included the null (Table 2.2). Results of logistic regression analyses using IPW were similar to the complete case analysis (Supplemental Table 2.2).

Table 2.2 Complete-case results of multivariable logistic regression [odds ratio (OR) and 95% CI][1] assessing the relation of prenatal exposure to a five-chemical mixture with Delis Kaplan Executive Function System (D-KEFS) Design Fluency[2] and Color-Word Interference[3] performance among adolescents in the main analysis group.

Exposure	**Performance**	**Design Fluency OR (95% CI)**	**Color Word OR (95% CI)**
	Best	*n=129*	*n=117*
	Poor	*n=244*	*n=256*
Log_2 DDE		0.85 (0.67, 1.09)	0.75 (0.55, 1.02)
Log_2 DDE^2		-	0.90 (0.83, 0.98)*
Log_2 HCB		1.00 (0.79, 1.26)	1.15 (0.90, 1.46)
Log_2 ΣPCB_4		1.16 (0.89, 1.53)	1.19 (0.90, 1.60)
Log_2 Pb		1.03 (0.84, 1.27)	0.77 (0.58, 0.97)*
Log_2 Mn		1.01 (0.63, 1.64)	1.72 (1.05, 2.86)*

[1]Exposures have been log2-transformed and models have been adjusted for child race, sex, age at exam, and HOME score; maternal marital status at child's birth, IQ, seafood consumption during pregnancy, and smoking during pregnancy; maternal and paternal education and annual household income at child's birth; and study examiner.
[2]Reference is best performance group (total correct raw scores > median and total errors raw score < median) compared to remaining participants (poor performance group).
[3]Reference is best performance group (total completion time raw score < median and total errors raw score < median) compared to remaining participants (poor performance group).
*$p<0.05$
Abbreviations: DDE: dichlorodiphenyldichloroethylene; HCB: hexachlorobenzene; ΣPCB_4: Sum of 4 PCB congeners (118, 138, 153, 180); Pb: lead; Mn: manganese.

We did not find any statistically significant chemical-sex interactions in the main logistic regression models (Table 2.3). However, in sex-specific findings, we did note that among males in *Set 1*, a two-fold increase in ΣPCB_4 exposure was associated with a higher odds of being in the poor Design Fluency performance group compared to the best performance group (OR=1.54; 95% CI: 1.05, 2.32). Meanwhile, among females in *Set 1*, a two-fold increase in prenatal Mn exposure was associated with an increased odds of being in the poor Color-Word Interference performance group compared to being in the best performance group (OR=2.23; 95% CI: 1.07, 4.86). This association was attenuated in males (OR=1.41; 95% CI: 0.66, 3.07). The results of sex-stratified analyses using IPW were similar to complete-cases results (Supplemental Table 2.3).

Table 2.3 Sex-stratified complete-case results of multivariable logistic regression analyses [odds ratio (OR) and 95% CI][1] assessing the relation of prenatal exposure to a five-chemical mixture with Delis-Kaplan Executive Function System (D-KEFS) Design Fluency[2] and Color-Word Interference[3] performance among adolescents in the main analysis group.

		Males	**Females**		**Males**	**Females**	
Exposure	**Performance**	**Design Fluency OR (95% CI)**	**Design Fluency OR (95% CI)**	**p for interaction**	**Color Word OR (95% CI)**	**Color Word OR (95% CI)**	**p for interaction**
	Best	*n=60*	*n=69*		*n=47*	*n=70*	
	Poor	*n=119*	*n=125*		*n=132*	*n=124*	
Log_2 DDE		0.71 (0.49, 1.02)	0.89 (0.59, 1.33)	0.4	0.84 (0.49, 1.55)	0.72 (0.45, 1.11)	0.6
Log_2 DDE^2		-	-	-	0.93 (0.82, 1.08)	0.88 (0.77, 1.00)	0.3
Log_2 HCB		1.08 (0.80, 1.48)	0.74 (0.48, 1.13)	0.2	1.05 (0.68, 1.45)	1.34 (0.89, 2.04)	0.5
Log_2 ΣPCB_4		1.54 (1.05, 2.32)*	1.13 (0.71, 1.84)	0.2	1.47 (0.94, 2.36)	1.02 (0.66, 1.59)	0.2
Log_2 Pb		0.97 (0.63, 1.51)	1.04 (0.80, 1.37)	0.6	0.69 (0.40, 1.14)	0.77 (0.55, 1.01)	0.4
Log_2 Mn		0.84 (0.41, 1.70)	1.37 (0.65, 2.95)	0.4	1.41 (0.66, 3.07)	2.23 (1.07, 4.86)*	0.8

[1]Exposures have been log2-transformed and models have been adjusted for child race, sex, age at exam, and HOME score; maternal marital status at child's birth, IQ, seafood consumption during pregnancy, and smoking during pregnancy; maternal and paternal education and annual household income at child's birth; and study examiner.
[2]Reference is best performance group (total correct raw scores > median and total errors raw score < median) compared to remaining participants (poor performance group).
[3]Reference is best performance group (total completion time raw score < median and total errors raw score < median) compared to remaining participants (poor performance group).
*$p<0.05$
Abbreviations: DDE: dichlorodiphenyldichloroethylene; HCB: hexachlorobenzene; ΣPCB_4: Sum of 4 PCB congeners (118, 138, 153, 180); Pb: lead; Mn: manganese.

Linear and negative binomial regression analyses of inhibition and prenatal chemical mixture exposures: Next we analyzed the association of the chemical mixture with each individual component of Design Fluency (total correct and set loss design raw scores) and Color-Word Interference (completion time and total error raw scores) to assess how each component contributed to observed associations with the combined binary measures (Table 2.4; Supplemental Table 2.4). In the main analyses (*Set 1*), a doubling of cord blood Mn was associated with 3.33 seconds longer (95% CI: 0.52, 6.15 seconds) Color-Word Interference completion times. The remaining results were imprecise and included the null with IPW results being similar to complete case results (Table 2.4; Supplemental Table 2.4).

Table 2.4 Complete case results of multivariable linear regression (difference and 95% CI)[1] and negative binomial regression [rate ratio (RR) and 95% CI][1] assessing the relation of prenatal exposure to a five-chemical mixture with Delis Kaplan Executive Function System (D-KEFS) Design Fluency and Color-Word Interference scores among 373 adolescents in the main analysis group.

Exposure	Design Fluency Total Correct[2] Difference (95% CI)	Design Fluency Set Loss Designs[2] RR (95% CI)	Color-Word Completion Time (seconds)[2] Difference (95% CI)	Color-Word Total Errors[2] RR (95% CI)
Log_2 DDE	0.10 (-0.26, 0.45)	0.85 (0.65, 1.12)	-0.78 (-2.53, 0.97)	0.91 (0.80, 1.04)
Log_2 DDE^2	-	-	-0.29 (-0.76, 0.19)	1.00 (0.96, 1.03)
Log_2 HCB	0.02 (-0.31, 0.35)	0.99 (0.78, 1.27)	-0.10 (-1.47, 1.27)	1.09 (0.97, 1.22)
Log_2 ΣPCB_4	-0.25 (-0.63, 0.14)	0.94 (0.72, 1.23)	1.09 (-0.51, 2.69)	1.13 (0.99, 1.28)
Log_2 Pb	-0.07 (-0.37, 0.23)	1.09 (0.86, 1.43)	-0.39 (-1.66, 0.87)	0.93 (0.84, 1.01)
Log_2 Mn	0.15 (-0.53, 0.82)	1.02 (0.63, 1.65)	3.33 (0.52, 6.15)*	1.10 (0.88, 1.36)

[1]Exposures have been log2-transformed and models have been adjusted for child race, sex, age at exam, and HOME score; maternal marital status at child's birth, IQ, seafood consumption during pregnancy, and smoking during pregnancy; maternal and paternal education and annual household income at child's birth; and study examiner.
[2]Raw scores
*$p<0.05$
Abbreviations: DDE: dichlorodiphenyldichloroethylene; HCB: hexachlorobenzene; ΣPCB_4: Sum of 4 PCB congeners (118, 138, 153, 180); Pb: lead; Mn: manganese.

Secondary analyses. The results of the secondary analyses (*Set 2*) were imprecise, though we observed suggestive evidence of an increased odds of poor Color-Word Interference performance per doubling of Mn concentrations (OR=1.73; 95% CI: 0.94, 3.24) and a decreased odds of poor Color-Word Interference performance per doubling of MeHg concentrations (OR=0.70; 95% CI: 0.51, 0.95) (Supplemental Table 2.5). IPW results were similar (Supplemental Table 2.6).

Discussion

Results of our main analyses provided evidence that prenatal exposure to Mn at the levels seen in the NBC may adversely impact inhibition among adolescents even with adjustment for exposure to multiple prevalent neurotoxic metals and organochlorines (Table 2.2). Specifically, in multi-exposure models, we observed adverse associations of cord blood Mn with the Color-Word Interference measure of inhibition. Results were largely unchanged when we used IPW to account for potential selection bias due to loss to follow-up. Color-Word Interference, a verbal task, appeared to be more sensitive to Mn exposure than Design Fluency, a non-verbal task. This is consistent with other studies that have found associations between increasing Mn concentrations and poorer performance on verbal cognitive tasks.[78–80] Prenatal Mn exposure was also adversely associated with the individual components of Color-Word Interference (completion time and total errors raw scores), though completion time appeared more sensitive to exposure impacts than total errors (Table 2.4). Although more imprecise due to a smaller sample size, the adverse impacts of Mn were still evident in our secondary analyses, which included MeHg and As as part of the chemical mixture (Supplemental Table 2.5). Prenatal exposure to Mn has also been associated with decrements in inhibition in a small exploratory study (n=27) where increasing tooth Mn concentrations, reflecting exposure in the 20th week of gestation, were associated with behavioral

disinhibition as measured by a Forbidden Toy Task, a CPT, and a Children's Stroop Test at 36 and 54 months.[38] Our findings provide evidence that prenatal exposures to relatively low-level Mn may have adverse impacts on inhibition that persist beyond early childhood into adolescence. Of note, there is no well-established industrial source of Mn exposure located near the NBC study communities. Therefore, NBC participants were likely exposed to Mn via multiple sources including diet, the most common source of Mn in the general population, and potentially, via ingestion of Mn-contaminated water.[81,82]

In addition, we observed adverse associations of cord serum ΣPCB_4 with performance on both Design Fluency and Color-Word Interference measures of inhibition, however these associations were imprecise and included the null. Once again, results were unchanged when we used IPW to account for loss to follow-up or when MeHg and As were included in the chemical mixture. Prenatal PCB exposure has previously been implicated as detrimental to inhibition in several studies among younger children.[27,28,31,83] In the Michigan-based prospective cohort study which had much higher PCB levels than those seen in the NBC,[56] increasing prenatal PCB exposure was associated with poorer response inhibition and greater impulsivity as measured by CPT errors of commission and WCST preservative errors among 11-year-olds who had not been breastfed.[27] In the Oswego-based cohort, which had similar PCB biomarker concentrations to the NBC, increasing prenatal PCB exposure was associated with lower inhibition at ages 4.5 and 9.5 years measured using CPT commission errors, as well as a Differential Reinforcement of Low Rates task administered at the older age.[28,31,83] Interestingly, an analysis of prenatal PCB exposure and CPT errors of commission in the NBC at age 8 years did not find an adverse association.[32] This discrepancy may be, in part, due to variation in CPT tests - by design, the CPT used in the NBC was more sensitive to skills in attention rather than inhibition.[57]

Among the remaining *Set 1* exposures (HCB, DDE, Pb), associations with inhibition outcomes were imprecise and crossed the null. We observed an adverse association of HCB with Color-Word Interference, while the association between HCB and Design Fluency was largely null (Table 2.2). A longitudinal cohort study in Greece found that HCB concentrations in maternal serum during the third trimester of pregnancy were associated with lower executive function and working memory scores on the MSCA among 4-year-olds.[35] However, participants in the NBC study had lower concentrations of HCB than participants in the Greek study. In addition, we assessed inhibition and other executive function measures in adolescence rather than early childhood. It is possible that prenatal HCB exposure does not adversely impact inhibition at the levels seen in the NBC, or that HCB-related impacts do not persist through adolescence.

We also observed associations of DDE and DDE^2 with better performance on both inhibition subtests (Table 2.2). An apparent beneficial association of DDE with inhibition could be due to residual negative confounding as could occur from co-exposure to beneficial nutrients in dietary sources of DDE exposure.[84] Although we adjusted for maternal seafood consumption during pregnancy, self-reported diet is imprecise and we did not have information on other potential dietary sources of DDE as well as beneficial nutrients, such as fruit and vegetable consumption.

Finally, Pb was not associated with Design Fluency and unexpectedly associated with decreased odds of being in the poor Color-Word Interference performance group (Table 2.2). This unexpected association may be due to negative residual confounding by socioeconomic position. For example, in the NBC study, participants with mothers who were older at the time of their birth tended to have higher cord blood Pb concentrations, however older mothers were also more likely to attain higher education levels and have a higher household income than younger mothers. Although we adjusted for maternal education and household income, these variables may not fully

capture sociodemographic and economic confounding. In addition, including maternal age at birth in models did not change Pb-inhibition associations. In addition, previous studies have not found strong evidence of associations between prenatal Pb exposure and executive functions.[37,85] One longitudinal study that measured cord blood Pb at birth and blood Pb at multiple time points thereafter found that executive function outcomes in mid-childhood were adversely impacted by recent Pb exposure, rather than Pb exposure at birth.[85] These findings suggest that exposure timing may play an important role in the association of Pb with inhibition or other executive functions.

When we stratified our results by sex, we found a significant adverse association between prenatal ΣPCB_4 exposure and inhibition among males and a significant adverse association between prenatal Mn exposure and inhibition among females (Table 2.3). There is other evidence of potential increased susceptibility of males to PCB-associated decrements in attention.[32,52] In addition, other studies have found an increased susceptibility of females to Mn-associated decrements in visuospatial learning, working memory, attention, and externalizing behavior.[29,86] However, none of the chemical-sex interactions in the main logistic regression models were statistically significant, potentially due to limitations in study power.

In secondary analyses, in which As and MeHg were included in the chemical mixture, we found that increasing prenatal MeHg exposure was associated with a higher odds of being in the poor Design Fluency performance group, yet a lower odds of being in the poor Color-Word Interference performance group (Supplemental Table 2.5). Studies of populations with high dietary MeHg exposure in the Faroe Islands and the Seychelles have not found evidence of adverse associations between prenatal exposure to MeHg and executive function.[39,58] The decreased odds of being in the poor performance group per doubling of MeHg observed in our study may be due to residual confounding by nutritional benefits of fish consumption, an important source of MeHg

exposure.[84] Although we accounted for maternal seafood consumption during pregnancy, there is likely measurement error in the use of self-reported fish intake as a proxy for nutritional confounding in this setting. Meanwhile, although As exposure was weakly adversely associated with both inhibition subtests, confidence limits bounding the effect estimates were wide and included the null (Supplemental Table 2.5). As previously noted, there are few studies of prenatal exposure to As and inhibition. However, two cross-sectional studies have found adverse associations of As exposure with working memory, another executive function in which inhibition plays a role, but at much higher levels of As than in our study population.[41,42] Therefore, further research is necessary to characterize the impact of low-level prenatal As exposures on inhibition.

In this study, the main analysis involved traditional logistic regression, while BKMR was used as an exploratory tool. The traditional method was used primarily to improve the interpretability of the results and to be able to compare results with other studies. One benefit of BKMR is the ability to visualize the joint association between the chemical mixture and the inhibition outcomes. In this study, we found an adverse joint association of the chemical mixture with Color-Word Interference, but not Design Fluency (Figure 2.5). It is possible that we did not see a joint adverse association of this chemical mixture with Design Fluency because some of the associations we found were positive, while others appeared negative, thus cancelling each other out when assessed jointly. Specifically, while a doubling of ΣPCB_4 concentrations was associated with a higher odds of being in the poor Design Fluency performance group, a doubling of DDE concentrations was associated with a lower odds of being in the poor Design Fluency performance group, and the associations with the remaining exposures and Design Fluency were largely null.

The NBC is a prospective study with biomarkers of prenatal exposure to multiple organochlorines and metals and detailed psychometric measures of adolescent inhibition which

enabled us to conduct this investigation. Having comprehensive psychometric assessments of executive function in adolescence is a relatively unique resource and was central to our analyses. However, there were some limitations. First, missing data resulting from a combination of loss to follow-up and missing covariate or exposure data among those adolescents who completed psychometric testing of inhibition may result in biased estimates. We were able to account for this using IPW and results were similar to unweighted analyses thereby supporting that bias in our findings was minimal. Furthermore, although we adjusted our analyses for maternal self-reported diet during pregnancy, this may not have been sufficient to account for the nutritional benefits of foods that are also sources of chemical exposure. The potential for residual negative confounding by diet may have resulted in underestimates of the impact of certain exposures such as PCBs and MeHg on inhibition. We did not adjust for multiple comparisons in this study as decreasing the frequency of type I errors, or rejecting the null hypothesis when it is true, may come at the cost of increasing type II errors, or failing to reject the null hypothesis when it is false.[87] However, this have led to the observation of some spurious associations. There are also some limitations in the use of cord blood Mn as a biomarker of Mn exposure. First, Mn concentrations in blood were detected by ICP-MS which detects ions based on their mass to charge ratio.[88] An isotope of Mn has a mass of 55 atomic mass units (amu) and is bordered by two isotopes of iron at 54 and 56 amu.[88] This may result in iron contributing to the Mn signal and therefore overestimating Mn concentrations among those with high iron levels.[88] However, the laboratory in which these analyses were conducted reported that although there was some inflation of Mn concentrations due to iron, the relative levels of Mn were unaffected. Second, there is not yet consensus about which biological matrix is the most valid biomarker of Mn exposure.[89,90] However, there is evidence that

cord blood Mn is a useful measure of fetal exposure and better-correlated with third-trimester dentin Mn levels than maternal biomarkers.[89,91]

In conclusion, this study is among the first to estimate the association between prenatal exposures to a prevalent chemical mixture and executive function among adolescents and provides new evidence of an adverse joint association between a chemical mixture and a verbal inhibition task. In addition, after accounting for multiple exposures, Mn appeared to be more consistently adverse than other chemicals in the mixture. Future studies assessing the impact of prenatal exposure to analogous chemical mixtures on inhibition in other populations of adolescents are needed to fully characterize the role of these prevalent exposures on critical aspects of adolescent neurodevelopment.

CHAPTER 3:

Prenatal exposure to chemical mixtures and working memory among adolescents

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Abstract

Background: Working memory is the ability to keep information in one's mind and mentally manipulate it. Decrements in working memory play a key role in many behavioral and psychiatric disorders, therefore identifying modifiable environmental risk factors for such decrements is important for mitigating these disorders. There is some evidence that prenatal exposure to individual chemicals may adversely impact working memory among children, but few studies have explored the association of co-exposure to multiple chemicals with this outcome in adolescence, a time when working memory skills undergo substantial development.

Methods: We investigated the association of biomarkers of prenatal exposure to organochlorines (DDE, HCB, PCBs) and metals (lead, manganese) with working memory measured with Wide Range Assessment of Memory and Learning, 2nd Edition among a socio-demographically diverse group of adolescents living near a superfund site in New Bedford, Massachusetts. We used Bayesian Kernel Machine Regression and linear regression analyses and assessed effect modification by sex and prenatal social disadvantage.

Results: In BKMR models, we observed an adverse joint association of the chemical mixture with Verbal, but not Symbolic, Working Memory. In co-exposure and covariate-adjusted linear regression models, a twofold increase in cord blood Mn was associated with lower working memory scaled scores, with a stronger association with Verbal Working Memory (difference=-0.75; 95% CI: -1.29, -0.20 points) compared to Symbolic Working Memory (difference=-0.44; 95% CI: -1.00, 0.12 points). There was little evidence of effect modification by sex and some

evidence associating organochlorine pesticides with poorer working memory among those with greater social disadvantage.

Conclusion: This study provided evidence of an adverse joint association of a chemical mixture with a verbal working memory task among adolescents, as well as an adverse association of prenatal Mn exposure with working memory.

Introduction

Working memory is the ability to not only keep information in one's mind temporarily, but also mentally manipulate or work with it.[1] It includes verbal and non-verbal memory, both of which are critical to numerous abilities such as mentally ordering information, adapting instructions into action, and updating plans in response to new information.[1] Along with inhibition and cognitive flexibility, working memory is a building block of executive function necessary for higher-order skills including planning, reasoning, problem-solving, and decision-making.[1] Although working memory skills begin to develop in childhood, and young children are able to hold some information in their minds, manipulating such information involves recruitment of the dorsolateral prefrontal cortex and superior parietal cortex, anatomical regions of the brain that do not undergo substantial development until adolescence.[92] Working memory deficits are associated with many psychiatric and behavioral disorders. For example, both verbal and spatial working memory impairments are core features of schizophrenia.[14,15] In addition, children with ADHD or autism generally perform worse on both verbal and visuospatial working memory tasks than controls.[16,17] Although it is not known whether working memory decrements are risk factors for these disorders or whether the pathology that leads to these disorders may also impact working

memory, understanding potential modifiable environmental risk factors is vital to better understanding and mitigating the functional impacts of these disorders.

Several epidemiologic studies have assessed the association of prenatal exposures to organochlorines such as dichlorodiphenyldichloroethylene (DDE), hexachlorobenzene (HCB), and polychlorinated biphenyls (PCBs) with childhood working memory. Specifically, higher DDE, HCB, and PCB levels measured either in cord serum or maternal serum during pregnancy have been associated with poorer memory scores (including working memory skills) on the McCarthy Scales of Children's Abilities (MSCA) among 4-year-olds in Spain and Greece, though these adverse associations did not always reach significance.[33,35,36] Associations of working memory with organochlorines have been less consistent across studies of older children (7-to-11-year-olds),[27,34] although in Michigan children born to mothers consuming PCB-contaminated Lake Michigan fish, higher cord serum PCB levels were associated with poorer working memory on the Sternberg Memory Paradigm and the Weschler Intelligence Scale for Children, Revised (WISC-R) digit span task.[27] Importantly, previous studies have not assessed the impact of prenatal exposures to organochlorines on working memory among adolescents, despite this being the time when working memory skills undergo substantial development.

Some studies have focused on the potential adverse impacts of metals on working memory among children and adolescents. Lead (Pb) levels measured in maternal erythrocytes during pregnancy and cord blood have been associated with lower working memory scores on the Behavior Rating Inventory of Executive Function (BRIEF) checklist and psychometric tests of working memory among older children (ages 7-11).[30,37] In an Italian cohort of children residing near ferro-manganese plants, adolescent girls with the highest deciduous tooth Mn concentrations reflecting prenatal exposure committed more working memory errors on the Virtual Radial Arm

Maze (VRAM) than those with intermediate levels of exposure, but tooth Mn was not associated with working memory in boys.[29] Only one study has analyzed the relation of prenatal methylmercury (MeHg) exposure with working memory in adolescence and there were no adverse associations observed.[39] Finally, several cross-sectional studies have found biomarkers of exposure to arsenic (As) and other metals to be associated with decrements in working memory skills among 6 to 12-year-old children.[41,78,93,94] However, the design of such studies does not allow for establishing directionality of the association.

Simultaneous exposure to multiple chemical contaminants is common.[25] Few studies, however, have examined the association of prenatal exposure to multiple pollutants with working memory among children or adolescents. In one of the few studies that has examined such an association – a prospective study based in the Faroe Islands – low cord blood MeHg concentrations combined with high cord blood Pb concentrations were associated with decrements in working memory among 14-year-olds as measured by Digit Span Backward scores.[45] Two cross-sectional studies have also assessed the impact of multiple metals on working memory in children. In an Italian cohort study of 6 to 12-year-olds, proximity to an industrial site from which airborne metals and other contaminants were emitted was used as an exposure proxy for multiple metal exposures, however biomarkers of exposure were used when assessing exposure to specific individual metals.[46] Participants who lived closer to the industrial site performed worse on psychometric tests of working memory than those who lived further away; blood Pb and urine cadmium were adversely associated with the working memory indices of the WISC-IV; and hair Mn and blood Pb were associated with more errors on the CANTAB Spatial Working Memory test.[46] Finally, in a cross-sectional study of 8 to 11-year-old children from Bangladesh, researchers found that in the

presence of As co-exposure, greater blood Mn concentrations were associated with lower WISC-IV Working Memory scores, but Mn-As interactions were not statistically significant.[47]

As substantial working memory development occurs during adolescence, this may be a key time when adverse impacts manifest despite few previous studies in this age group. In addition, given that exposure to organochlorines and metals seldom occurs independently, the purpose of this study was to assess the impact of prenatal exposure to a chemical mixture on working memory in adolescents. In the main analysis, we focused on a chemical mixture composed of DDE, HCB, PCBs, Pb, and Mn based on likely neurodevelopmental toxicity, prevalence of exposure, and availability of exposure biomarkers in our study target population. In a number of previous studies, associations of prenatal exposures to neurotoxicants with executive functions varied by sex[29,33,34,51,52] and socioeconomic stress indicators.[46] Therefore, we also assessed the potential for sex and social disadvantage to modify the impact of exposure mixtures on working memory. Secondary analyses included MeHg and As as additional components of the chemical mixture.

Methods

Study population. The New Bedford Cohort (NBC) is a longitudinal birth cohort study of 788 mother-infant pairs recruited shortly after the infant's birth at St. Luke's Hospital in New Bedford, Massachusetts. The NBC was established to assess the effects of prenatal chemical exposures on child development among families residing in communities near the New Bedford Harbor. Potential chemical exposures to residents of this region were of particular concern as the New Bedford Harbor was designated a Superfund site in 1982 due to PCB and metal contamination from local industrial emissions.[60] Enrollment eligibility criteria for mothers included age ≥ 18 years, speaking English or Portuguese, and living in one of the four towns surrounding the New

Bedford Harbor for the duration of the pregnancy. Infants born via cesarean section or too ill to undergo neonatal examination were excluded from the study. Biomarkers of prenatal chemical exposure were collected on study infants at birth or in the peripartum period. NBC children have been followed periodically since birth for detailed neurodevelopmental assessments.

The focus of this analysis is the subset of 528 NBC children who participated in follow up during adolescence, which took place between 2008 and 2014 (median age 15.5, range 13-17 years). A total of 373 of these adolescents had complete data on working memory outcomes, covariates, and biomarkers of prenatal exposure to DDE, HCB, PCBs, Pb, and Mn. 235 participants had complete data on working memory outcomes, covariates, and biomarkers of prenatal exposure to DDE, HCB, PCBs, Pb, Mn, MeHg, and As.

Chemical exposure assessment. DDE, HCB, and PCBs were measured in cord blood samples collected at birth. The samples were centrifuged, and the serum fraction was removed before being stored at -20 degrees Celsius prior to analysis. The cord serum samples were analyzed for DDE, HCB, and 51 PCB congeners at the Harvard T.H. Chan School of Public Health Organic Chemistry Laboratory (Boston, MA) using gas chromatography with electron capture detection.[60,62,63] The sum of the 4 most prevalent PCB congeners (ΣPCB_4) including 118, 138, 153, and 180 were used for this analysis due to their minimal measurement error and common usage to assess congener-specific effects in other population-based studies. The limits of detections (LODs) of DDE, HCB, and PCBs ranged from 0.001 ng/g to 0.07 ng/g serum with within-sample coefficients of variation over 5 years ranging from 5% to 7.5%, and between-batch coefficients of variation ranging from 20% to 39%, reflecting high reproducibility for organics analyses.[62]

Pb and Mn were measured in cord whole blood collected at birth. The samples were analyzed by the Harvard T.H. Chan School of Public Health Metals Laboratory (Boston, MA) using isotope dilution inductively coupled plasma mass spectrometry (ICP-MS, Sciex Elan 5000, Perkin Elmer, Norwalk, CT) and external calibration on a dynamic reaction cell-inductively coupled plasma-mass spectrometer (DRC-ICP-MS, Elan 6100, Perkin Elmer, Norwalk, CT), respectively, and concentrations were reported as the mean of 5 replicate measurements. For quality control (QC) monitoring, procedural blanks, duplicates, spiked samples, standard reference material (NIST SRM 955b Pb in blood; NIST SRM 1643d trace elements in water), biological reference material (ICP03B-05 and ICP03B-02 multi-elements in human blood from INSPQ/ Laboratoire de Toxicologie, Quebec) and certified reference material (GBW 09101 human hair, Shanghai Institute of Nuclear Research, Academia Sinica, China) were used. Recovery rates for QC and spiked samples were 90-110%, precision was >95%. The LOD was 0.02 μg/dL.

Total Hg and As were measured in maternal hair and toenail samples, respectively, collected, on average, 10 days postpartum. Hair samples were cut from the occiput and, where identifiable, the 3 centimeters closest to the scalp were analyzed as reflective of exposures during the last trimester of pregnancy. Both hair and toenail samples were cleaned using sonication, rinsed with distilled deionized water, and dried for 24 hours prior to analysis. Hair was then analyzed for total Hg, a reasonable proxy for MeHg,[65] using a DM-80 Direct Mercury analyzer at the Harvard T.H. Chan School of Public Health Trace Metals Analysis Laboratory (Boston, MA).[64] Toenails were weighed and digested with 1 ml of HNO_3 acid for 24 hours at room temperature then analyzed using an external calibration method on a dynamic reaction cell-inductively coupled plasma-mass spectrometer (Agilent 7700x ICP-MS) at the Dartmouth Trace Elements Analysis Laboratory (Hanover, NH). QC procedures for both analyses included daily calibration verification,

procedural blanks, and certified reference material. Recovery rates for QC standards were 90-110% and precision >95% for hair total Hg, while the coefficients of variation for reference standards were < 15% for toenail As.[64,67] The average LODs for total Hg and As were 50 ng/g of hair and 0.03 ng/g of toenails, respectively.

Working Memory assessment. A trained study examiner administered the Wide Range Assessment of Memory and Learning, 2nd Edition (WRAML2) Verbal Working Memory and Symbolic Working Memory subtests[50] to study participants at the adolescent assessment. For Verbal Working Memory, participants were read a list of words containing both animal and non-animal words. Next, participants were asked to recall the animal words in size order from smallest to largest followed by the non-animal words in any order. Finally, participants were asked to recall the animal words in size order from smallest to largest, followed by the non-animal words also in size order. For Symbolic Working Memory, the examiner first dictated a series of numbers then asked the participant to identify the listed numbers in correct numerical order on a Number Stimulus Card. Next, the examiner dictated a series of numbers and letters and asked the participant to identify the listed numbers in numerical order and the letters in alphabetical order on the Number-Alphabet Stimulus Card. Performance on each of the tests was based on the total correct raw score that was then age-standardized to a scaled score with a mean of 10 and standard deviation of 3. The Verbal and Symbolic Working Memory scaled scores were then combined to create the Working Memory Index, which was age-standardized to a mean of 100 with a standard deviation of 15.

Covariate assessment. The infant's race/ethnicity, birthweight, gestational age, and newborn exam as well as the mother's pregnancy and delivery course were obtained via review of hospital medical records from the birth. Ten days postpartum, a questionnaire eliciting information regarding maternal socio-demographics, medical history, diet, smoking, alcohol, and drug use, and infant feeding was completed during a study home visit. Periodic pediatric medical record reviews and parental and child self-reported questionnaires were administered at study follow-up visits to update demographic and health information for study participants. Follow-up assessments at ages 8 and 15 years also included a home visit to assess the quality of the child's home environment and parenting using the Home Observation for Measurement of the Environment (HOME)[68] and assessment of maternal IQ using the Kaufman Brief Intelligence Test (KBIT)[69]. We also constructed a measure of social disadvantage, the prenatal social disadvantage index (PNSDI), as the sum of five adverse social or economic exposures at the time of the child's birth where presence of each risk factor was assigned a value of 1, absence a value of 0: mother unmarried, mother's education as high school graduate or less, father's education as high school graduate or less, annual household income less than $20,000, and mother's age at birth less than 20 years.

Statistical analysis. For all statistical analyses, to reduce the influence of extreme values, chemical exposures were $\log_2$-transformed so associations are based on a two-fold increase in exposure concentrations. For all formal statistical modeling, model covariates were selected using Directed Acyclic Graphs (DAGs) based on a literature review regarding potential confounders of the relationship of prenatal organochlorine and metal exposures with cognition and covariates that had been previously found to predict cognitive outcomes in the NBC. All models were adjusted for adolescent race/ethnicity, sex, age at exam, year of birth, and 15-year HOME score; test examiner;

maternal IQ, maternal pregnancy seafood consumption and smoking; and family characteristics at the child's birth (maternal marital status, parental education, household income). Characteristics of participants who were included in the analyses were compared to those who were excluded using t-tests and chi-square tests where appropriate.

In exploratory analyses, we examined potential non-linear relationships and interactions of exposure to a chemical mixture composed of DDE, HCB, ΣPCB_4, Pb, and Mn with working memory outcomes using Bayesian Kernel Machine Regression (BKMR). BKMR is an exposure-response surface estimation technique that models the relationship between a large number of exposures and an outcome using a flexible exposure-response function.[70] In BKMR, this function is estimated using a Gaussian Kernel, which is capable of capturing many underlying functional forms.[70] BKMR implements an iterative estimation algorithm (Markov Chain Monte Carlo) to estimate the function. The resulting graphics helped us to identify non-linear exposure-outcome associations and interactions between exposures. To assess non-linearities, we visually inspected plots of the estimated exposure-response functions and 95% credible intervals of DDE, HCB, ΣPCB_4, Pb, and Mn with working memory scaled scores while assigning the remaining exposures to their median values. To assess interactions, we visually inspected plots of the estimated exposure-response functions between one of the five main exposures and working memory scaled scores, where a second exposure was fixed at varying levels and all of the remaining exposures were assigned to their median value. We assumed no interaction in plots where the slope of each chemical was similar at varying levels of the second chemical. The results of BKMR analysis informed specification of standard parametric regression models as described below. We also used BKMR to assess the joint association of the chemical mixture with each of the working memory outcomes. BKMR analyses were conducted using the R *bkmr* package.[72]

As Verbal Working Memory, Symbolic Working Memory, and the Working Memory Index scores were normally distributed and the exploratory BKMR results supported linear models with no interactions, we fit multivariable linear regression models using Ordinary Least Squares (OLS) to estimate the association between chemical exposures and working memory outcomes. All five (DDE, HCB, ΣPCB_4, Pb, Mn) exposures were included in all regression models simultaneously along with the aforementioned covariates. We then assessed potential effect modification of exposure-outcome associations by sex and PNSDI using interaction terms in the models followed by sex- and PNSDI-stratified linear regression models. In PNSDI-stratified models, we compared chemical associations among participants who had a PNSDI of 3 or more to those had a PNSDI of less than 3. This cut-off was selected as it was correlated with other indicators of social disadvantage such as the HOME score and allowed for sufficient numbers of participants in each group to maintain enough power for PNSDI-stratified analyses.

To account for potential selection bias due to loss to follow-up, we used inverse probability weighting (IPW).[73] IPW weights participants included in the analyses based on the inverse of the probability of their being in the study given their particular set of exposures and covariates, thereby creating a pseudo-population that represents the original source population. The particular set of exposures and covariates used to create the pseudo-population in this analysis included DDE, HCB, ΣPCB_4, and Pb and socio-demographics at birth such as maternal education, household income, and obstetric risk score (a score that summarizes adverse factors that occur during the perinatal period)[95] and child characteristics such as race/ethnicity and sex. We used stabilized IP weights[73] to improve efficiency, trimmed at the 2.5^{th} and 97.5^{th} percentile.

Because maternal hair and toenail samples were collected at a home visit ten days postpartum, rather than at birth, there were many participants missing MeHg and As exposure

biomarkers. Thus, in a secondary analysis, we added biomarkers of MeHg and As to our chemical mixture and assessed the impact of DDE, HCB, ΣPCB_4, Pb, Mn, MeHg, and As on working memory. Once again, we used BKMR to explore non-linear relationships, interactions, and joint associations of this mixture with working memory as well as standard parametric multivariable linear regression models. All statistics were conducted using R version 3.6.0.[71]

Results

Study population. Table 3.1 describes the outcome, exposure, and covariate measures of the NBC subset assessed as adolescents who had complete data on working memory outcomes, biomarkers of exposure to DDE, HCB, ΣPCB_4, Pb, and Mn, and covariates (n=373) and those were excluded from the analysis. Included NBC study participants were socio-demographically diverse with 29% non-white, 51% with mothers having less than or equal to a high school education at the time of their birth, and 31% having an annual household income of < $20,000 at the time of their birth (Table 3.1). Those who were included in the analysis had greater sociodemographic and economic advantage compared to those with missing data. Compared to adolescents excluded from the analyses, mothers of included adolescents were more likely to be married at birth and have a higher IQ. Included parents were more likely to have more than a high school education and an annual household income ≥$20,000. Participants in the main analytic group had slightly higher cord serum DDE and ΣPCB_4 levels and lower cord blood Pb levels than those who were excluded due to loss to follow-up or missing data. They also performed slightly better on tests of working memory than those who were excluded.

Table 3.1 Characteristics of New Bedford Cohort (NBC) participants who were evaluated as adolescents and included in the main analysis group[1] and those who were excluded.

Descriptive Characteristic	Main analysis group, n=373			Excluded group, n=415			
Working Memory Measures[3]	**n(%)**	**Mean (SD)**	**Range**	**n(%)**	**Mean (SD)**	**Range**	**p-value[2]**
Verbal Working Memory	373	9.0 (2.7)	1-17	155	8.3 (2.9)	1-17	0.008*
Symbolic Working Memory	373	9.8 (2.8)	1-19	154	9.2 (2.6)	1-14	0.02*
Working Memory Index	373	96.6 (13.2)	55-142	154	93.1 (13.1)	60-128	0.006*
Exposure Measures[4]							
Cord serum DDE (ng/g)	373	0.6 (1.2)	0.02-14.9	378	0.4 (0.5)	0-4.2	0.003*
Cord serum HCB (ng/g)	373	0.03 (0.02)	0-0.1	378	0.03 (0.05)	0-0.7	0.2
Cord serum ΣPCB_4 (ng/g)	373	0.3 (0.3)	0.01-4.4	378	0.2 (0.2)	0.01-1.9	0.05
Cord blood Pb (µg/dL)	373	1.4 (0.9)	0-9.4	375	1.7 (1.7)	0-17.4	<0.001*
Cord blood Mn (µg/dL)	373	4.2 (1.6)	0.7-14.6	335	4.3 (2.0)	0.2-22.1	0.6
Covariate Measures[5]							
Child Characteristics							
Race/Ethnicity							0.09
Non-Hispanic White	263 (70.5)			268 (64.6)			
Hispanic	33 (8.8)			56 (13.5)			
Other	77 (20.6)			89 (21.4)			
Missing	0			2 (0.5)			
Sex							0.05
Male	179 (48.0)			229 (55.2)			
Female	194 (52.0)			186 (44.8)			
Age at Exam	373	15.5 (0.6)	14.4-17.8	155	15.7 (0.7)	14.0-17.9	<0.001*
Home Score	373	43.9 (6.3)	21-56	118	42.7 (6.0)	27-53	0.07
Year of Birth							
1993-1994	100 (26.8)			159 (38.3)			0.003*
1995-1996	153 (41.0)			147 (35.4)			
1997-1998	120 (32.2)			109 (26.3)			
Maternal Characteristics							
Marital status at birth							<0.001*
Not married	136 (36.5)			195 (47.0)			
Married	237 (63.5)			165 (39.8)			
Missing	0			55 (13.3)			
Maternal IQ	373	99.4 (10.4)	57-124	262	95.8 (10.2)	72-126	<0.001*

Table 3.1 (Continued)

Seafood during pregnancy (serv/day)	373	0.5 (0.6)	0-5.3	260	0.6 (0.7)	0-6	0.6
Smoking during pregnancy							0.1
No	272 (72.9)			210 (50.6)			
Yes	101 (27.1)			103 (24.8)			
Missing	0			102 (24.6)			
Household Characteristics at Birth							
Maternal education							<0.001*
≤ High School	190 (50.9)			231 (55.7)			
> High School	183 (49.1)			127 (30.6)			
Missing	0			57 (13.7)			
Paternal Education							0.002*
≤ High School	246 (66.0)			266 (64.1)			
> High School	127 (34.0)			81 (19.5)			
Missing	0			68 (16.4)			
Annual Household Income							0.001*
< $20,000	115 (30.8)			150 (36.1)			
≥ $20,000	258 (69.2)			201 (48.4)			
Missing	0			64 (15.4)			
Examination Characteristics							
Examiner							0.4
1	277 (74.3)			121 (78.1)			
2	96 (25.7)			34 (21.9)			

[1]Main analysis group: complete working memory outcome, covariate and exposure data for DDE, HCB, PCBs, Pb and Mn, n=373. [2]P-values represent results comparing characteristics between participants included in *Set 1* and those excluded from *Set 1* using t-tests and chi-square tests. [3]NBC participants with missing working memory measures: Verbal Working Memory n=260; Symbolic Working Memory n=261; Working Memory Index n=261. [4]NBC participants with missing exposure measures: DDE n=37; HCB n=37; ΣPCB_4 n=37; Pb n= 40; Mn n=80. [5]NBC participants with missing covariate measures: age at exam n=260; HOME score n= 297; maternal IQ n=153; seafood during pregnancy n= 155.*p<0.05

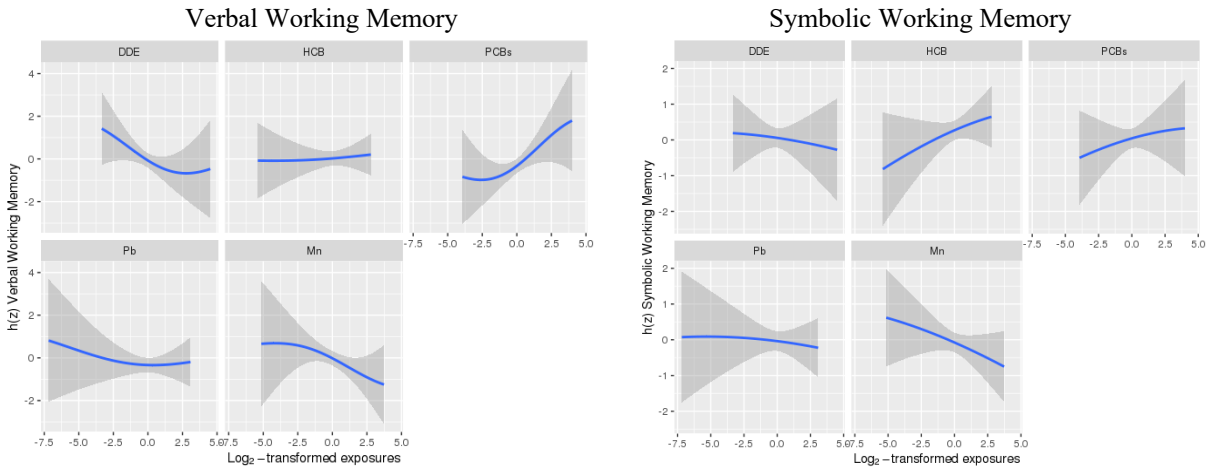
Abbreviations: DDE: dichlorodiphenyldichloroethylene; HCB: hexachlorobenzene; ΣPCB_4: Sum of 4 PCB congeners (118, 138, 153, 180); Pb: lead; Mn: manganese.

Exposure measures. Among participants in the main analytic group, exposures to DDE, HCB, and ΣPCB_4 were moderately correlated (Spearman r=0.4-0.6) and exposure to Pb was weakly correlated with the organochlorines and Mn (Spearman r=0.1-0.2). Mn was not correlated with the organochlorines. Despite the residential proximity of NBC participants to the New Bedford Harbor Superfund site, chemical biomarker levels in the NBC study participants were similar to the general populations of the U.S. and Canada,[56,74–76] with the exception of total hair Hg concentrations which were similar to those observed in high fish-eating populations.[77]

Working memory measures. WRAML2 Verbal and Symbolic Working Memory were moderately correlated with one another (Spearman r=0.6). NBC WRAML2 working memory scores were lower than the standardized sample [mean (SD) of Verbal and Symbolic Working Memory: 10 (3) and Working Memory Index: 100 (3)] (Table 3.1).

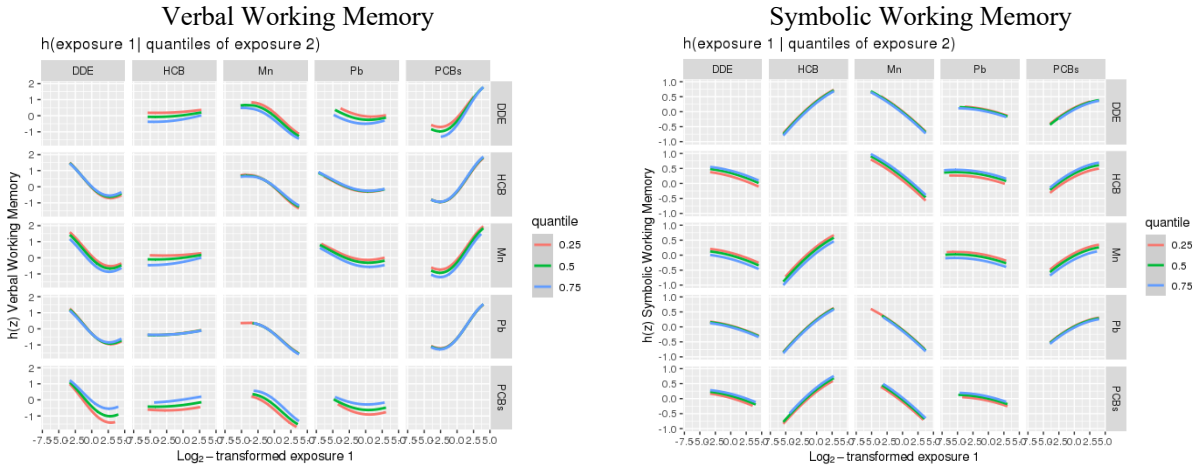
BKMR model results. Visual inspection of exploratory BKMR analyses of the association of a chemical mixture composed of DDE, HCB, ΣPCB_4, Pb, and Mn with Verbal and Symbolic Working Memory did not indicate any non-linear relationships or interactions between exposures (Figures 3.1-3.2). Therefore, we did not include higher-order terms or interactions between chemicals in the parametric models. BKMR results suggested an adverse association of the joint chemical mixture with Verbal Working Memory for joint exposures up to the ~60th percentile when compared to their median levels (Figure 3.3). There was no evidence of a joint association of the exposures with the Symbolic Working Memory subtest (Figure 3.3).

Figure 3.1 Estimated exposure-response functions and 95% credible intervals[1] of each of the 5 main exposures with the Wide Range Assessment of Memory and Learning 2nd Edition working memory scaled scores, where all remaining exposures are assigned to their median value among adolescents in the main analysis group[2].



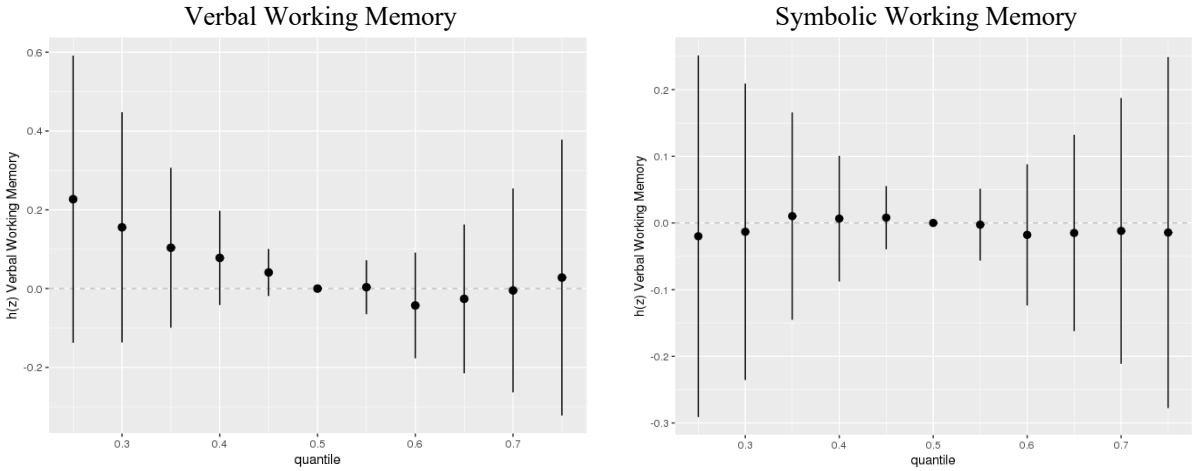
[1]Exposures have been log2-transformed and models have been adjusted for all listed exposures, child race, sex, age at exam, and HOME score; maternal marital status at child's birth, IQ, seafood consumption during pregnancy, and smoking during pregnancy; maternal and paternal education and annual household income at child's birth; study examiner.
[2]Main analysis group: complete working memory outcome, covariate and exposure data for DDE, HCB, PCBs, Pb and Mn, n=373.
Abbreviations: DDE: dichlorodiphenyldichloroethylene; HCB: hexachlorobenzene; PCBs: Sum of 4 PCB congeners (118, 138, 153, 180); Pb: lead; Mn: manganese.

Figure 3.2 Exposure-response functions[1] associating each of the 5 main exposures and a second exposure fixed at various quantiles with the Wide Range Assessment of Memory and Learning 2nd Edition working memory scaled scores, while the remaining exposures are assigned to their median value among adolescents in the main analysis group[2].



[1]Exposures have been log2-transformed and models have been adjusted for all listed exposures, child race, sex, age at exam, and HOME score; maternal marital status at child's birth, IQ, seafood consumption during pregnancy, and smoking during pregnancy; maternal and paternal education and annual household income at child's birth; study examiner.
[2]Main analysis group: complete working memory outcome, covariate and exposure data for DDE, HCB, PCBs, Pb and Mn, n=373.
Abbreviations: DDE: dichlorodiphenyldichloroethylene; HCB: hexachlorobenzene; ΣPCB_4: Sum of 4 PCB congeners (118, 138, 153, 180); Pb: lead; Mn: manganese.

Figure 3.3 Joint association (estimates and 95% credible intervals[1]) of the five-chemical mixture with the Wide Range Assessment of Memory and Learning 2nd Edition working memory scaled scores among adolescents in the main analysis group[2]. Chemical mixture levels at each percentile are compared to each component at its median level.



[1]Exposures have been log2-transformed and models have been adjusted for all listed exposures, child race, sex, age at exam, and HOME score; maternal marital status at child's birth, IQ, seafood consumption during pregnancy, and smoking during pregnancy; maternal and paternal education and annual household income at child's birth; study examiner.
[2]Main analysis group: complete working memory outcome, covariate and exposure data for DDE, HCB, PCBs, Pb and Mn, n=373.

Linear regression model results. Table 3.2 shows the results of covariate-adjusted linear regression analyses of the association of prenatal concentrations of DDE, HCB, ΣPCB_4, Pb, and Mn with Verbal Working Memory, Symbolic Working Memory, and the Working Memory Index, where all five exposures were included in the models simultaneously. We found that a twofold increase in cord blood Mn concentration was associated with lower working memory scores (Verbal Working Memory: difference=-0.75; 95% CI: -1.29,-0.20 points; Symbolic Working Memory: difference=-0.44; 95% CI: -1.00, 0.12 points; Working Memory Index: difference=-3.23; 95% CI: -5.87,-0.59 points). Findings also included an unexpected positive association between a twofold increase in ΣPCB_4 and Verbal Working Memory (difference=0.32; 95% CI: 0.01, 0.63 points). Associations of the remaining exposures and working memory outcomes were modest and imprecise with confidence limits that included the null.

Table 3.2 Complete-case results of multivariable linear regression analyses (difference in points associated with a twofold increase in exposure and 95% CI)[1] assessing the relation of prenatal exposure to a five-chemical mixture with Wide Range Assessment of Memory and Learning, 2nd Edition working memory scaled scores among adolescents in the main analysis group[2].

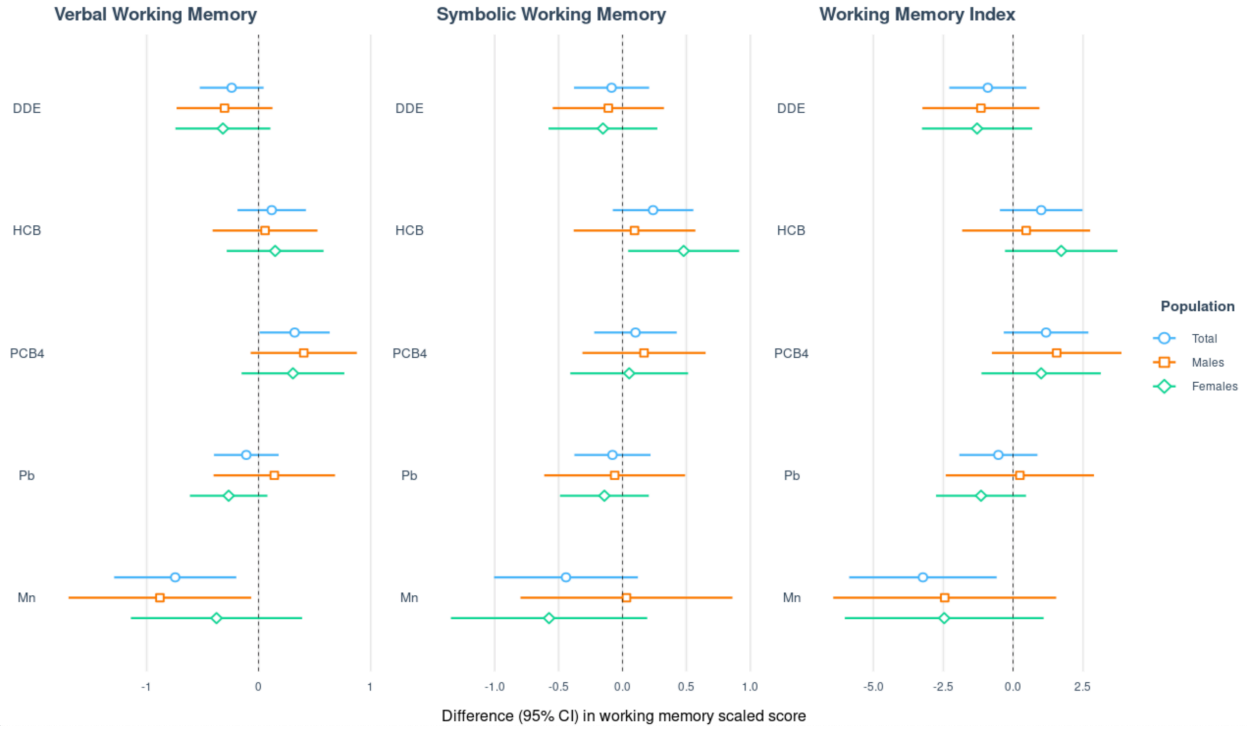
Exposure	Verbal Working Memory Difference (95% CI)	Symbolic Working Memory Difference (95% CI)	Working Memory Index Difference (95% CI)
Log_2 DDE	-0.24 (-0.53, 0.04)	-0.09 (-0.38, 0.21)	-0.91 (-2.28, 0.47)
Log_2 HCB	0.12 (-0.19, 0.42)	0.24 (-0.08, 0.55)	1.00 (-0.47, 2.48)
Log_2 ΣPCB_4	0.32 (0.01, 0.63)*	0.10 (-0.22, 0.42)	1.18 (-0.34, 2.69)
Log_2 Pb	-0.11 (-0.40, 0.18)	-0.08 (-0.38, 0.22)	-0.53 (-1.92, 0.87)
Log_2 Mn	-0.75 (-1.29, -0.20)*	-0.44 (-1.00, 0.12)	-3.23 (-5.87, -0.59)*

[1]Exposures have been log2-transformed and models have been adjusted for all listed exposures, child race, sex, age at exam, and HOME score; maternal marital status at child's birth, IQ, seafood consumption during pregnancy, and smoking during pregnancy; maternal and paternal education and annual household income at child's birth; study examiner.
[2]Main analysis group: complete working memory outcome, covariate and exposure data for DDE, HCB, PCBs, Pb and Mn, n=373.
*$p<0.05$
Abbreviations: DDE: dichlorodiphenyldichloroethylene; HCB: hexachlorobenzene; ΣPCB_4: Sum of 4 PCB congeners (118, 138, 153, 180); Pb: lead; Mn: manganese.

Assessment of effect modification. In sex-stratified analyses (Figure 3.4; Supplemental Table 3.1), a doubling of cord blood Mn was associated with lower Verbal Working Memory scores among both sexes, though the association was stronger among males (difference=-0.88; 95% CI: -1.70, -0.07 points) than females (difference=-0.38; 95% CI: -1.14, 0.39 points). In contrast, a Mn-Symbolic Working Memory association was stronger in females than males. There was also an unexpected positive association between HCB concentrations and Symbolic Working Memory scores among females (difference=0.48; 95% CI: 0.04, 0.91 points). There was little evidence of other chemical-sex interactions.

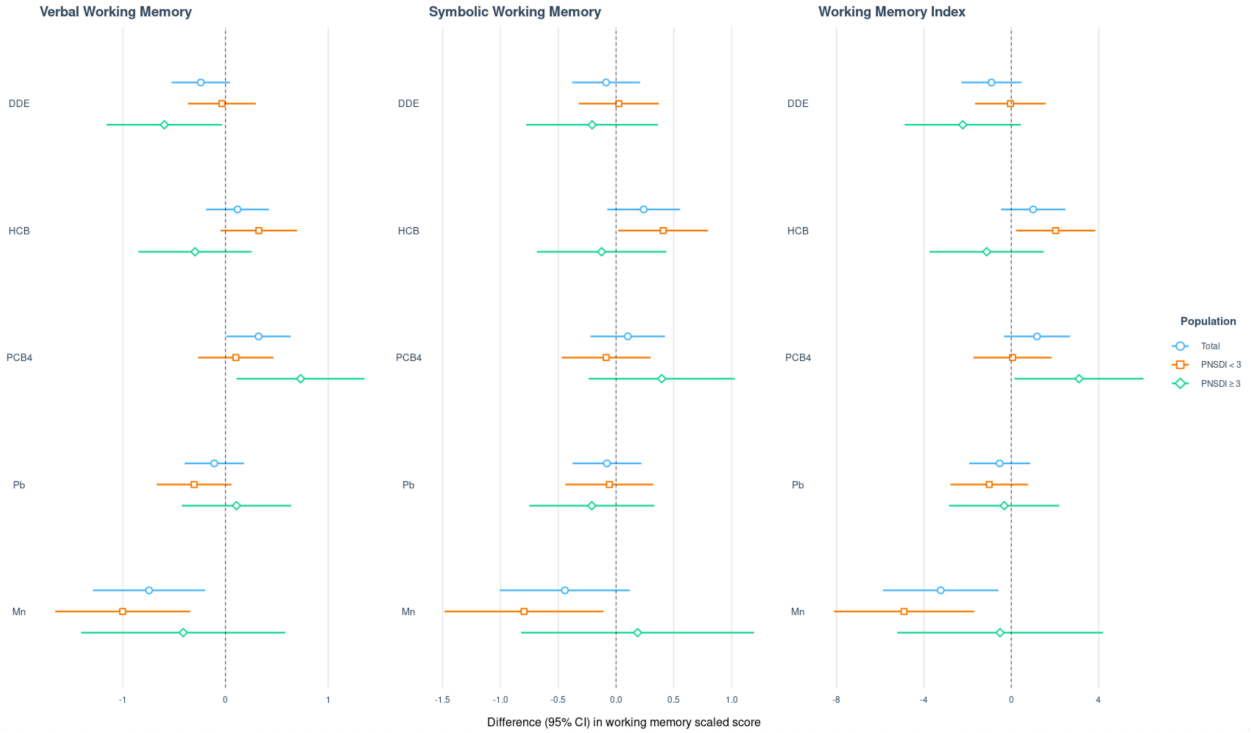
Of the 373 study participants, 241 had a PNSDI < 3 (less prenatal social disadvantage), while 132 had a PNSDI ≥ 3 (more social disadvantage). We found suggestive evidence of an interaction between PNSDI and DDE for Verbal Working Memory, with a stronger negative association among those with more social disadvantage (difference = -0.60; 95% CI: -1.16 points, -0.03 vs. difference = -0.04; 95% CI: -0.37, 0.30 points) (Figure 3.5; Supplemental Table 3.2). In general, we noted stronger negative associations of DDE and HCB with the working memory measures among those with a PNSDI ≥ 3 (Figure 3.5). Unexpectedly, ΣPCB_4 exposure was positively associated with working memory outcomes for both strata of PNSDI (Figure 3.5; Supplemental Table 3.2), with the strongest evidence of a positive association with Verbal Working Memory where PNSDI ≥ 3 (difference = 0.73; 95% CI: 0.11, 1.35 points). Also unexpected was suggestive evidence that the strongest negative associations of Mn with working memory were where PNSDI was less than 3. IPW results of the main, sex-stratified, and PNSDI-stratified analyses were similar to the results of complete-case analyses (Supplemental Tables 3.3-3.5).

Figure 3.4 Sex-stratified and overall results of multivariable linear regression analyses (difference in points associated with a twofold increase in exposure and 95% CI)[1] assessing the relation of prenatal exposure to a five-chemical mixture with Wide Range Assessment of Memory and Learning, 2nd Edition working memory scaled scores among adolescents in the main analysis group[2].



[1]Exposures have been log2-transformed and models have been adjusted for all listed exposures, child race, sex, age at exam, and HOME score; maternal marital status at child's birth, IQ, seafood consumption during pregnancy, and smoking during pregnancy; maternal and paternal education and annual household income at child's birth; study examiner.
[2]Main analysis group: complete working memory outcome, covariate and exposure data for DDE, HCB, PCBs, Pb and Mn. Total n=373; Males n= 179; Females n=194.
Abbreviations: DDE: dichlorodiphenyldichloroethylene; HCB: hexachlorobenzene; ΣPCB_4: Sum of 4 PCB congeners (118, 138, 153, 180); Pb: lead; Mn: manganese

Figure 3.5 Prenatal social disadvantage index (PNSDI)[1]-stratified and overall results of multivariable linear regression analyses (difference in points associated with a twofold increase in exposure and 95% CI)[2] assessing the relation of prenatal exposure to a five-chemical mixture with Wide Range Assessment of Memory and Learning, 2nd Edition working memory scaled scores among adolescents in the main analysis group[3].



[1]Prenatal social disadvantage index (PNSDI) was constructed as the sum of five adverse social or economic exposures at the time of the child's birth where presence of each risk factor was assigned a value of 1, absence a value of 0: mother unmarried, mother's education as high school graduate or less, father's education as high school graduate or less, annual household income less than $20,000, and mother's age at birth less than 20 years.

[2]Exposures have been log2-transformed and models have been adjusted for all listed exposures, child race, sex, age at exam, and HOME score; maternal marital status at child's birth, IQ, seafood consumption during pregnancy, and smoking during pregnancy; maternal and paternal education and annual household income at child's birth; study examiner.

[3]Main analysis group: complete working memory outcome, covariate and exposure data for DDE, HCB, PCBs, Pb and Mn. Total n=373; PNSDI < 3 n= 241; PNSDI ≥ 3 n=132.

Abbreviations: DDE: dichlorodiphenyldichloroethylene; HCB: hexachlorobenzene; ΣPCB_4: Sum of 4 PCB congeners (118, 138, 153, 180); Pb: lead; Mn: manganese.

Secondary analyses. In secondary analyses, we added biomarkers of MeHg and As to our chemical mixture and assessed associations of DDE, HCB, ΣPCB_4, Pb, Mn, MeHg, and As with working memory (n= 235). Similar to participants in the main analyses, those who were included in the secondary analyses performed better on all tests of working memory, had higher cord serum DDE levels and lower cord Pb levels, and had characteristics consistent with greater sociodemographic advantage (Supplemental Table 3.6). Among participants in the secondary analytic group, MeHg was moderately correlated with the organochlorines (Spearman r=0.2-0.5), while As was not correlated with other exposures.

The results of exploratory BKMR analyses of the association of the seven-chemical exposure mixture with Verbal and Symbolic Working Memory did not indicate any non-linear relationships or interactions between the exposures in determining working memory outcomes (Supplemental Figures 3.1-3.2). Similarly, BKMR did not demonstrate an adverse joint association of the chemical mixture with either Verbal or Symbolic Working Memory (Supplemental Figure 3.3). The results of covariate-adjusted linear regression showed similar patterns of associations as were observed in the main analyses, though the effect estimates were typically attenuated and less precise (Supplemental Table 3.7). MeHg and As were not associated with working memory measures. IPW results were similar to complete case (Supplemental Table 3.8).

Discussion

The purpose of this study was to examine the hypothesized association of prenatal exposure to a five-chemical mixture composed of DDE, HCB, ΣPCB_4, Pb, and Mn with working memory among adolescents from the NBC study. The most consistent finding across the main linear regression analyses was an association of cord blood Mn with lower working memory scores

(Table 3.2). This association was strongest for Verbal Working Memory and these findings remained when we used IPW to account for potential selection bias due to loss to follow-up. They were also observed when we included MeHg and As in the chemical mixture, though the effect estimates were somewhat attenuated especially for Symbolic WM (Supplemental Table 3.7). In sex-stratified results, we found a stronger adverse association of Mn with Verbal Working Memory among males, but a stronger adverse association of Mn with Symbolic Working Memory among females, though the interaction with sex was not statistically significant (Figure 3.4). In PNSDI-stratified analyses, we observed Mn to be more strongly adversely associated with all working memory outcomes among those who had a lower PNSDI (Figure 3.5). This may be due to a type of saturation effect in which it is harder to detect subtle chemical associations when other risk factors such as high PNSDI predominate. There is not a well-established point source for Mn exposure located near New Bedford, therefore exposure to Mn was likely via diet, the most common source of Mn in the U.S. general population.[82]

Consistent with our results, high prenatal exposure to Mn measured in teeth was previously found to be associated with working memory errors on the Virtual Radial Arm Maze among adolescent girls in Italy.[29] In addition, a small exploratory study found that tooth Mn levels reflecting prenatal exposures were associated with poor inhibition, another executive function related to working memory, among young children ages 36-54 months.[38] Within the NBC study, we previously found suggestive evidence of an adverse association of prenatal Mn exposure with a verbal measure of inhibition.[96] Two cross-sectional studies of children ages 7-12 in Brazil have also found adverse associations between hair Mn and verbal tasks, specifically verbal working memory and verbal memory.[86,97]

We also observed evidence of an unexpected positive association between ΣPCB_4 exposure and Verbal Working Memory scores (Table 3.2). It remained when accounting for loss to follow-up using IPW and was present (though attenuated) when including MeHg and As in the chemical mixture. The attenuation of this association in secondary analyses when MeHg and As were included in the model may be due to less precision due to a smaller sample. It may also be due to population differences between the two groups – when we accounted for potential loss to follow-up using IPW, we did see a slightly stronger positive association between ΣPCB_4 and Verbal Working Memory than in the complete case results (Supplemental Table 3.8). This positive association may be due to negative confounding by diet.[84] Although we accounted for seafood consumption in our models, it had been measured with a food frequency questionnaire which may not have adequately captured this information resulting in residual confounding by beneficial dietary nutrients. Our results were not consistent with those in the Michigan cohort, where researchers found an adverse association between prenatal PCB exposure and working memory among 11-year-olds.[27] A number of differences in the two studies may contribute to the discordant findings: PCB exposure levels were much lower in the NBC cohort than in Michigan; Michigan study participants were younger than those in the NBC; Michigan participants were selected based on contaminated fish consumption while in the NBC study, PCB exposure was associated with not only fish consumption, but other dietary and demographic factors as well, which may have led to differing exposure patterns; working memory was assessed using different psychometric tests in the two studies; and the Michigan study did not account for other environmental exposures which may have resulted in unmeasured confounding.[27,56,60]

Although results crossed the null, associations of working memory outcomes with DDE and Pb trended in a negative direction, while associations with HCB appeared weakly positive

(Table 3.2). In secondary analyses when MeHg and As were added into the chemical mixtures, we found that associations of MeHg and As with working memory outcomes were largely null. This may be due to the smaller sample size and therefore a less power to detect effects.

For DDE, our results were consistent with those in California and Greece in which working memory among 10 year-olds and 4-year-olds, respectively, was weakly adversely impacted by prenatal exposure to DDE at levels higher than those in the NBC, without reaching significance.[34,35] One Spanish cohort, which had also higher levels of DDE than the NBC cohort, did find an adverse association between prenatal DDE and McCarthy Scales of Children's Abilities Total Memory scores which encompassed working memory among young children, however they did not assess working memory as a distinct outcome.[33] Of note, while the aforementioned studies focused on just one exposure at a time, our study assessed multiple exposures to both organochlorines and metals in the same models, which accounted for potential confounding by these other exposures.

Our Pb results were consistent with those found in eastern Massachusetts, where associations between prenatal Pb exposure at levels somewhat lower than those found in the NBC and working memory among 7-year-olds trended in an adverse direction without reaching significance.[37] A study of 11-year-old children in Quebec found evidence of adverse associations between cord blood Pb and working memory at higher levels of Pb than those found in the NBC study.[30] A study in Boston previously found that general executive function outcomes among 10-year-olds were adversely impacted by recent or concurrent Pb exposure, rather than prenatal Pb exposure, so these may be important windows of susceptibility to consider.[85] Finally, the one study that examined maternal serum pregnancy HCB concentrations and working memory among four-year-old children in Greece found an adverse association at the highest levels of exposure,[35]

however the NBC study had lower levels of HCB than those found in Greece (mean (SD): 0.1 (0.1) ng/ml).

The NBC cohort is a diverse population with a substantial proportion of participants exposed to economic and sociodemographic disadvantages (Table 3.1). When we assessed effect modification by a prenatal social disadvantage index, we observed stronger adverse associations of DDE and HCB with working memory measures among participants with a PNSDI ≥ 3 compared to those who had a lower PNSDI (Figure 3.5). We also observed stronger positive associations between ΣPCB_4 and working memory among participants with more social disadvantage though, as previously mentioned, positive PCB-working memory associations may be due to residual negative confounding by diet which cannot be excluded in this study. Our results point to the importance of examining effect modification by socio-demographic stressors in the association between prenatal exposure to chemical pollutants and working memory or other executive function outcomes.

Exploratory BKMR analyses did not support non-linear associations between the prenatal exposures of interest and working memory outcomes, nor did they show evidence of interactions between chemicals (Figures 3.1-3.2). The BKMR results suggested an adverse joint association of the chemical mixture with Verbal Working Memory but not Symbolic Working Memory (Figure 3.3). In a previous analysis within the NBC study, we examined the association of the same chemical mixture with inhibition, another building block of executive function closely related to working memory.[96] Using BKMR, we also observed an adverse joint association of the chemical mixture with a verbal inhibition task, but not a non-verbal inhibition task.[96]

Of the 788 original participants recruited to the NBC, 528 completed the adolescent follow-up assessment and 373 had complete data on exposures, outcomes, and covariates of interest. To

account for this loss to follow-up and other missing data, we used IPW to weight participants that were included in our study so they would represent those who were excluded. The IPW results were very similar to the complete-case results, giving reassurance that selection bias due to censoring was unlikely. We were also limited in the number of participants who had data on prenatal exposure to MeHg and As as samples for these measures required an extra study visit. Therefore, we may have been underpowered to observe effects of these exposures. In addition, it is possible that we observed some spurious associations as we did not adjust for multiple comparisons in this study. However, decreasing the frequency of type I errors, or rejecting the null hypothesis when it is true, may increase the risk of type II errors, or failing to reject the null hypothesis when it is false.[87]A limitation of using cord blood Mn as a biomarker of Mn exposure is that there is some disagreement about which biological specimen is the most valid biomarker of Mn exposure, however cord blood Mn has been found to be a useful measure of fetal exposure and well-correlated with other biomarkers of prenatal exposure.[89–91] Finally, a limitation of using ICP-MS to measure cord blood Mn is that an isotope of Mn has a similar mass to two isotopes of iron which may result in an overestimation of Mn concentrations among those with high iron levels.[88] However, the laboratory in which these analyses were conducted reported that although some inflation of Mn levels occurred, relative Mn concentrations were unaffected.

Despite these limitations, the NBC is a diverse, prospective study with data on biomarkers of prenatal exposure to organochlorines and metals, comprehensive psychometric measures of working memory, and sociodemographic, dietary, and lifestyle information. These comprehensive data resources allowed us to conduct an investigation of the association of prenatal exposure to a chemical mixture with working memory among adolescents while adjusting for potential confounders. We found suggestive evidence of an adverse impact of prenatal Mn on adolescent

working memory, particularly on a Verbal Working Memory task, as well as an adverse joint association of the chemical mixture with Verbal Working Memory. We also observed different associations of prenatal exposures with working memory outcomes among participants who had greater sociodemographic/socioeconomic disadvantage, supporting the importance of social and economic stressors as potential source of altered susceptibility to chemical exposure risk. Given that working memory undergoes considerable development during adolescence and deficiencies may be associated with numerous psychiatric and behavioral disorders, further research should examine the impact of environmental exposures on working memory in this particular age group, as well as the potential socioeconomic disparities in vulnerability to environmental chemical exposures.

CHAPTER 4:

Prenatal exposure to chemical mixtures and cognitive flexibility among adolescents

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Abstract

Background: Cognitive flexibility, the ability to smoothly adapt to changing circumstances, is a skill that is vital to higher level executive functions such as problem-solving, planning, and reasoning. Because it undergoes substantial development during adolescence, decrements in cognitive flexibility may not become apparent until this time. There is evidence that prenatal exposure to individual chemicals may adversely impact cognitive flexibility in children, but few studies have explored the association of co-exposure to multiple chemicals with these skills in adolescents.

Methods: We investigated this association among a diverse group of adolescents living near a superfund site in New Bedford, Massachusetts. Specifically, using Bayesian Kernel Machine Regression (BKMR) and traditional regression analyses, we investigated the association of biomarkers of prenatal exposure to organochlorines (DDE, HCB, PCBs) and metals (lead, manganese) with cognitive flexibility, measured with four subtests of the Delis-Kaplan Executive Function System.

Results: In BKMR models, we observed adverse joint associations of the chemical mixture with three of the four subtests. In covariate-adjusted linear regression models, a twofold increase in cord blood manganese was associated with lower scaled scores on two of the subtests: Trail-making (completion time scaled score: difference=-0.59; 95% CI: -1.15, -0.04 points) and Color-Word Interference (completion time scaled score: difference=-0.52; 95% CI: -1.06, 0.02 points). There was little evidence of effect modification by sex and some evidence of effect modification by

social disadvantage particularly with organochlorine chemicals in their associations with cognitive flexibility.

Conclusion: This study was among the first to provide evidence of an adverse joint association of a chemical mixture with cognitive flexibility among adolescents.

Introduction

Cognitive flexibility is one of the core executive functions along with inhibition and working memory that serve as building blocks of higher-level cognitive processes such as problem-solving, planning, and reasoning.[1] It involves the ability to shift perspectives, adapt to changing circumstances, and switch flexibly between tasks.[1] Development of the core executive function skills such as inhibitory control and working memory begins during early childhood, however, because cognitive flexibility builds on these skills, it evolves later with substantial development occurring during adolescence.[1,98] Impairment in a range of executive function skills play a role in multiple behavioral and psychiatric disorders including substance use disorder, attention-deficit/hyperactivity disorder (ADHD), conduct disorder, depression, obsessive compulsive disorder, and schizophrenia.[8–13] Therefore, identifying modifiable environmental risk factors associated with executive function decrements is vital to reducing the functional burden of these disorders.

In utero, the developing brain undergoes a period of rapid neurological growth, yet it is not well-protected from environmental toxicants during this time and is therefore highly sensitive to potential long-term injury from such exposures.[22,54,55] There is some evidence of associations of prenatal exposures to polychlorinated biphenyls (PCBs) and lead (Pb) with poor performance on tasks assessing cognitive flexibility in mid-childhood.[27,37] Specifically, Pb levels measured in

maternal erythrocytes during pregnancy were adversely associated with cognitive flexibility among 7-year-olds as measured by a parent-rated behavioral checklist.[37] In addition, cord serum PCB concentrations were associated with decrements in the Wisconsin Card Sorting Test (WCST), a psychometric test of cognitive flexibility and problem-solving, among 11-year-olds in a high fish-eating population who had been breastfed less than 6 weeks.[27]

Prenatal exposure to chemical contaminants has also been studied in relation to other components of executive function and general executive function in childhood and adolescence. Prenatal exposure to dichlorodiphenyldichloroethylene (DDE), hexachlorobenzene (HCB), and PCBs has been adversely associated with both psychometric testing and behavioral checklist assessments of inhibition and working memory in early to mid-childhood, though these findings have not been consistent across studies.[27,28,31–36] Prenatal exposure to metals such as Pb and manganese (Mn) have also been implicated as harmful to inhibition and working memory in age groups ranging from early childhood through adolescence.[29,37,38] There are few studies of the association of prenatal methylmercury (MeHg) or arsenic (As) exposure with executive function. However, cross-sectional studies have found adverse correlations between As exposure and working memory, switching attention, and problem-solving among children ranging from ages 3 through 16.[40–43]

Finally, there is evidence that exposure to chemical contaminants rarely occurs independently and chemicals may interact to induce differing health effects than individual exposures.[26] Despite this, there are no studies investigating the association of a chemical mixture with cognitive flexibility specifically. Of the few studies that have assessed the relation of multiple chemical exposures on other executive functions, some have found evidence of interactions

between chemicals, such as Pb and mercury in their association with inhibition,[44,45] though others have not, such as Mn and As in their association with working memory.[47]

In summary, many studies have linked prenatal exposures to organochlorines and metals with decrements in executive function skills in childhood, but few have assessed the impact of co-exposure to these chemicals and none specifically on cognitive flexibility. Given that substantial development of cognitive flexibility occurs in adolescence and therefore altered executive function may not become apparent until this time, the goal of this study is to assess the relation of prenatal exposure to a chemical mixture composed of organochlorines and metals with cognitive flexibility among adolescents. In addition, given that previous studies of the association between prenatal exposures to neurotoxicants and executive function have found evidence of effect modification by sex and socioeconomic stressors in exposure-related impacts,[29,33,34,46,51,52] we assessed effect modification by sex and measures of social disadvantage in this study.

Methods

Study Population. The New Bedford Cohort (NBC) study is a longitudinal birth cohort study designed to assess the impacts of prenatal chemical pollutant exposures on child health and development among families living near the New Bedford Harbor. The New Bedford Harbor was designated a Superfund site in 1982 due to PCB and metal contamination from local industry.[60] Between 1993 and 1998, 788 mother-infant pairs were recruited into the NBC study shortly after the infant's birth at St. Luke's Hospital in New Bedford, Massachusetts. Recruited mothers were at least 18 years old, spoke English or Portuguese, and were living in one of the four towns surrounding the NBH throughout their pregnancy. Infants born by cesarean section or who were too ill to undergo neonatal examination were excluded from the study. Study participants have

been followed periodically since birth. At an adolescent follow-up which occurred between 2008 and 2014, 528 children (median age 15.5, range 13-17 years) completed neurodevelopmental assessments including measures of cognitive flexibility. A subset of 373 of the NBC adolescents had complete data on cognitive flexibility outcomes, covariates, and biomarkers of prenatal exposure to DDE, HCB, PCBs, Pb, and Mn. 235 participants had additional data on biomarkers of prenatal exposure to MeHg and As.

Chemical exposure assessment. DDE, HCB, and 51 PCB congeners were measured in cord serum, Pb and Mn were measured in cord blood, and total Hg and As were measured in maternal hair and toenails, respectively. Cord blood was collected at birth, while maternal hair and toenails were collected, on average, 10 days postpartum. PCB exposure was estimated using the sum of the 4 most prevalent congeners (ΣPCB_4: 118, 138, 153, 180) due to their minimal measurement error and common usage to assess congener-specific effects in other population-based studies. Details regarding collection, storage, and exposure analysis methods of these biomarkers have been described elsewhere.[62,64] Briefly, cord serum was analyzed for DDE, HCB, and PCBs using gas chromatography with electron capture detection,[62] while cord blood was analyzed for Pb using isotope dilution inductively coupled plasma mass spectrometry and for Mn using external calibration on a dynamic reaction cell-inductively coupled plasma-mass spectrometer. Hair was analyzed for total Hg, a reasonable proxy for MeHg,[65] using a DM-80 Direct Mercury analyzer [64] and toenails were analyzed for As using an external calibration method on a dynamic reaction cell-inductively coupled plasma-mass spectrometer. For the organochlorines, within-sample coefficients of variation over 5 years ranged from 5% to 7.5% and between-batch coefficients of variation ranged from 20% to 39%, reflecting high reproducibility for organics analyses.[62] For Pb

and Mn, quality control (QC) monitoring, procedural blanks, duplicates, spiked samples, standard reference material (NIST SRM 955b Pb in blood; NIST SRM 1643d trace elements in water), biological reference material (ICP03B-05 and ICP03B-02 multi-elements in human blood from INSPQ/ Laboratoire de Toxicologie, Quebec) and certified reference material (GBW 09101 human hair, Shanghai Institute of Nuclear Research, Academia Sinica, China) were used. Recovery rates for QC and spiked samples were 90-110% and precision was >95%. Finally, for total Hg and As, QC procedures included daily calibration verification, procedural blanks, and certified reference material. Recovery rates for QC standards were 90-110% and precision >95% for total Hg, while the coefficients of variation for reference standards were < 15% for toenail As.[64,67] The detection limit for DDE, HCB, and PCBs ranged from 0.001 ng/g to 0.07 ng/g serum, for Pb and Mn was 0.02 µg/dL, for total Hg was 50 ng/g of hair, and for As was 0.03 ng/g.

Cognitive Flexibility Assessment. Cognitive flexibility was measured using four subtests of the Delis-Kaplan Executive Function System (D-KEFS),[49] which were administered by a trained study examiner at the NBC study adolescent (~15-year) follow-up visit. The four subtests included: Trail-making: Number-Letter Switching condition, Verbal Fluency: Category Switching condition, Design Fluency: Filled Dots and Empty Dots Switching condition, and Color-Word Interference: Inhibition/Switching condition. In Trail-making: Number-Letter Switching, participants connected numbers then letters switching in correct numeric and alphabetical order (e.g., from A to 1 to B to 2). Performance was measured by the completion time scaled score, total errors raw score (the sum of set loss, sequencing, and time-discontinue errors), and an overall performance measure that combined both completion time and total errors raw scores. Specifically, the overall performance measure was dichotomized where the best performance group included

those who performed better than population median score for both dimensions (total completion time raw score < 67 seconds and total errors raw score < 1 error) and the poor performance group included the remaining participants. In Verbal Fluency: Category Switching, participants alternated between saying words from two different categories as quickly as possible for 60 seconds. Performance was measured with the switching accuracy scaled score and the total errors raw score, which was created as the sum of the total set loss errors and the total repetition errors. In Design Fluency: Filled Dots and Empty Dots Switching, participants drew designs by switching connections between filled and empty dots. Performance was measured using the total correct scaled score and the total errors raw score, which was created as the sum of the total set loss designs and the total repeated designs raw scores. In Color-Word Interference: Inhibition/Switching, participants switched between naming font colors and reading words that named a color but were printed in a discordant font color. Similar to Trail-making, performance was measured using the completion time scaled score, total errors raw score (the sum of uncorrected and self-corrected errors), and a dichotomized performance measure that combined completion time and total errors raw scores where the best performance group included those who performed better than the population median for both dimensions (total completion time raw score < 59 seconds and total errors raw score < 2 errors) and the poor performance group included the remaining participants. Scaled scores were age-standardized raw scores scaled to a mean of 10, standard deviation of 3. Of note, higher scaled scores mean better performance. Raw scores were used when scaled scores were unavailable. Higher error and completion time raw scores mean worse performance.

Covariate Assessment. Participant demographic, health, lifestyle, and exposure information were obtained via periodic medical record review and parental and child self-reported questionnaires.

Hospital medical records were reviewed to obtain information regarding the infant's race/ethnicity, birthweight, gestational age, and newborn exam as well as the mother's pregnancy and delivery course. At a home visit that took place approximately ten days postpartum, participating mothers completed a questionnaire regarding maternal socio-demographics, medical history, diet, smoking, alcohol, and drug use, and infant feeding. At a 15-year follow-up assessment, in addition to updating medical and demographic information, a home visit took place to assess the quality of the child's home environment and parent-child relationship using the Home Observation for Measurement of the Environment (HOME) questionnaire[68] and maternal IQ was measured using the Kaufman Brief Intelligence Test (KBIT).[69]

In this study, we defined social disadvantage using a prenatal social disadvantage index (PNSDI) composed of the sum of five adverse social or economic exposures at the time of the child's birth where the presence of each of the following risk factors was assigned a value of 1 and absence a value of 0: mother unmarried, mother's education as high school graduate or less, father's education as high school graduate or less, annual household income less than $20,000, and mother's age at birth less than 20 years.

Statistical analysis. We used Directed Acyclic Graphs (DAGs) to select potential covariates. The DAG was informed with a literature review of potential confounders of the relationships of prenatal organochlorine and metal exposures with neurodevelopment, as well as covariates that had previously predicted cognition outcomes in the NBC. The following covariates were included in the final models: adolescent race/ethnicity, sex, age at exam, and HOME score; maternal marital status at birth, IQ, seafood consumption and smoking during pregnancy; maternal and paternal education and household income at child's birth; and examiner. Characteristics of participants who

were included in analyses were compared to those not included using t-tests, Wilcoxon Rank Sum tests, and chi-square tests where appropriate.

To analyze the association between a five-chemical mixture composed of DDE, HCB, ΣPCB_4, Pb, and Mn and multiple measures of cognitive flexibility, we first log_2-transformed exposures to reduce the influence of extreme values. Next, we used Bayesian Kernel Machine (BKMR) as an exploratory technique to examine potential non-linear relationships and interactions between chemical exposures and continuous measures of cognitive flexibility using the scaled scores, but not the error scores. The error scores for D-KEFS cognitive flexibility measures have distributions consistent with count data which cannot currently be accommodated as an outcome in BKMR analyses. BKMR, an exposure-response surface estimation technique, can model the relationship between multiple exposures and an outcome using a flexible exposure-response function, *h*.[70] In this analysis, *h* was estimated using a Gaussian Kernel, which can capture many underlying functional forms.[70]

Using BKMR, we were able to identify non-linear exposure-outcome associations and interactions between exposures, which were then used to inform the specification of standard parametric linear regression models. Specifically, to assess non-linearities, we visually inspected plots of the estimated exposure-response functions and 95% credible intervals of DDE, HCB, ΣPCB_4, Pb, and Mn with cognitive flexibility scaled scores while the remaining exposures were assigned their median value. Where the exposure-response function appeared non-linear, we formally tested for the utility of a quadratic term for the chemical with both a Wald test and by fitting covariate-adjusted linear regression models with and without a quadratic term and comparing model fit using a likelihood ratio test for nested models. To assess interactions, we visually inspected plots of the estimated exposure-response functions between one of the five main

exposures (DDE, HCB, ΣPCB_4, Pb, Mn) and cognitive flexibility scaled scores, where a second exposure was fixed at varying levels and all of the remaining exposures were assigned to their median value. We assumed no interaction in cases where the slope of each chemical was similar at varying levels of the other chemicals. In most but not all cases, BKMR supported the use of linear regression models with no interactions between exposures. We also used BKMR to assess the joint association between the chemical mixture and each cognitive flexibility scaled score by measuring the association between the outcomes and chemical mixture levels at various percentiles compared to their median levels. BKMR analyses were conducted using the *bkmr* package in R.[72]

As Trail-making completion time, Verbal Fluency switching accuracy, Design Fluency total correct, and Color-Word completion time scaled scores were normally distributed, we used OLS to fit multivariable linear regression models to estimate their association with the five-chemical mixture. All five exposures (DDE, HCB, ΣPCB_4, Pb, and Mn) were simultaneously included in the linear regression models along with the previously mentioned covariates. We then assessed effect modification of exposure-outcome associations by sex and prenatal social disadvantage using sex- and social disadvantage-stratified linear regression models. In PNSDI-stratified models, we compared chemical associations among participants who had a PNSDI of 3 or more (more prenatal social disadvantage) to those who had a PNSDI of less than 3 (less prenatal social disadvantage). A cut-off of 3 was selected as it was correlated with other indicators of social disadvantage such as the HOME score, while maintaining enough power to conduct stratified analyses.

Next, we analyzed the association between the five-chemical mixture and cognitive flexibility error scores, specifically Trail-making, Verbal Fluency, Design Fluency, and Color-Word total errors raw scores. Once again, all five exposures of interest were included in the models

together with covariates. All error scores had distributions consistent with count data, but Trail-making, Verbal Fluency, and Design Fluency total error raw scores were consistent with over-dispersed count data. As negative binomial regression is a more flexible approach than Poisson regression that is appropriate for both count data and over-dispersed count data, it was used to assess the relationship of all error scores with the chemical mixtures. Finally, logistic regression was used to assess the relationship of the chemical mixture with overall Trail-making and Color-Word interference performance. To account for both speed and accuracy, these two subtests were assessed using a binary outcome that integrated two different scoring criteria for each task -- completion time and total errors. This method allowed us to create a more comprehensive representation of overall performance that may not be possible by assessing completion time and total errors separately. In logistic regression, we assessed the odds of being in the poor compared to the best performance (reference) group.

Finally, we used inverse probability weighting (IPW) for censoring to account for potential selection bias due to loss to follow-up.[73] In IPW, individuals in the analysis group are weighted based on the inverse of the probability of their being included in the analysis given their particular exposure and covariate values, creating a pseudo-population that represents the original source population that was recruited to the NBC at birth. Biomarker levels of DDE, HCB, ΣPCB_4 and Pb and socio-demographic characteristics of the mother at birth such as education and household income and child characteristics such as race/ethnicity and sex were used in the IPW missingness model based on how well they predicted loss to follow-up in this analysis and in other studies reported in the literature. In this analysis, we used stabilized weights[73] trimmed at the 2.5^{th} and 97.5^{th} percentile to improve efficiency and minimize the influence of extreme weights.

As a secondary analysis, we added biomarkers of MeHg and As to our chemical mixture and analyzed the association of DDE, HCB, ΣPCB_4, Pb, Mn, MeHg, and As with cognitive flexibility. Many participants had missing information for these two exposure biomarkers as maternal hair and toenail samples were collected at a home visit ten days postpartum, rather than at birth like the remaining exposures. To assess the relationship between the seven-chemical mixture and cognitive flexibility, we once again used BKMR to assess non-linear associations, interactions, and joint associations and to inform subsequent parametric models; multivariable linear regression to assess cognitive flexibility scaled scores and IPW for censoring to account for potential selection bias due to loss to follow-up. All statistics were conducted using R version 3.6.0.[71]

Results

Study population. Table 4.1 describes the outcome, exposure, and covariate measures of all NBC participants included in our main analysis [those who had complete 15-year data on cognitive flexibility outcomes, biomarkers of prenatal exposure to DDE, HCB, ΣPCB_4, Pb, and Mn, and covariates (n=373)], and those who were excluded from the main analysis. Participants in the main analysis group were socio-demographically diverse with 29% identifying as non-white and 31% having a household income of less than $20,000 at the time of their birth. Included participants had higher cord serum DDE and lower cord blood Pb levels than those who were excluded due to loss to follow-up or missing data. They also performed better on some tests of cognitive flexibility than those who were excluded. In general, those who were included in the analysis had characteristics consistent with greater sociodemographic and economic advantage compared to those with missing data. For example, included adolescents had parents who were more likely to

have more than a high school education and an annual household income at or above $20,000 at the time of their birth than those who were excluded from the analyses.

Table 4.1 Characteristics of New Bedford Cohort participants who were evaluated as adolescents and included in the main analysis group[1] and those who were excluded from the main analysis.

Descriptive Characteristic	**Main analysis group, n=373**			**Excluded group, n=415**			
Cognitive Flexibility Measures[3]	**n(%)**	**Mean (SD)**	**Range**	**n(%)**	**Mean (SD)**	**Range**	**p-value[2]**
Trail-making							
Completion time scaled score	373	9.6 (2.8)	1-14	155	9.1 (2.8)	1-14	0.1
Total errors	373	0.9 (1.1)	0-5	154	1.0 (1.4)	0-13	0.5
Overall Trail-making performance							
Best performance	113 (30.3)			45 (29.0)			0.9
Poor performance	260 (69.7)			109 (70.3)			
Missing	0			1 (0.6)			
Verbal Fluency							
Total switching accuracy scaled score	373	9.2 (2.8)	2-17	155	9.0 (2.8)	1-17	0.3
Total errors	373	0.8 (1.2)	0-7	155	0.9 (1.3)	0-7	0.4
Design Fluency							
Total correct scaled score	373	9.9 (2.8)	2-18	155	9.6 (2.6)	2-17	0.3
Total errors	373	2.6 (3.1)	0-22	155	2.6 (2.6)	0-16	0.2
Color-Word Interference							
Completion time scaled score	373	9.9 (2.6)	1-15	154	9.8 (2.7)	1-14	0.7
Total errors	373	2.6 (2.4)	0-19	154	2.8 (2.4)	0-11	0.5
Overall Color-Word Interference performance							
Best performance	83 (22.3)			34 (21.9)			1.0
Poor performance	290 (77.7)			120 (77.4)			
Missing	0			1 (0.6)			
Exposure Measures[4]							
Cord serum DDE (ng/g)	373	0.6 (1.2)	0.02-14.9	378	0.4 (0.5)	0-4.2	0.003*
Cord serum HCB (ng/g)	373	0.03 (0.02)	0-0.1	378	0.03 (0.05)	0-0.7	0.1
Cord serum ΣPCB_4 (ng/g)	373	0.3 (0.3)	0.01-4.4	378	0.2 (0.2)	0.01-1.9	0.05
Cord blood Pb (µg/dL)	373	1.4 (0.9)	0-9.4	375	1.7 (1.7)	0-17.4	<0.001*
Cord blood Mn (µg/dL)	373	4.2 (1.6)	0.7-14.6	335	4.3 (2.0)	0.2-22.1	0.6
Covariate Measures[5]							
Child Characteristics							
Race/Ethnicity							0.09
Non-Hispanic White	263 (70.5)			268 (64.6)			

Table 4.1 (Continued)

Hispanic	33 (8.8)			56 (13.5)			
Other	77 (20.6)			89 (21.4)			
Missing	0			2 (0.5)			
Sex							0.05
Male	179 (48.0)			229 (55.2)			
Female	194 (52.0)			186 (44.8)			
Age at Exam	373	15.5 (0.6)	14.4-17.8	155	15.7 (0.7)	14.0-17.9	<0.001*
Home Score	373	43.9 (6.3)	21-56	118	42.7 (6.0)	27-53	0.07
Year of Birth							0.003*
1993-1994	100 (26.8)			159 (38.3)			
1995-1996	153 (41.0)			147 (35.4)			
1997-1998	120 (32.2)			109 (26.3)			
Maternal Characteristics							
Marital status at birth							0.006*
Not married	136 (36.5)			195 (47.0)			
Married	237 (63.5)			165 (39.8)			
Missing	0			55 (13.3)			
Maternal IQ	373	99.4 (10.4)	57-124	262	95.8 (10.2)	72-126	<0.001*
Seafood during pregnancy (serv/day)	373	0.5 (0.6)	0-5.3	260	0.6 (0.7)	0-6	0.6
Smoking during pregnancy							0.1
No	272 (72.9)			210 (50.6)			
Yes	101 (27.1)			103 (24.8)			
Missing	0			102 (24.6)			
Household Characteristics at Birth							
Maternal education							<0.001*
≤ High School	190 (50.9)			231 (55.7)			
> High School	183 (49.1)			127 (30.6)			
Missing	0			57 (13.7)			
Paternal Education							0.002*
≤ High School	246 (66.0)			266 (64.1)			
> High School	127 (34.0)			81 (19.5)			
Missing	0			68 (16.4)			
Annual Household Income							0.001*
< $20,000	115 (30.8)			150 (36.1)			
≥ $20,000	258 (69.2)			201 (48.4)			
Missing	0			64 (15.4)			
Examination Characteristics							
Examiner							0.4

Table 4.1 (Continued)

1	277 (74.3)	121 (78.1)	
2	96 (25.7)	34 (21.9)	

[1]Main analysis group: complete outcome, covariate and prenatal exposure biomarker data for DDE, HCB, ΣPCB_4, Pb and Mn, n=373. [2]P-values represent results comparing characteristics between participants included in main analysis and those excluded from main analysis using t-tests, chi-square tests, and Wilcoxon rank sum tests where appropriate. P-values reflect comparisons between groups with non-missing data. [3]NBC participants with missing cognitive flexibility measures: Trail-making completion time n=260, total errors n = 261; Verbal Fluency total switching accuracy n = 260, total errors n= 260; Design Fluency total correct n= 260, total errors n=260; Color-Word Interference completion time n = 261, total errors n=261. [4]NBC participants with missing exposure measures: DDE n=37; HCB n=37; ΣPCB_4 n=37; Pb n= 40; Mn n=80. [5]NBC participants with missing covariate measures: age at exam n=260; HOME score n= 297; maternal IQ n=153; seafood during pregnancy n= 155. *$p<0.05$.
Abbreviations: DDE: dichlorodiphenyldichloroethylene; HCB: hexachlorobenzene; ΣPCB_4: Sum of 4 PCB congeners (118, 138, 153, 180); Pb: lead; Mn: manganese.

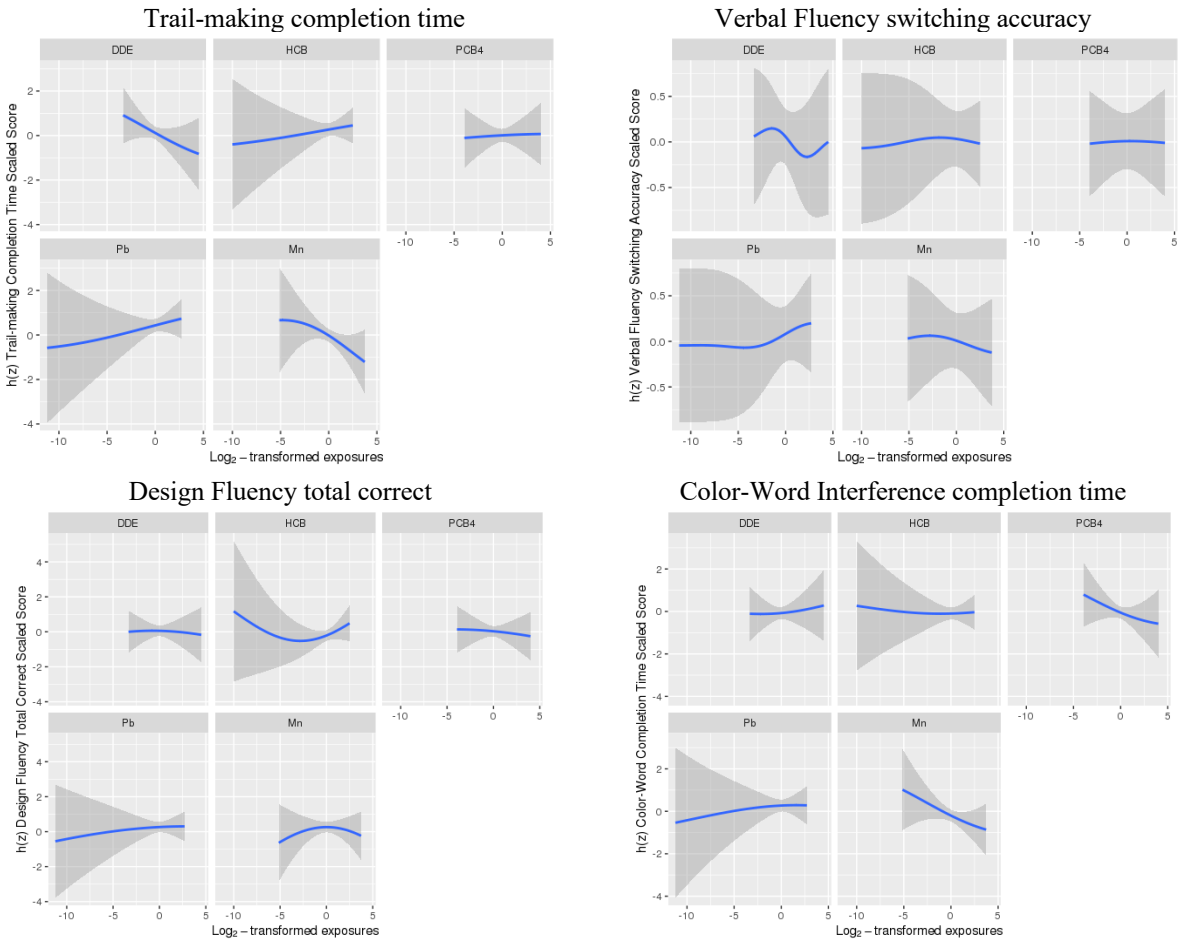
Chemical exposure measures. Despite its residential proximity to the NBH Superfund site, DDE, HCB, ΣPCB_4, Pb, and Mn levels in NBC study participants were similar to the general populations of the U.S. and Canada.[56,74,75] Among the 373 participants in the main analysis group, exposures to DDE, HCB, and ΣPCB_4 were moderately correlated (Spearman r=0.4-0.6), however Pb and Mn exposures were not well correlated with the organochlorines or with each other (Spearman r=0-0.2).

Executive Function Measures. D-KEFS Trail-making, Verbal Fluency, Design Fluency, and Color-Word Interference scaled scores were weakly positively correlated with each other (Spearman r=0.2-0.4). As expected, total error raw scores were negatively correlated with scaled scores in which a better score meant better performance (e.g., Trail-making completion time and total errors Spearman r=-0.5). NBC D-KEFS cognitive flexibility scaled scores were lower than the standardized sample (mean (SD) of cognitive flexibility scaled scores = 10 (3)) (Table 4.1).

BKMR analysis of cognitive flexibility scaled scores and prenatal chemical mixture exposures. Exploratory BKMR analyses of the association of a chemical mixture composed of DDE, HCB, ΣPCB_4, Pb, and Mn with the cognitive flexibility outcomes indicated a potential non-linear relationship between HCB and the Design Fluency total correct scaled score (Figure 4.1), which was confirmed with formal statistical testing. We did not find evidence of any other non-linear relationships or any interactions between chemicals (Figures 4.1-4.2). Therefore, a quadratic term for HCB was included in the linear regression model for Design Fluency. We then used BKMR to assess the joint association of the chemicals with cognitive flexibility scaled scores and found evidence of adverse joint associations between the five-chemical mixture and Trail-making

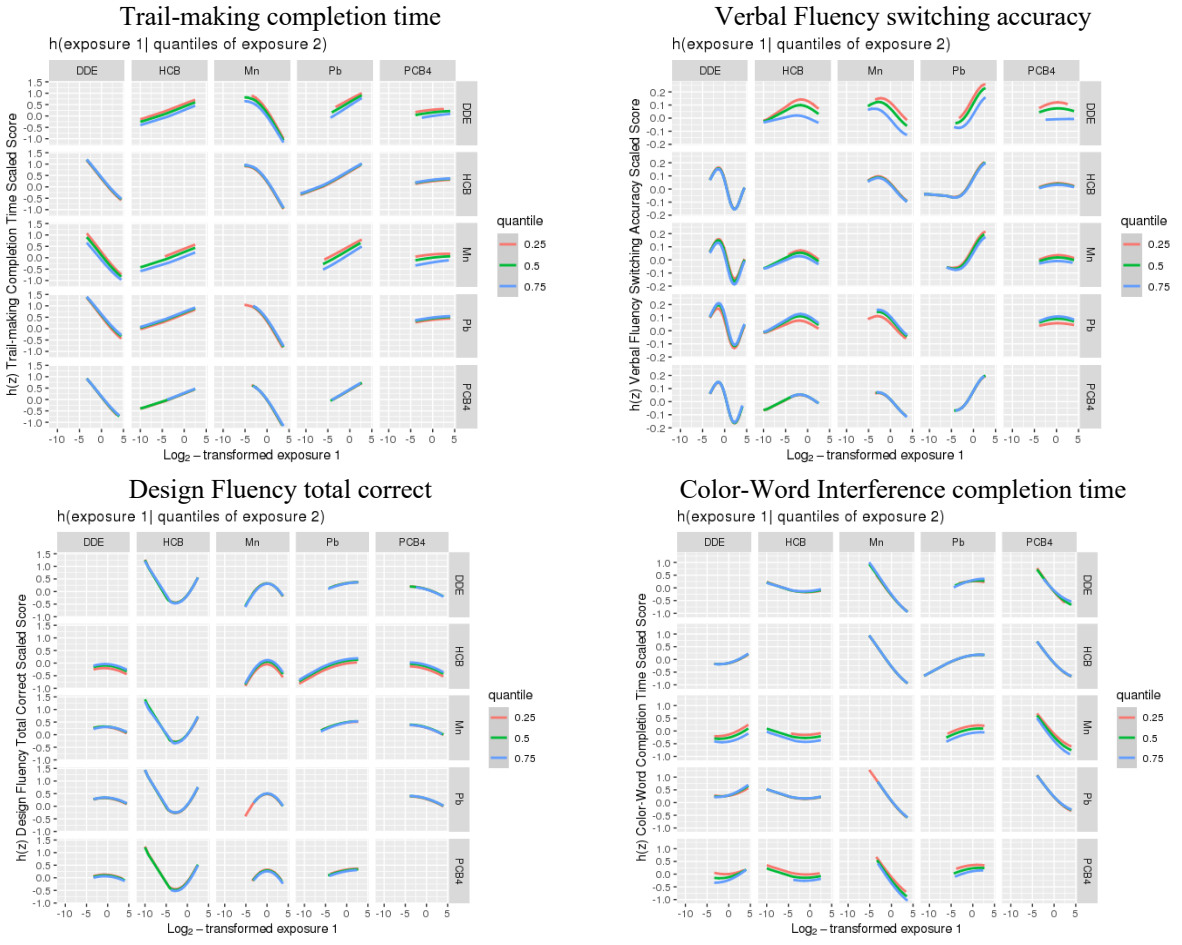
completion time, Color-Word completion time, and Verbal Fluency switching accuracy scaled scores (Figure 4.3). There was an unexpected suggestion of improved performance on the Design Fluency total correct scaled scores with joint exposure.

Figure 4.1 Estimated covariate-adjusted exposure-response functions and 95% credible intervals[1] between DDE, HCB, ΣPCB$_4$, Pb, and Mn and Delis Kaplan Executive Function System (D-KEFS) cognitive flexibility scaled scores among adolescents in the main analysis group[2]. In each plot, all remaining exposures are assigned their median value.



[1]Exposures have been log2-transformed and models have been adjusted for child race, sex, age at exam, year of birth, and HOME score; maternal marital status at child's birth, IQ, seafood consumption during pregnancy, and smoking during pregnancy; maternal and paternal education and annual household income at child's birth; and study examiner.
[2]Main analysis group: complete outcome, covariate and prenatal exposure biomarker data for DDE, HCB, ΣPCB$_4$, Pb and Mn, n=373.
Abbreviations: DDE: dichlorodiphenyldichloroethylene; HCB: hexachlorobenzene; ΣPCB$_4$: Sum of 4 PCB congeners (118, 138, 153, 180); Pb: lead; Mn: manganese.

Figure 4.2 Covariate-adjusted exposure-response functions[1] between one of the 5 main exposures (DDE, HCB, ΣPCB$_4$, Pb, Mn) and Delis-Kaplan Executive Function System (D-KEFS) cognitive flexibility scaled scores, where a second exposure is fixed at various quantiles, among adolescents in the main analysis group[2]. In each plot, all remaining exposures are assigned to their median value.

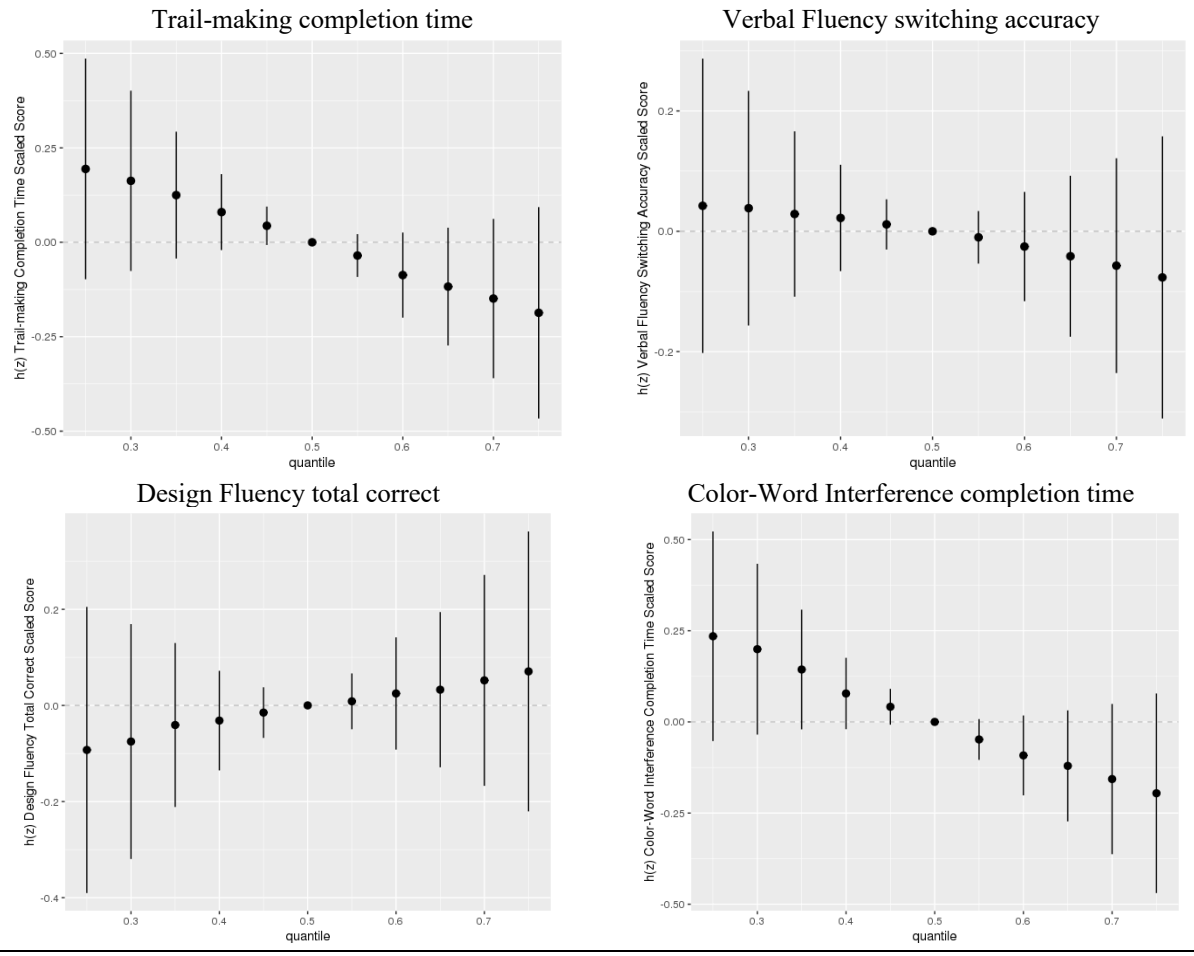


[1]Exposures have been log2-transformed and models have been adjusted for child race, sex, age at exam, year of birth, and HOME score; maternal marital status at child's birth, IQ, seafood consumption during pregnancy, and smoking during pregnancy; maternal and paternal education and annual household income at child's birth; and study examiner.

[2]Main analysis group: complete outcome, covariate and prenatal exposure biomarker data for DDE, HCB, ΣPCB$_4$, Pb and Mn, n=373.

Abbreviations: DDE: dichlorodiphenyldichloroethylene; HCB: hexachlorobenzene; ΣPCB$_4$: Sum of 4 PCB congeners (118, 138, 153, 180); Pb: lead; Mn: manganese

Figure 4.3 Joint association between the chemical mixture composed of DDE, HCB, PCBs, Pb, and Mn (estimates and 95% credible intervals[1]) and the Delis-Kaplan Executive Function System (D-KEFS) cognitive flexibility scaled scores, comparing chemical mixture levels at various percentiles compared to their median levels, among adolescents in the main analysis group[2].



[1]Exposures have been log2-transformed and models have been adjusted for child race, sex, age at exam, year of birth, and HOME score; maternal marital status at child's birth, IQ, seafood consumption during pregnancy, and smoking during pregnancy; maternal and paternal education and annual household income at child's birth; and study examiner.

[2]Main analysis group: complete outcome, covariate and prenatal exposure biomarker data for DDE, HCB, ΣPCB_4, Pb and Mn, n=373.

Abbreviations: DDE: dichlorodiphenyldichloroethylene; HCB: hexachlorobenzene; ΣPCB_4: Sum of 4 PCB congeners (118, 138, 153, 180); Pb: lead; Mn: manganese

Linear regression analyses of cognitive flexibility and prenatal chemical mixture exposures. In linear regression models adjusted for covariates and the remaining exposures, a twofold increase in cord blood Mn concentrations was associated with lower cognitive flexibility scaled scores on two subtests: Trail-making (completion time scaled score: difference=-0.59; 95% CI: -1.15, -0.04 points) and Color-Word Interference (completion time scaled score: difference=-0.52; 95% CI: -1.06, 0.02 points) (Table 4.2). The associations of Mn with Verbal and Design Fluency were also negative, though weaker than other measures and with wide confidence intervals that crossed the null. We did not find evidence of any other consistent adverse associations between the remaining chemicals and cognitive flexibility outcomes. Including a quadratic term for HCB in the Design Fluency model based on the BKMR results, we found a positive association of both HCB and HCB^2 with Design Fluency scaled scores (HCB: total correct scaled score difference = 1.36; 95% CI: 0.44, 2.28 points); HCB^2: total correct scaled score difference= 0.08; 95% CI: 0.02, 0.14 points). When we accounted for potential selection bias using IPW, the results were largely unchanged, the only exception being the results of the Color-Word Interference analyses (Supplemental Table 4.1). In the complete case analysis, ΣPCB_4 and Mn were negatively associated with Color-Word Interference while associations with the remaining chemicals were largely null. In the IPW results, ΣPCB_4 was associated with slightly better performance on Color-Word Interference (though the association was imprecise) and the adverse association between Mn and Color-Word was attenuated.

Table 4.2 Complete-case results of multivariable linear regression analyses (difference in points associated with a twofold increase in exposure and 95% CI)[1] assessing the relation of prenatal exposure to a five-chemical mixture with Delis-Kaplan Executive Function System (D-KEFS) cognitive flexibility scaled scores among adolescents in the main analysis group[2].

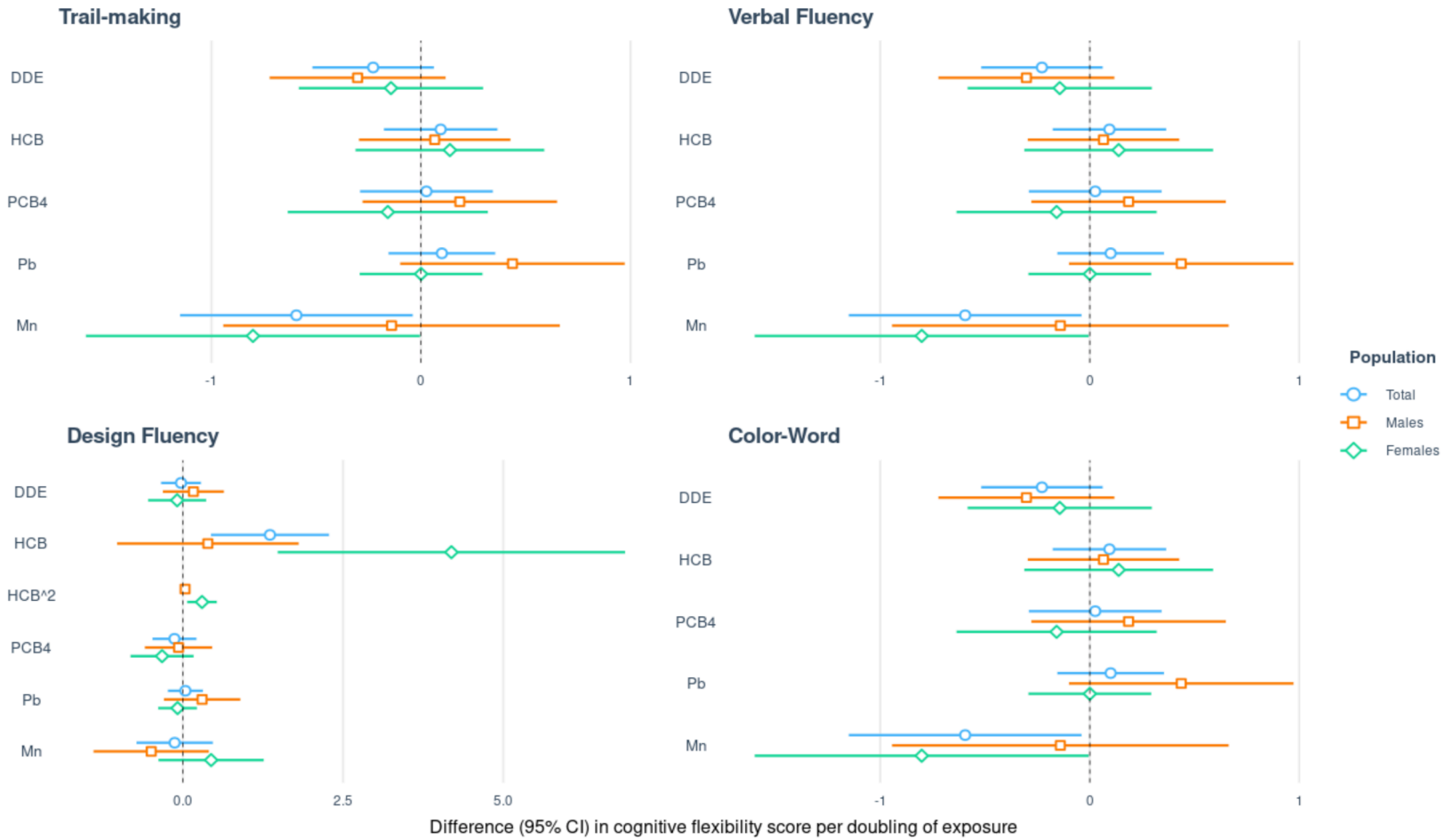
Exposure	Trail-making completion time Difference (95% CI)	Verbal Fluency switching accuracy Difference (95% CI)	Design Fluency total correct Difference (95% CI)	Color-Word Interference completion time Difference (95% CI)
Log_2 DDE	-0.23 (-0.52, 0.06)	-0.11 (-0.40, 0.19)	-0.03 (-0.34, 0.28)	0.06 (-0.22, 0.35)
Log_2 HCB	0.09 (-0.18, 0.36)	-0.04 (-0.31, 0.24)	1.36 (0.44, 2.28)*	0.01 (-0.25, 0.28)
Log_2 HCB^2	-	-	0.08 (0.02, 0.14)*	-
Log_2 ΣPCB_4	0.03 (-0.29, 0.34)	0.05 (-0.28, 0.37)	-0.13 (-0.47, 0.21)	-0.20 (-0.51, 0.11)
Log_2 Pb	0.10 (-0.15, 0.35)	0.25 (-0.01, 0.51)	0.04 (-0.23, 0.31)	0.02 (-0.23, 0.27)
Log_2 Mn	-0.59 (-1.15, -0.04)*	-0.29 (-0.86, 0.28)	-0.13 (-0.72, 0.47)	-0.52 (-1.06, 0.02)

[1]Exposures have been log2-transformed and models have been adjusted for child race, sex, age at exam, year of birth, and HOME score; maternal marital status at child's birth, IQ, seafood consumption during pregnancy, and smoking during pregnancy; maternal and paternal education and annual household income at child's birth; and study examiner.
[2]Main analysis group: complete outcome, covariate and prenatal exposure biomarker data for DDE, HCB, ΣPCB_4, Pb and Mn, n=373.
*$p<0.05$
Abbreviations: DDE: dichlorodiphenyldichloroethylene; HCB: hexachlorobenzene; ΣPCB_4: Sum of 4 PCB congeners (118, 138, 153, 180); Pb: lead; Mn: manganese.

Assessment of effect modification by sex and PNSDI. In sex-stratified analyses, there was little evidence of sexual dimorphism in associations (Figure 4.4, Supplemental Table 4.2). There was suggestive evidence of sexual dimorphism in some associations of Mn with cognitive flexibility, but the potentially more susceptible sex varied by outcome. Unexpectedly, Pb was associated with better performance on some measures of cognitive flexibility in males but most associations were imprecise. The only statistically significant chemical-sex interactions that we observed were of sex with HCB and HCB^2 in their associations with Design Fluency (p for interaction = 0.02 and 0.04 respectively): specifically, the positive HCB-Design Fluency and HCB^2-Design Fluency associations were stronger among females than males.

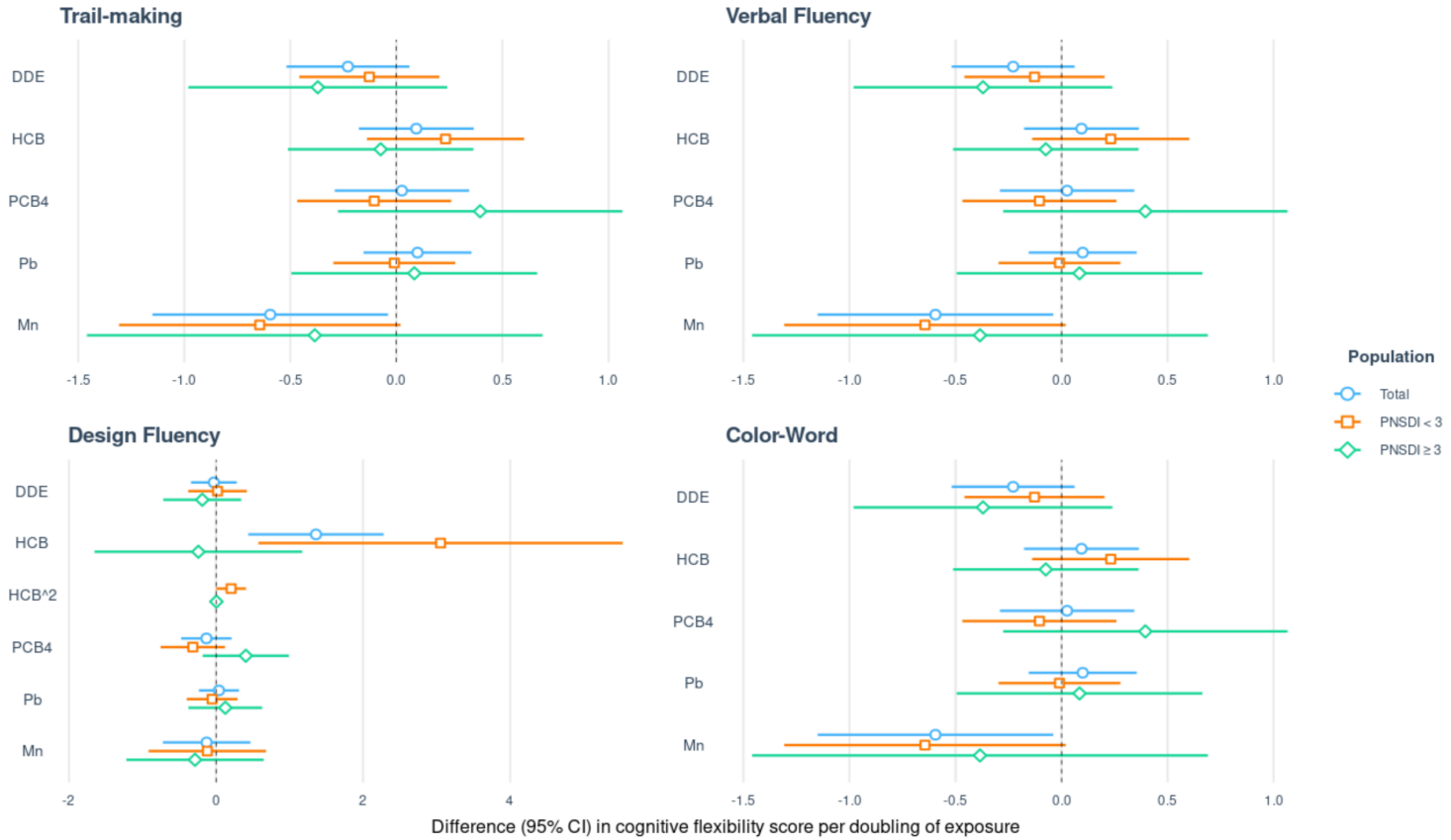
We found some evidence of interactions between PNSDI and the organochlorine chemicals (Figure 4.5; Supplemental Table 4.3). Specifically, DDE exposure had a stronger adverse association with cognitive flexibility scaled scores among those with a PNSDI ≥ 3 compared to those with PNSDI< 3. In addition, while the associations between HCB and cognitive flexibility outcomes were positive among those with a PNSDI < 3, they trended negative for those with a PNSDI ≥ 3. Unexpectedly, we observed positive associations between ΣPCB_4 and cognitive flexibility outcomes among participants with more prenatal social disadvantage and negative associations between ΣPCB_4 and cognitive flexibility among those with less prenatal social disadvantage. We did not observe evidence of interactions with PNSDI and the metals in their associations with cognitive flexibility although Mn was consistently (albeit imprecisely) negatively associated with cognitive flexibility regardless of PNSDI category. The sex-stratified and PNSDI-stratified IPW results were similar to the complete case results (Supplemental Tables 4.4 and 4.5).

Figure 4.4 Sex-stratified and overall complete-case results of multivariable linear regression analyses (difference in points associated with a twofold increase in exposure and 95% CI)[1] assessing the relation of prenatal exposure to a five-chemical mixture with Delis-Kaplan Executive Function System (D-KEFS) cognitive flexibility scaled scores among adolescents in the main analysis group[2].



[1]Exposures have been log2-transformed and models have been adjusted for child race, sex, age at exam, year of birth, and HOME score; maternal marital status at child's birth, IQ, seafood consumption during pregnancy, and smoking during pregnancy; maternal and paternal education and annual household income at child's birth; and study examiner.
[2]Main analysis group: complete outcome, covariate and prenatal exposure biomarker data for DDE, HCB, ΣPCB_4, Pb and Mn. Total n=373; Males n= 179; Females n=194.
Abbreviations: DDE: dichlorodiphenyldichloroethylene; HCB: hexachlorobenzene; PCB_4: Sum of 4 PCB congeners (118, 138, 153, 180); Pb: lead; Mn: manganese

Figure 4.5 Prenatal social disadvantage index (PNSDI)[1]-stratified and overall complete-case results of multivariable linear regression analyses (difference in points associated with a twofold increase in exposure and 95% CI)[2] assessing the relation of prenatal exposure to a five-chemical mixture with Delis-Kaplan Executive Function System (D-KEFS) cognitive flexibility scaled scores among adolescents in the main analysis group[3].



[1]Prenatal social disadvantage index (PNSDI) was constructed as the sum of five adverse social or economic exposures at the time of the child's birth where presence of each risk factor was assigned a value of 1, absence a value of 0: mother unmarried, mother's education as high school graduate or less, father's education as high school graduate or less, annual household income less than $20,000, and mother's age at birth less than 20 years.[2]Exposures have been log2-transformed and models have been adjusted for child race, sex, age at exam, year of birth, and HOME score; maternal marital status at child's birth, IQ, seafood consumption during pregnancy, and smoking during pregnancy; maternal and paternal education and annual household income at child's birth; and study examiner. [3]Main analysis group: complete outcome, covariate and prenatal exposure biomarker data for DDE, HCB, ΣPCB_4, Pb and Mn. Total n=373; PNSDI < 3 n= 241; PNSDI ≥ 3 n=132.Abbreviations: DDE: dichlorodiphenyldichloroethylene; HCB: hexachlorobenzene; PCB_4: Sum of 4 PCB congeners (118, 138, 153, 180); Pb: lead; Mn: manganese.

Negative binomial and logistic regression analyses of cognitive flexibility and prenatal chemical mixture exposures. When we analyzed the association between the five-chemical mixture and cognitive flexibility error raw scores, we found that a doubling of prenatal Mn concentrations was significantly associated with an increased rate of Trail-making errors (rate ratio =1.31; 95% CI: 1.02, 1.69) and a doubling of prenatal DDE concentrations was significantly associated with an increased rate of Verbal Fluency errors (rate ratio=1.23; 95% CI: 1.05, 1.45) (Table 4.5). The remaining associations analyzed were not consistently in the same direction for a given exposure and had confidence limits that crossed the null.

When we combined speed and accuracy to create binary outcomes representing overall Trail-making and Color-Word Interference performance, we found evidence of an adverse association of cord blood Mn with both subtests (Table 4.6). Specifically, a twofold increase in Mn concentrations was associated with an increased odds of being the in poor Trail-making performance group (OR=1.54; 95% CI: 0.93, 2.61) and the poor Color-Word Interference performance group (OR=1.72; 95% CI: 0.97, 3.10). In addition, we found evidence of adverse associations of ΣPCB_4 and Pb with Color-Word Interference (ΣPCB_4 OR=1.50; 95% CI: 1.08, 2.14; Pb OR=1.32; 95% CI: 1.00, 1.80). The IPW results of both the negative binomial regression and logistic regression analyses were similar to the complete case analyses (Supplemental Tables 4.6 and 4.7).

Table 4.5 Complete-case results of negative binomial regression analyses [rate ratio (RR) and 95% CI][1] assessing the relation of prenatal exposure to a five- chemical mixture with Delis-Kaplan Executive Function System (D-KEFS) cognitive flexibility error raw scores among adolescents in the main analysis group[2].

Exposure	Trail-making total errors RR (95% CI)	Verbal Fluency total errors RR (95% CI)	Design Fluency total errors RR (95% CI)	Color-Word Interference total errors RR (95% CI)
Log_2 DDE	1.00 (0.88, 1.14)	1.23 (1.05, 1.45)*	0.99 (0.88, 1.11)	0.97 (0.89, 1.07)
Log_2 HCB	1.01 (0.89, 1.15)	0.90 (0.80, 1.00)	1.06 (0.94, 1.20)	1.04 (0.94, 1.14)
Log_2 ΣPCB_4	1.01 (0.88, 1.17)	0.96 (0.80, 1.14)	0.99 (0.87, 1.13)	1.08 (0.97, 1.19)
Log_2 Pb	0.96 (0.87, 1.06)	1.14 (0.97, 1.35)	1.01 (0.89, 1.13)	1.09 (0.99, 1.20)
Log_2 Mn	1.31 (1.02, 1.69)*	0.79 (0.58, 1.06)	0.82 (0.65, 1.03)	0.98 (0.81, 1.17)

[1]Exposures have been log2-transformed and models have been adjusted for child race, sex, age at exam, year of birth, and HOME score; maternal marital status at child's birth, IQ, seafood consumption during pregnancy, and smoking during pregnancy; maternal and paternal education and annual household income at child's birth; and study examiner.
[2]Main analysis group: complete outcome, covariate and prenatal exposure biomarker data for DDE, HCB, ΣPCB_4, Pb and Mn, n=373.
*$p<0.05$
Abbreviations: DDE: dichlorodiphenyldichloroethylene; HCB: hexachlorobenzene; ΣPCB_4: Sum of 4 PCB congeners (118, 138, 153, 180); Pb: lead; Mn: manganese.

Table 4.6 Complete-case results of logistic regression analyses [odds ratio (OR) and 95% CI][1] assessing the relation of prenatal exposure to a five-chemical mixture with Delis-Kaplan Executive Function System (D-KEFS) Trail-making and Color-Word Interference performance[2] among adolescents in the main analysis group[3].

Exposure	Trail-making Overall Performance OR (95% CI)	Color-Word Interference Overall Performance OR (95% CI)
Log_2 DDE	1.13 (0.86, 1.49)	0.94 (0.68, 1.31)
Log_2 HCB	0.95 (0.73, 1.20)	1.10 (0.83, 1.42)
Log_2 ΣPCB_4	0.94 (0.71, 1.26)	1.50 (1.08, 2.14)*
Log_2 Pb	1.05 (0.82, 1.32)	1.32 (1.00, 1.80)
Log_2 Mn	1.54 (0.93, 2.61)	1.72 (0.97, 3.10)

[1]Exposures have been log2-transformed and models have been adjusted for child race, sex, age at exam, year of birth, and HOME score; maternal marital status at child's birth, IQ, seafood consumption during pregnancy, and smoking during pregnancy; maternal and paternal education and annual household income at child's birth; and study examiner.
[2]Performance takes into account both completion time and total errors raw scores. Those in the best performance group include anyone with < median level completion time and < median total errors with the remaining observations in the poor performance group.
[3]Main analysis group: complete outcome, covariate and prenatal exposure biomarker data for DDE, HCB, ΣPCB_4, Pb and Mn, n=373.
*$p<0.05$
Abbreviations: DDE: dichlorodiphenyldichloroethylene; HCB: hexachlorobenzene; ΣPCB_4: Sum of 4 PCB congeners (118, 138, 153, 180); Pb: lead; Mn: manganese.

Secondary analyses. In secondary analyses, we assessed the relationship between prenatal biomarkers of MeHg and As in addition to DDE, HCB, ΣPCB_4, Pb, and Mn with cognitive flexibility among 235 NBC adolescents who had complete exposure, covariate, and outcome data. Chemical biomarker levels of MeHg in the NBC study participants were similar to those observed in high fish-eating populations[77] and As levels were similar to the general population[76] (Supplemental Table 4.8). Participants who were included in the secondary analyses performed better on Trail-making than those who were excluded, had higher cord serum DDE levels and lower cord blood Pb levels, and had characteristics consistent with greater sociodemographic advantage (Supplemental Table 4.8). Among participants in the secondary analysis group, DDE, HCB, and ΣPCB_4 were moderately correlated with each other and with MeHg (Spearman r=0.2-0.7), while the remaining metals were not well-correlated with each other or with the organochlorines.

The results of exploratory BKMR analyses of the association between the seven-chemical mixture and cognitive flexibility scaled scores indicated a potential non-linear relationship between Mn and Design Fluency (Supplemental Figure 4.1), which was confirmed with formal statistical testing. Therefore, a quadratic term for Mn was included for the Design Fluency linear regression model. We did not observe any interactions between the exposures in their association with the cognitive flexibility outcomes (Supplemental Figure 4.2). When we used BKMR to assess the joint association of the chemicals on cognitive flexibility scaled scores, we found evidence of adverse joint associations between the seven-chemical mixture and Trail-making and Color-Word completion time scaled scores (Supplemental Figure 4.3). However, the adverse associations we had observed between the five-chemical mixtures and Verbal Fluency switching accuracy scaled scores was attenuated (Supplemental Figure 4.3).

Similar to the main linear regression results, the secondary linear regression results provided some evidence of a negative association between Mn and cognitive flexibility scaled scores though with less precision due to a smaller sample size (Supplemental Table 4.9). Specifically, compared to the main results, the secondary results showed a similar strength of association of Mn with Trail-making, a stronger association of Mn with Verbal Fluency, and a slightly attenuated association of Mn with Color-Word Interference. Including a quadratic term for Mn in the Design Fluency model based on the results of BKMR, we found a positive association of Mn with Design Fluency and a negative association of Mn^2 with Design Fluency (Mn: total correct scaled score difference = 6.67; 95% CI 2.25, 11.09 points; Mn^2: total correct scaled score difference = -1.64 95% CI: -2.69, -0.59 points;) (Supplemental Table 4.9). We did not find evidence of adverse associations of MeHg or As with cognitive flexibility. We did observe a statistically significant adverse association between ΣPCB_4 and Color-Word Interference that was stronger than what we had observed in the main analyses (completion time scaled score difference: -0.51; 95% CI: -0.96, -0.51 points), however it became null when we accounted for potential selection bias using IPW (Supplemental Table 4.10).

Discussion

In this study, we examine the hypothesized association of prenatal exposure to a five-chemical mixture composed of DDE, HCB, ΣPCB_4, Pb, and Mn with cognitive flexibility among adolescents. Our analyses provided suggestive evidence that after adjusting for other neurotoxic exposures and covariates, cord blood Mn levels were adversely associated with cognitive flexibility among adolescents in the NBC. The completion time scaled scores for Trail-making and Color-Word Interference tasks were most impacted, though we observed a pattern of adverse associations between Mn and all cognitive flexibility scaled scores (Table 4.2). Results were

largely unchanged when we used IPW to account for potential selection bias due to loss to follow-up with the exception of the association between Mn and Color-Word Interference, which was attenuated (Supplemental Table 4.1). In addition, Mn was associated with an increased risk of committing Trail-making errors (Table 4.5) and an increased odds of being the poor performance group for both Trail-making and Color-Word Interference (Table 4.6), the two outcomes that accounted for both speed and accuracy. Although more imprecise due to a smaller sample size, the potential adverse association between Mn and cognitive flexibility was still evident in the secondary analyses which included MeHg and As as part of the chemical mixture.

Mn is an essential trace element that has been found to be neurotoxic at higher levels.[24] As there is no industrial source of Mn exposure located near the NBC study communities, participants were likely exposed to Mn via multiple sources including diet, the most common source of Mn in the general population.[82] Although this is the first prospective study to analyze the association between prenatal Mn exposure and cognitive flexibility specifically, there have been other studies that have explored the relation of prenatal Mn with other executive functions. Within the NBC study, we previously found evidence of adverse associations of cord blood Mn with inhibition on a verbal task measured by the D-KEFS Color-Word Interference inhibition condition[96] and working memory measured by the Wide Range Assessment of Memory and Learning, 2nd Edition (WRAML2) verbal and symbolic working memory tasks.[99] In addition, a small exploratory study found that tooth Mn concentrations representative of prenatal exposure were associated with decrements in behavioral inhibition as measured by a Forbidden Toy Task, CPT errors of commission, and Stroop Test among young children.[38] Finally, tooth Mn concentrations reflecting prenatal exposures were associated with decrements in working memory among adolescent girls, but not boys, living near ferro-manganese industries in Italy.[29]

We did not observe consistent patterns of adverse associations between the other individual chemicals (DDE, HCB, ΣPCB_4, Pb) and cognitive flexibility outcomes in the standard linear regression, logistic regression, or negative binomial regression analyses (Tables 4.2, 4.5, and 4.6). This may be due to lower exposure levels among NBC participants than those seen in other studies[56] or a limited sample size. In the case of the organochlorines that were assessed in this study, this may be due to negative confounding by diet.[84] In this study, a food frequency questionnaire was used to measure maternal seafood consumption during pregnancy, which may not fully capture dietary intake of seafood resulting in some residual confounding by diet especially for findings related to common seafood contaminants such as MeHg and PCBs.

Using BKMR we noted adverse joint associations of the five-chemical mixture with Trail-making completion time, Verbal Fluency switching accuracy, and Color-Word completion time scaled scores (Figure 4.3). These joint associations were still present but somewhat attenuated when MeHg and As were included in the chemical mixture (Supplemental Figure 4.3). This provides suggestive evidence that there may be an adverse relation between simultaneous exposure to these multiple chemicals and cognitive flexibility that is not apparent when assessing the impact of each exposure in multiple exposure parametric regression models. This combination of exposures has not been studied previously in relation to cognitive outcomes outside of the NBC. However, within the NBC, we previously observed adverse joint associations between these five chemicals and psychometric tests of inhibition and working memory.[96,99]

We did not observe an adverse joint association of the chemical mixtures with Design Fluency total correct scaled scores (Figure 4.3, Supplemental Figure 4.3). In addition, exploratory BKMR analyses indicated potential non-linear associations of HCB with Design Fluency in the main analyses (Figure 4.1) and of Mn with Design Fluency in the secondary analyses (Supplemental

Figure 4.1). It is unclear why Design Fluency associations appeared distinct from those observed for the other measures of cognitive flexibility, though it is possible that as the nature of this task may more open-ended than the others, it may reflect a different aspect of cognitive flexibility than the other tasks.

When we assessed effect modification by sex, we found evidence of a significant interaction of HCB and HCB^2 with sex in their relation with Design Fluency total correct scaled scores (p for interaction=0.02, 0.04 respectively) (Figure 4.4; Supplemental Table 4.2). However, evidence of an HCB-sex interaction was not found in the only prior study of HCB and executive function.[35] Whether age at assessment (age four compared to adolescents) or other factors may be important to potential sexual dimorphic associations of HCB with executive function is uncertain and should be explored further. We did not observe evidence of sexual dimorphism in associations between the remaining chemicals and cognitive flexibility outcomes.

In PNSDI-stratified analyses, we observed stronger adverse associations between the organochlorine pesticides (DDE and HCB) and cognitive flexibility outcomes among those with more prenatal social disadvantage (Figure 4.5; Supplemental Table 4.3). Unexpectedly, we observed positive associations between ΣPCB_4 and cognitive flexibility outcomes among participants with greater prenatal social disadvantage as compared to those with lesser disadvantage where effect estimates tended to be adverse. However, all of these unexpected associations were very imprecise and therefore limit possible inferences. We did not observe significant interactions between PNSDI and metals, however the differences in organochlorine-related impacts between the low and high-PNSDI groups underscore the importance of examining effect modification by socio-demographic stressors.

This study had some limitations including loss to follow-up which may result in selection bias, limited data on MeHg and As exposures, potential residual confounding by diet, multiple comparisons, and issues regarding Mn measurement in cord blood. To account for loss to follow-up, we used IPW for censoring and found that, in most cases, results were very similar to the complete-case results, meaning selection bias was unlikely to have impacted study findings. Because MeHg and As samples were collected ten days post-partum at a home visit, rather than in the hospital at the child's birth, we were not able to capture exposure among many participants resulting in potential loss of power to observe effects of these exposures. Therefore, the relation between prenatal exposure to MeHg and As should be explored further in larger samples. As previously mentioned, although we were able to account for maternal seafood consumption during pregnancy, this information was obtained with a food frequency questionnaire which may not fully capture diet. This could result in residual negative confounding by seafood consumption and an underestimation of the adverse association between certain chemicals such as the organochlorines and cognitive flexibility. In addition, the possibility of observing some spurious associations cannot be excluded as we did not adjust for multiple comparisons. However, adjusting for multiple comparisons may increase the frequency of type II errors or failing to observe a true association and was therefore not done in this study.[87]A Finally, there were two limitations in using cord blood Mn as a biomarker of Mn exposure. First, there is not consensus about which biological matrix is the most valid biomarker of prenatal Mn exposure but there is evidence that cord blood Mn correlates well with third trimester tooth Mn.[89–91] Second, Mn concentrations were detected in cord blood with ICP-MS which detects ions based on their mass to charge ratio.[88] An Mn isotope has a mass that is similar to two isotopes of iron which may result in iron contributing to the Mn signal among those with high iron levels.[88] However, although Mn levels were potentially inflated,

relative Mn concentrations were unaffected, according to the laboratory in which these analyses took place.

This study also had important strengths including a socio-demographically diverse study population, a prospective study design, several biomarkers of prenatal exposure to organochlorines and metals, comprehensive psychometric measures of cognitive flexibility in adolescence (an age group for whom manifestations of adverse executive function development may be most evident), and detailed sociodemographic, dietary, and lifestyle information. These factors allowed us to conduct an in-depth investigation of the association of prenatal exposure to a chemical mixture composed of neurotoxic organochlorines and metals with cognitive flexibility among adolescents while adjusting for important potential confounders and exploring effect modifiers, including indicators of social disadvantage. Using parametric regression analyses, we found suggestive evidence of an adverse impact of prenatal Mn on adolescent cognitive flexibility, while accounting for other chemicals and covariates. Using BKMR, we also observed an adverse joint association between the full chemical mixture on multiple measures of cognitive flexibility. Finally, we observed stronger adverse associations between prenatal exposures to organochlorine pesticides and cognitive flexibility outcomes among participants with more prenatal social disadvantage, highlighting the potential for exposure to sociodemographic stressors to alter susceptibility to adverse neurodevelopmental impacts of prenatal chemical exposures. The study findings contribute to the limited literature assessing the relation of chemical mixtures and executive function among adolescents, while underscoring the importance of analyzing the impact of multiple exposures simultaneously and focusing on socially disadvantaged populations .

CHAPTER 5:

Building blocks of executive function as mediators of the association of prenatal manganese with problem-solving skills.

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Abstract

Background: Problem-solving skills build upon three core executive functions: inhibition, working memory, and cognitive flexibility. There is evidence of adverse associations of prenatal exposure to manganese (Mn) with core executive functions, but less is known about Mn associations with problem-solving. This study aimed to investigate the association of prenatal Mn exposure with problem-solving and to identify potential neuropsychological mechanisms through which this association may be mediated.

Methods: Study participants were 379 adolescents from the New Bedford Cohort (NBC) who have undergone periodic evaluations since their birth (1993-1998) to mothers residing near a Massachusetts Superfund site. We investigated the association of cord blood Mn with problem-solving measured by the Delis-Kaplan Executive Function System (D-KEFS) Sorting and Tower subtests [scores scaled to a mean (SD) of 10 (3)] using multivariable linear regression. Inhibition and cognitive flexibility were also measured by the D-KEFS; working memory was measured with the Wide Range Assessment of Memory and Learning, 2nd edition. Regression-based causal mediation was used to assess the proportion of the Mn-problem-solving association mediated by inhibition, working memory, and cognitive flexibility.

Results: NBC adolescents (age 13-17 years) were diverse with 29% non-white and 31% in a low-income household at birth. Their cord blood Mn concentrations were similar to other general population samples. Mn was associated with Sorting but not Tower scores. Specifically, a doubling

of cord blood Mn concentrations was associated with -0.66 points lower (95% CI: -1.26, -0.06) Sort Recognition score. In mediation analyses, inhibition, working memory, and cognitive flexibility combined mediated 44% of the total effect of Mn on Sorting. When analyzed individually, working memory mediated a larger proportion (37%) of the effect than inhibition (14%) or cognitive flexibility (23%).

Conclusion: We observed adverse associations of cord blood Mn with problem-solving among adolescents. Working memory was an important mediator of this association.

Introduction

Executive functions are mental processes involved in goal-directed behavior.[100] Problem-solving is a higher-order executive function essential to social development, school performance, and other everyday functions.[1,3,101,102] Higher-order executive functions build upon the three core executive functions: inhibition, working memory, and cognitive flexibility.[2] Inhibition is the ability to resist impulse and focus one's attention, working memory is the ability to mentally manipulate information in one's mind, and cognitive flexibility is the ability to shift perspectives and switch flexibly between tasks.[1] As the three core executive functions are employed in problem-solving, decrements in these core skills may in turn lead to lower problem-solving capabilities.[3] The developmental trajectories of the three core executive functions differ: for example, inhibition skills largely develop in early childhood with smaller improvements through adolescence, while working memory and cognitive flexibility have more linear trajectories from early childhood through adolescence.[100] Because of these distinct developmental trajectories, children draw on different skills at different ages to produce goal-oriented behavior such as completing problem-solving tasks.[3] Adolescence is a critical time for development in the prefrontal cortex, one of the

brain regions directly involved in higher-order executive functions.[4] Specifically, synapse proliferation and pruning in the prefrontal cortex, as well as myelination in multiple brain regions that occur leading up to and throughout adolescence likely play a role in executive function development.[4] Due to the extensive development of higher-order executive functions that occur during adolescence, it is an optimal time to differentiate between executive function skills and to identify potential decrements.[3]

As the developing brain undergoes rapid neurological growth in utero, it is highly sensitive to potential damage from neurotoxic chemicals that may result in long-term impacts, including those that affect executive function. Manganese (Mn) is an essential trace metal necessary for proper brain functioning and therefore actively transferred across the placenta[103] and both deficient and excess Mn exposure have been associated with neurodevelopmental toxicity.[23,24] There are no studies of the relation of prenatal exposure to Mn with higher-level executive functions such as problem-solving, however, there is some evidence that prenatal exposure to manganese (Mn) may be associated with decrements in core executive function skills from early childhood through adolescence.[29,38,96,99,104] Cross-sectional studies have also provided evidence of adverse associations between blood and hair Mn levels and psychometric tests of executive function in mid-childhood, though temporality cannot be established in such studies.[46,47] In the study population assessed in the current analysis, the New Bedford Cohort (NBC), we have previously found adverse associations of cord blood Mn concentrations with all three core executive functions: inhibition, working memory, and cognitive flexibility among adolescents.[96,99,104] The present analysis builds on our previous work by assessing the relation of Mn with problem-solving skills and conducting a mediation analysis to elucidate the neuropsychological mechanisms through which cord blood Mn may impact these higher-level skills. Given previous findings of

sexual dimorphism in Mn-related associations with executive functions and other cognitive skills,[29,105,106] we also conducted analyses stratified by sex.

In summary, problem-solving is an essential skill that may be impacted by prenatal exposure to neurotoxic chemicals such as Mn, yet no studies have investigated this association in adolescence, a time of substantial executive function development. Inhibition, working memory, and cognitive flexibility are employed when solving complex problems and have been found to be associated with prenatal Mn exposure, therefore these neuropsychological functions may act as potential mediators of the association of prenatal Mn exposure with problem-solving skills. Our target study population has undergone extensive standardized psychometric testing of multiple facets of executive function, including problem solving skills, which few, if any, prior studies have assessed in adolescents. We used this unique resource to first assess associations of Mn with adolescents' problem-solving skills and then to explore potential neurodevelopmental pathways through which prenatal exposure to Mn may impact those skills.

Methods

Study population. The New Bedford Cohort (NBC) is a longitudinal birth cohort study established to investigate the effects of chemical exposures on the health and development of children living near the New Bedford Harbor in Massachusetts. The New Bedford Harbor was contaminated with polychlorinated biphenyls (PCBs) and metals by local industrial emissions and designated a Superfund site in 1982.[60] Between 1993 and 1998, 788 mother-infant pairs were recruited shortly after the infant's birth at St. Luke's Hospital in New Bedford, Massachusetts. Enrollment eligibility criteria for mothers included age ≥ 18 years, speaking English or Portuguese, and living in one of the four towns surrounding the New Bedford Harbor for at least the duration of the

pregnancy. Infants born via cesarean section or too ill to undergo neonatal examination were excluded from the study. Between 2008 and 2014, 528 study participants completed the adolescent (~15-year) follow-up assessment which included detailed psychometric testing of executive function. This analysis focuses on the 379 adolescents who had complete data on all executive function outcomes, biomarkers of prenatal Mn exposure, and covariates of interest.

Exposure assessment. Mn concentrations were measured in cord whole blood samples which were collected at birth and refrigerated prior to analyses at the Harvard T.H. Chan School of Public Health Trace Metals Analysis Laboratory (Boston, MA). Blood Mn was measured using external calibration on a dynamic reaction cell-inductively coupled plasma-mass spectrometer (DRC-ICP-MS, Elan 6100, Perkin Elmer, Norwalk, CT) and concentrations reported as the mean of five replicate measurements. The limit of detection was 0.02 μg/dL. Quality control (QC) monitoring included procedural blanks, duplicates, spiked samples, and standard reference material (NIST SRM 955b Pb in blood; NIST SRM 1643d trace elements in water); biological reference material (ICP03B-05 and ICP03B-02 multi-elements in human blood from INSPQ/ Laboratoire de Toxicologie, Quebec); and certified reference material (GBW 09101 human hair, Shanghai Institute of Nuclear Research, Academia Sinica, China). Recovery rates for QC and spiked samples were 90-110% and precision >95%.

Executive function assessment. At the 15-year follow-up visit, a trained study examiner administered the Delis-Kaplan Executive Function System (D-KEFS)[49] including the Trail-making, Verbal Fluency, Design Fluency, Color-Word Interference, Sorting, and Tower subtests and the Wide Range Assessment of Memory and Learning, 2nd (WRAML2)[50] including the Verbal and Symbolic Working Memory subtests. Performance on each subtest was measured using scaled

scores. For all D-KEFS and WRAML2 subtests, age-standardized scaled scores with mean of 10 and standard deviation of 3 are based on each instrument's standardization population sample.

Inhibition was measured using two of the D-KEFS subtests: Design Fluency: Empty Dots Only condition and Color-Word Interference: Inhibition condition. In Design Fluency: Empty Dots Only, the examinee had 60 seconds to create as many different designs as possible by connecting only empty dots in response boxes that contained both filled and empty dots. Performance was measured with the total correct scaled score. In Color-Word Interference: Inhibition, the examinee named the font color of words denoting colors, rather than the words that were printed. For example, if the word '*red'* was printed in green font, the examinee was to say the word '*green',* rather than the word '*red*'. Performance was measured by the completion time scaled score.

Working memory was measured with the WRAML2 Verbal Working Memory and Symbolic Working Memory subtests. In Verbal Working Memory, participants were read a list of animal and non-animal words and asked to recall the animal words in size order followed by non-animal words in any order. Next, they were asked to recall both the animal words and non-animal words both in size order. In Symbolic Working Memory, participants were read a series of numbers then asked to identify the listed numbers in correct numerical order on a Number Stimulus Card. Next, participants were read a series of numbers and letters and asked to identify the numbers in numerical order and the letters in alphabetical order on a Number-Alphabet Stimulus Card. Performance on the working memory subtests was measured using total correct scaled scores.

Cognitive flexibility was measured using four subtests of the D-KEFS including Trail-making: Number-Letter Switching condition, Verbal Fluency: Category Switching condition, Design Fluency: Filled Dots and Empty Dots Switching condition, and Color-Word Interference: Inhibition/Switching condition. In Trail-making: Number-Letter Switching, participants were

asked to connect numbers then letters switching in correct alphabetical and numeric order (e.g., from A to 1 to B to 2). Performance was measured with the completion time scaled score. In Verbal Fluency: Category Switching, participants alternated between saying words from two different categories (e.g., fruit and furniture) as quickly as possible for 60 seconds. Performance was measured with the switching accuracy scaled score. In Design Fluency: Filled Dots and Empty Dots Switching, participants drew designs by alternating connecting filled and empty dots. Performance was measured using the total correct scaled score. In Color-Word Interference: Inhibition/Switching, participants switched between reading words denoting colors and naming the discordant font color in which a color word is printed. Performance was measured using the completion time scaled score.

Finally, problem-solving was measured using three subtests of the D-KEFS including Sorting: Free Sorting, Sorting: Sort Recognition, and Tower. In Sorting: Free Sorting, participants sorted 6 mixed up cards into two groups with three cards per group according to as many rules as possible. For example, sorting rules should reflect common features such as grouping cards by color (blue versus yellow) or by the nature of the images they contain (animals versus vehicles). In Sorting: Sort Recognition, the examiner sorted the same set of cards into two groups and asked the participant to identify the rule that the examiner used to sort. For both Sorting subtests, performance was measured with the description scaled score. In Tower, participants moved one disc at a time across three pegs to build pictured towers composed of stacked discs in the fewest number of moves possible. Performance was measured with the total achievement scaled score.

Covariate assessment. Hospital medical records from the mothers' labor and delivery and participants' birth were reviewed to obtain each infant's race/ethnicity, birthweight, gestational

age, and newborn exam as well as the mother's pregnancy and delivery course. Approximately, ten days later, at a study home visit, mothers completed a questionnaire regarding maternal socio-demographics, medical history, diet, smoking, alcohol, and drug use. Pediatric medical records were reviewed periodically and parental and child self-reported questionnaires were administered at study follow-up visits, including at age 15 years, to update demographic and health information. The follow-up also included home visits to assess the quality of the child's home environment and the child-parent relationship using the Home Observation for Measurement of the Environment (HOME)[68] and assessment of maternal IQ using the Kaufman Brief Intelligence Test (KBIT).[69]

Statistical analysis. Our analysis included assessment of the potential for the building blocks of higher order executive function to mediate any associations of Mn with problem-solving skills. Having previously demonstrated associations of Mn with these building blocks (inhibition, working memory and cognitive flexibility),[96,99,104] we began by assessing the hypothesized direct association of Mn with problem-solving. We then assessed the role of inhibition, working memory, and cognitive flexibility as mediators of the hypothesized association of Mn with problem-solving.

For all analyses, Mn exposures were $\log_2$-transformed to reduce the influence of extreme values, therefore associations are based on a two-fold increase in exposure concentrations. Covariates were selected using Directed Acyclic Graphs (DAGs) based on a literature review regarding potential confounders of the relationship of Mn exposure with problem-solving (exposure-outcome confounders), confounders of the relationship of Mn exposure with inhibition, working memory, and cognitive flexibility (exposure-mediator confounders), and confounders of the relationship of inhibition, working memory, and cognitive flexibility with problem-solving (mediator-outcome confounders). All models were adjusted for adolescent race/ethnicity, sex, age

at exam, year of birth, and 15-year HOME score; test examiner; maternal IQ, maternal pregnancy seafood consumption and smoking; and family characteristics at the child's birth (maternal marital status, parental education, household income). T-tests and chi-square tests were used to compare characteristics of participants who were included in the analyses to those who were excluded. Regression diagnostics confirmed the appropriateness of fitting multivariable linear regression models using Ordinary Least Squares (OLS) to estimate the association between Mn exposure and problem-solving outcomes.

To assess the extent to which the building blocks of executive function might mediate the association between cord blood Mn and problem-solving, we first selected which subtests would represent the mediators and outcomes of interest. We based these decisions in part on a literature review of the association between Mn exposure and cognitive outcomes, which supported focusing on verbal skills,[78–80,96,99,104] as well as the strength of the associations of Mn exposure with the potential mediators, and of the potential mediators with the problem-solving outcomes. As the requirements that need to be satisfied for a variable to be considered a potential mediator are a significant association between the exposure and the mediator and a significant association between the mediator and the outcome,[107] any inhibition, working memory, or cognitive flexibility subtests that did not fit these criteria were not considered potential mediators.

We used a regression-based causal mediation approach[108] to explore four mediating neuropsychological pathways for the association of Mn with problem-solving: inhibition, working memory, cognitive flexibility, and joint mediation by inhibition, working memory, and cognitive flexibility. For all mediation analyses we assumed no unmeasured exposure-outcome confounders, mediator-outcome confounders, or exposure-mediator confounders as well as no mediator-outcome confounders affected by the exposure given the set of covariates included in the model.[107]

We also formally tested for and found no evidence of exposure-mediator interactions in multivariable linear regression models; therefore, all mediation analyses assumed no exposure-mediator interaction. We estimated the total effect of cord blood Mn on problem-solving skills, the natural indirect effect through all three mediators jointly and through each mediator independently, and the natural direct effect of prenatal Mn on problem-solving skills through any pathway outside of the mediator(s). In this analysis, we have continuous exposure, mediators, and outcome, therefore, the causal effects are estimated on the difference scale. For a continuous outcome, the natural indirect effect (NIE) would generally be interpreted as the difference in mean problem-solving scaled scores that would result if Mn exposure was fixed to a level $x+1$, but where the mediator takes on the level that it would naturally have under exposure level $x+1$ compared to the level it would have naturally have under exposure level x.[109] However, because we $\log_2$-transformed our exposure, the NIE can be interpreted as the difference in mean problem-solving scaled scores that would result if Mn exposure was fixed to a level $2x$, but where the mediator takes on the level it would naturally have under exposure level $2x$ compared to the level it would naturally have under exposure level x. Meanwhile, the natural direct effect (NDE) is the difference in mean problem-solving scaled scores that would result if we compared Mn exposure at level x to level $2x$, while the mediator takes on the level it would naturally take if the Mn exposure level was x. Therefore, in causal mediation analyses, the NIE is the amount of the total effect of prenatal Mn on problem-solving explained by inhibition, working memory, and cognitive flexibility (individually or jointly) and the NDE is the amount of the total effect that is not explained by the mediator, but rather through other pathways. The total effect is the sum of the NIE and NDE and the proportion mediated is the indirect effect divided by the total effect. To assess sexual dimorphism in Mn-related effects on executive function, we conducted a secondary mediation

analysis stratified by sex. Standard errors and confidence intervals were calculated using the bootstrap method with 1000 simulations. All analyses were conducted using R version 3.6.0[71] and all mediation analyses were conducted using the *CMAverse* package.[110]

We also conducted a set of sensitivity analyses to assess the extent to which an unmeasured confounder would have to affect both the mediator and the outcome to fully explain away the NIE and the NDE. To do this, we calculated and reported the e-value.[111] The e-value is calculated on the risk ratio scale and defined as the minimum strength of the association that an unmeasured mediator-outcome confounder would have to have with both the mediator and the outcome to invalidate the NIE and the NDI.[111] To directly compare the e-value to the results of the mediation analyses, we converted the mediation results to the risk ratio scale using the method described by VanderWeele and Ding.[111]

Results

Table 5.1 compares the executive function, Mn exposure biomarker concentrations, and covariate measures of NBC adolescents who were included in the analyses and those who were excluded. Those who were included had complete data on inhibition, working memory, cognitive flexibility, and problem-solving outcomes, cord blood Mn concentrations, and covariates (n=379). Overall, included NBC study participants were socio-demographically diverse: 29% were non-white, 51% had a mother with less than or equal to a high school education, 66% had a father with less than or equal to a high school education, and 31% were born into households with an annual income of < $20,000 (Table 5.1). Compared to those excluded, those included in the analysis had higher scores on the WRAML2 Verbal and Symbolic Working Memory subtests and the D-KEFS Trail-making and Sorting subtests. They also tended to be younger and have a higher HOME score

than excluded participants. Parents of included adolescents were more likely to be married, have more than a high school education, and an annual household income ≥$20,000. Cord blood Mn concentrations did not vary by inclusion or exclusion in the analysis and, in the NBC study participants, were similar to levels observed in other cohort studies in the United States and Canada.[75,112] All executive function outcomes were positively correlated with one another (Figure 5.1). Inhibition subtests had a Spearman correlation coefficient of 0.2, working memory subtests had a Spearman correlation coefficient of 0.6, cognitive flexibility subtests had Spearman correlation coefficients ranging from 0.2 to 0.4, and problem-solving subtests had Spearman correlation coefficients ranging from 0.2 to 0.6.

Table 5.1 Characteristics of all New Bedford Cohort (NBC) participants included in the analysis[1] and those excluded.

Descriptive Characteristic	Analysis group, n=379		Excluded group, n=409		
	n(%)	Mean (SD)	n(%)	Mean (SD)	p-value[2]
Mediator/Outcome Measures[4]					
D-KEFS[3] Inhibition scaled scores					
Design Fluency: Empty Dots Only total correct	379	9.6 (2.7)	149	9.2 (3.0)	0.2
Color-Word Interference: Inhibition completion time		9.9 (2.9)	149	9.8 (2.7)	0.7
WRAML2[4] Working Memory scaled scores					
Verbal Working Memory	379	9.0 (2.7)	149	8.3 (2.9)	0.007*
Symbolic Working Memory	379	9.8 (2.7)	149	9.2 (2.6)	0.01*
D-KEFS[3] Cognitive Flexibility scaled scores					
Trail-making: Number-Letter Switching completion time	379	9.6 (2.7)	149	9.1 (2.8)	0.07
Verbal Fluency: Category Switching total switching accuracy	379	9.2 (2.8)	149	8.9 (2.8)	0.2
Design Fluency: Filled & Empty Dots Switching total correct	379	9.9 (2.8)	149	9.6 (2.6)	0.4
Color-Word Interference: Inhibition/Switching completion time	379	9.9 (2.6)	149	9.9 (2.6)	1.0
D-KEFS[3] Problem-solving scaled scores					
Sorting: Free description	379	9.1 (2.4)	149	8.2 (2.7)	<0.001*
Sorting: Sort Recognition description	379	7.1 (3.1)	149	6.5 (3.0)	0.04
Tower: total achievement	379	9.7 (2.4)	149	9.8 (2.1)	0.6
Exposure Measures[5]					
Cord blood manganese (μg/dL)	379	4.2 (1.6)	329	4.3 (2.0)	0.7
Covariate Measures[6]					
Child Characteristics					
Race/Ethnicity					0.06
Non-Hispanic White	268 (70.7)		263 (64.3)		
Hispanic	33 (8.7)		56 (13.7)		
Other	78 (20.6)		88 (21.5)		
Missing	0		2 (0.5)		
Sex					0.04*
Male	181 (47.8)		227 (55.5)		
Female	198 (52.2)		182 (44.5)		
Age at Exam	379	15.5 (0.6)	149	15.7 (0.7)	0.4
Home Score	379	43.9 (6.3)	149	42.7 (6.0)	0.07
Year of Birth					0.008*
1993-1994	104 (27.4)		155 (37.9)		

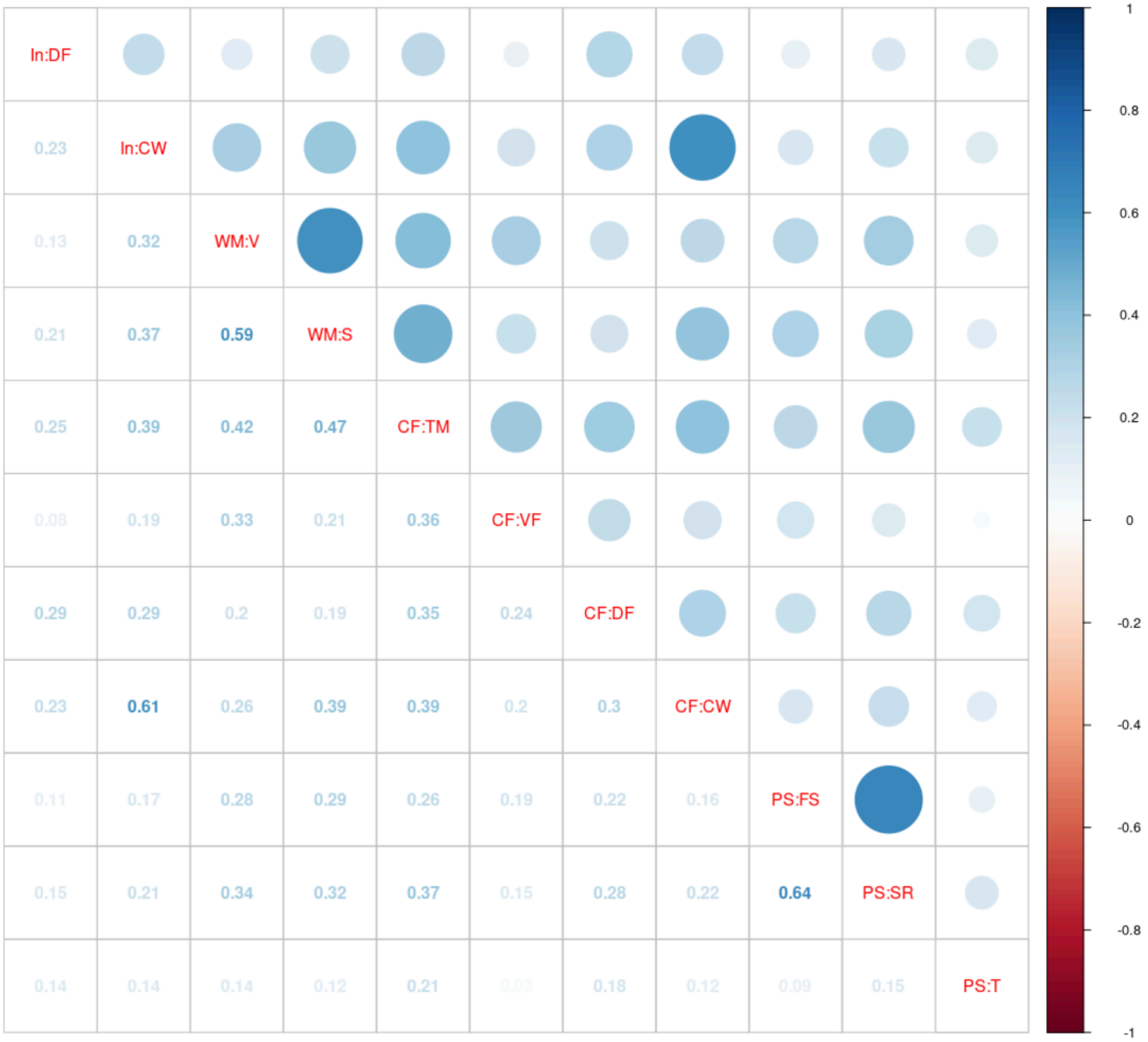
Table 5.1 (Continued)

1995-1996	155 (40.9)		145 (35.5)		
1997-1998	120 (31.7)		109 (26.7)		
Maternal Characteristics					
Marital status at birth					<0.001*
Not married	139 (36.7)		192 (46.9)		
Married	240 (63.3)		162 (39.6)		
Missing	0		55 (13.4)		
Maternal IQ	379	99.3 (10.4)	262	95.8 (10.2)	<0.001*
Seafood during pregnancy (serv/day)	379	0.5 (0.6)	260	0.6 (0.7)	0.8
Smoking during pregnancy					0.1
No	276 (72.8)		206 (50.4)		
Yes	103 (27.2)		101 (24.7)		
Missing	0		102 (24.9)		
Maternal education at birth					<0.001*
≤ High School	194 (51.2)		227 (55.5)		
> High School	185 (48.8)		125 (30.6)		
Missing	0		57 (13.9)		
Household Characteristics at Birth					
Paternal Education					0.002*
≤ High School	250 (66.0)		262 (64.1)		
> High School	129 (34.0)		79 (19.3)		
Missing	0		68 (16.6)		
Annual Household Income					0.001*
< $20,000	117 (30.9)		148 (36.2)		
≥ $20,000	262 (69.1)		197 (48.2)		
Missing	0		64 (15.6)		
Examination Characteristics					
Examiner					0.6
1	283 (74.7)		115 (77.2)		
2	96 (25.3)		34 (22.8)		

[1]Those included in the analysis had complete inhibition, working memory, cognitive flexibility, & problem-solving outcome, covariate, and exposure data for cord blood manganese, n=379.[2]P-values represent results comparing characteristics between included and excluded participants using t-tests and chi-square tests. P-values reflect comparisons between non-missing data between groups.[3]D-KEFS: Delis-Kaplan Executive Function System[49]; [4]WRAML2: Wide Range Assessment of Memory and Learning, 2nd Edition.[50] [4]NBC participants with missing mediator/outcome measures: n=260. [4]NBC participants with missing exposure measures: Mn n=80. [5]NBC participants with missing covariate measures: age at exam n=260; HOME score n= 297; maternal IQ n=153; seafood during pregnancy n= 155. *p<0.05.

Abbreviations: DDE: dichlorodiphenyldichloroethylene; HCB: hexachlorobenzene; ΣPCB_4: Sum of 4 PCB congeners (118, 138, 153, 180); Pb: lead; Mn: manganes

Figure 5.1 Spearman correlation coefficients between inhibition, working memory, cognitive flexibility, and problem-solving scaled scores measured with the Delis-Kaplan Executive Function System (D-KEFS) and Wide Range Assessment of Memory and Learning, 2nd Edition (WRAML2) in 379 New Bedford Cohort adolescents.



Abbreviations:
In:DF – Inhibition: D-KEFS Design Fluency: Empty Dots Only total correct scaled score
In:CW – Inhibition: D-KEFS Color-Word Interference: Inhibition completion time scaled score
WM:V – Working memory: WRAML2 Verbal Working Memory
WM:S – Working memory: WRAML2 Symbolic Working Memory
CF:TM – Cognitive flexibility: D-KEFS Trail-making: Number-Letter Switching completion time scaled score
CF:VF – Cognitive flexibility: D-KEFS Verbal Fluency Category Switching total switching accuracy scaled score
CF:DF – Cognitive flexibility: D-KEFS Design Fluency Filled & Empty Dots Switching total correct scaled score
CF:CW – Cognitive flexibility: D-KEFS Color-Word Interference: Inhibition/Switching completion time scaled score
PS: FS – Problem-solving: D-KEFS Sorting Free Sort description scaled score
PS: SR – Problem-solving: D-KEFS Sorting Sort Recognition description scaled score
PS: T – Problem-solving: D-KEFS Tower total achievement scaled score

Table 5.2 shows the results of covariate-adjusted linear regression analyses of the association of cord blood Mn concentrations with problem-solving scaled scores. A doubling of cord blood Mn concentrations was associated with lower scaled scores on both Sorting subtests (Free Sort difference = -0.34 points; 95% CI: -0.82, 015; Sort Recognition difference=-0.66 points; 95% CI: -1.26, -0.06), but Mn was not associated with Tower scores.

Table 5.2 Results of multivariable linear regression analyses of difference (95% confidence intervals)[1] in problem-solving scaled scores per doubling of cord blood manganese concentrations among 379 New Bedford Cohort adolescents.

Problem-solving Measure (scaled scores)	Difference (95% CI)
Sorting: Free Sort[2]	-0.34 (-0.82, 0.15)
Sorting: Sort Recognition[2]	-0.66 (-1.26, -0.06)**
Tower: Total Achievement[2]	0.10 (-0.41, 0.61)

[1]Manganese exposure has been log2-transformed and models have been adjusted for child race, sex, age at exam, HOME score; maternal marital status at child's birth, IQ, seafood consumption during pregnancy, smoking during pregnancy; maternal and paternal education and annual household income at child's birth; study examiner.
[2]Delis-Kaplan Executive Function System (D-KEFS) scaled score
**p<0.05*p<0.10

Table 5.3 shows the results of covariate-adjusted linear regression analyses of the association of cord blood Mn concentrations with the potential mediators: inhibition, working memory, and cognitive flexibility scaled scores. The associations between prenatal Mn and the core executive function measures generally trended negative. Specifically, for inhibition, a twofold increase in cord blood Mn concentrations was associated with lower scaled scores on Color-Word Interference (difference=-0.64 points; 95% CI: -1.22,-0.05), but not Design Fluency. Both Verbal and Symbolic Working Memory were negatively associated with cord blood Mn, though Verbal Working Memory appeared more sensitive (Verbal difference=-0.81 points; 95% CI: -1.34,-0.28; Symbolic difference=-0.43 points; 95% CI: -0.97, 0.12). All cognitive flexibility scaled scores were negatively associated with cord blood Mn with Trail-making: Number-Letter Switching and Color-Word Interference: Inhibition/Switching having the strongest associations (Trail-making difference=-0.49 points (95% CI: -1.03, 0.06); Color-Word Interference difference = -0.43 points (95% CI: -0.96, 0.11).

Table 5.3 Results of multivariable linear regression analyses of difference (95% confidence intervals)[1] in inhibition, working memory, and cognitive flexibility scaled scores per doubling of cord blood manganese concentrations among 379 New Bedford Cohort adolescents.

Executive Function Measure (scaled scores)	Difference (95% CI)
Inhibition	
Design Fluency: Empty Dots Only[2]	0.17 (-0.39, 0.73)
Color-Word Interference: Inhibition[2]	-0.64 (-1.22, -0.05)**
Working Memory	
Verbal Working Memory[3]	-0.81 (-1.34, -0.28)**
Symbolic Working Memory[3]	-0.43 (-0.97, 0.12)
Cognitive Flexibility	
Trail-making: Number-Letter Switching[2]	-0.49 (-1.03, 0.06)*
Verbal Fluency Category Switching[2]	-0.22 (-0.76, 0.33)
Design Fluency Filled & Empty Dots Switching[2]	-0.05 (-0.63, 0.53)
Color-Word Interference: Inhibition/Switching[2]	-0.43 (-0.96, 0.11)

[1]Manganese exposure has been log2-transformed and models have been adjusted for child race, sex, age at exam, HOME score; maternal marital status at child's birth, IQ, seafood consumption during pregnancy, smoking during pregnancy; maternal and paternal education and annual household income at child's birth; study examiner.
[2]Delis-Kaplan Executive Function System (D-KEFS) scaled score
[3]Wide Range Assessment of Memory and Learning, 2nd Edition (WRAML2) scaled score
**p<0.05*p<0.10

The results of covariate-adjusted linear regression analyses of the association of inhibition, working memory, and cognitive flexibility scaled scores with problem-solving scaled scores are in Table 5.4. Scaled scores for all core executive function measures were positively associated with problem-solving measures and, in most cases, these associations were statistically significant. Both Sorting subtest scaled scores were most strongly associated with the D-KEFS Trail-making: Number-Letter Switching and the WRAML2 Verbal and Symbolic Working Memory scaled scores. The Tower total achievement scaled scores were most strongly associated with the D-KEFS Trail-making: Number-Letter Switching and Design Fluency: Filled and Empty Dots Switching scaled scores.

Table 5.4 Results of multivariable linear regression analyses of difference (95% confidence interval)[1] in problem-solving scaled scores per one point increase in inhibition, working memory, and cognitive flexibility scaled scores among 379 New Bedford Cohort adolescents.

	Problem-solving Measure (scaled score)		
Executive Function Measure (scaled scores)	Sorting: Free Sort Description[2] Difference (95% CI)	Sorting: Sort Recognition Description[2] Difference (95% CI)	Tower: Total Achievement[2] Difference (95% CI)
Inhibition			
Design Fluency: Empty Dots Only total correct[2]	0.13 (0.05, 0.22)**	0.17 (0.06, 0.28)**	0.14 (0.04, 0.23)**
Color-Word Interference: Inhibition completion time[2]	0.12 (0.03, 0.20)**	0.15 (0.04, 0.25)**	0.12 (0.03, 0.21)**
Working Memory			
Verbal Working Memory[3]	0.24 (0.15, 0.33)**	0.29 (0.18, 0.40)**	0.11 (0.01, 0.21)**
Symbolic Working Memory[3]	0.26 (0.17, 0.35)**	0.27 (0.16, 0.38)**	0.09 (-0.01, 0.18)*
Cognitive Flexibility			
Trail-making: Number-Letter Switching completion time[2]	0.21 (0.12, 0.30)**	0.30 (0.19, 0.41)**	0.19 (0.10, 0.29)**
Verbal Fluency Category Switching total switching accuracy[2]	0.11 (0.02, 0.20)**	0.09 (-0.03, 0.20)	0.02 (-0.08, 0.11)
Design Fluency Filled & Empty Dots Switching total correct[2]	0.17 (0.09, 0.26)**	0.25 (0.14, 0.35)**	0.18 (0.09, 0.27)**
Color-Word Interference: Inhibition/Switching completion time[2]	0.11 (0.02, 0.20)**	0.17 (0.06, 0.29)**	0.13 (0.03, 0.23)**

[1]Models have been adjusted for child race, sex, age at exam, and HOME score; maternal marital status at child's birth, IQ, seafood consumption during pregnancy, and smoking during pregnancy; maternal and paternal education and annual household income at child's birth; and study examiner.
**p<0.05*p<0.10

Next, we conducted a mediation analysis using Sorting: Sort Recognition as our main outcome (problem-solving) and the following mediators: Color-Word Interference: Inhibition (inhibition), Verbal Working Memory (working memory), and Trail-making: Number-Letter Switching (cognitive flexibility) (Table 5.5). Sorting: Sort Recognition was selected as the outcome to represent problem-solving in the mediation analysis as it was more strongly associated with Mn than the other problem-solving outcomes. The mediators selected to represent the core executive functions were selected based on the strength of their association with both the exposure (Mn) and outcome (Sorting: Sort Recognition). Among all study adolescents included in the mediation analysis, the combination of inhibition, working memory, and cognitive flexibility mediated 44% of the total effect of prenatal Mn on problem-solving. The NIE through the pathway including all three mediators showed a significant decrease in problem-solving scaled scores per doubling of Mn (difference=-0.27 points; 95% CI: -0.49, -0.08), while the NDE showed a non-significant decrease (difference = -0.34 points; 95% CI: -0.89, 0.20). When we explored the indirect effects of the mediators one-at-a-time, working memory appeared to be the most important mediator of the association between prenatal Mn and problem-solving with an NIE of -0.23 points (95% CI: -0.40, -0.07) and an NDE of -0.39 points (95% CI: -0.97, 0.15) thereby mediating 37% of the total effect. By contrast, the pathways through inhibition or cognitive flexibility alone mediated 14% and 23% of the total effect, respectively.

Table 5.5 Results of analyses assessing potential mediation of the association between cord blood manganese and problem-solving skills by measures of inhibition, working memory and cognitive flexibility among 379 New Bedford Cohort adolescents[1]. Problem-solving is measured by the Sorting: Sort Recognition description scaled score of the Delis Kaplan Executive Function System.

Model	Mediator	Natural Indirect Effect (95% CI)[1]	Natural Direct Effect (95% CI)[1]	Total Effect[1] (95% CI)	Proportion Mediated
Model 1	Joint mediation by inhibition, working memory, and cognitive flexibility[2,3]	-0.27 (-0.49, -0.08)**	-0.34 (-0.89, 0.20)	-0.61 (-1.18, -0.02)**	0.44
Model 2	Inhibition:				
	Color-Word Interference: Inhibition[2]	-0.08 (-0.24, 0.002)*	-0.53 (-1.10, 0.04)*	-0.61 (-1.17, -0.02)**	0.14
Model 3	Working Memory:				
	Verbal Working Memory[3]	-0.23 (-0.40, -0.07)**	-0.39 (-0.97, 0.15)	-0.61 (-1.17, -0.02)**	0.37
Model 4	Cognitive Flexibility:				
	Trail-making: Number Letter Switching[2]	-0.14 (-0.35, 0.02)*	-0.47 (-1.01, 0.12)	-0.61 (-1.18, 0.01)*	0.23

[1]Manganese exposure has been log2-transformed and models have been adjusted for child race, sex, age at exam, HOME score; maternal marital status at child's birth, IQ, seafood consumption during pregnancy, smoking during pregnancy; maternal and paternal education and annual household income at child's birth; study examiner.
[2]Delis-Kaplan Executive Function System (D-KEFS) scaled score
[3]Wide Range Assessment of Memory and Learning, 2nd Edition (WRAML2) scaled score
**p<0.05*p<0.10

Next, we conducted a sensitivity analysis in which we calculated the e-values on the risk ratio scale to examine the strength of the association that a hypothetical unmeasured confounder would have to have with both the exposure and the outcome to invalidate the total effect and with the mediator(s) and the outcome to invalidate the indirect and direct effects. In Table 5.6, for interpretation purposes, we converted the results of Table 5.5 to the risk ratio scale using the method described by VanderWeele and Ding.[111] This conversion dichotomizes problem-solving scaled scores into "high" and "low" groups. Using this scale, the risk ratio for the total effect of 0.84 represents a 16% decreased risk of being in the "high" problem-solving group compared to the "low" problem-solving group. The e-values of the NIEs ranged from 0.73-0.84 and NDEs ranged from 0.62-0.69 (Table 5.6). For example, in Model 3 in which working memory was assessed as the mediator of the Mn-problem-solving association, the e-value of the natural indirect effect was 0.75, meaning that the observed risk ratio of the indirect effect of 0.94 could be explained away by an unmeasured mediator-outcome confounder that decreased the risk of "high" working memory scores by 1-0.75=0.25 or 25% and also decreased the risk of "high" problem-solving scores by 25%, but weaker confounding could not. Similar interpretations can be applied to the remaining e-values.

Table 5.6 Results of analyses assessing potential mediation of the association between cord blood manganese and problem-solving skills by measures of inhibition, working memory and cognitive flexibility among 379 New Bedford Cohort adolescents[1] on the risk ratio (RR) scale[2] with e-values[4] for the natural indirect and natural direct effects.

Model	Mediator	Natural Indirect Effect RR (95% CI)[2]	E-value for Natural Indirect Effect	Natural Direct Effect RR (95% CI)[2]	E-value for Natural Direct Effect	Total Effect[1] RR (95% CI)
Model 1	Joint mediation by inhibition, working memory, and cognitive flexibility[4,5]	0.92 (0.87, 0.98)	0.73	0.90 (0.77, 1.07)	0.69	0.84 (0.70, 0.99)
Model 2	Inhibition					
	Color-Word Interference: Inhibition[4]	0.98 (0.94, 1.01)	0.84	0.86 (0.72, 1.02)	0.62	0.84 (0.70, 0.99)
Model 3	Working Memory					
	Verbal Working Memory[5]	0.94 (0.89, 0.98)	0.75	0.89 (0.75, 1.06)	0.67	0.84 (0.70, 0.99)
Model 4	Cognitive Flexibility					
	Trail-making: Number Letter Switching[4]	0.96 (0.91, 1.01)	0.80	0.87 (0.74, 1.02)	0.64	0.84 (0.70, 0.99)

[1]Manganese exposure has been log2-transformed and models have been adjusted for child race, sex, age at exam, HOME score; maternal marital status at child's birth, IQ, seafood consumption during pregnancy, smoking during pregnancy; maternal and paternal education and annual household income at child's birth; study examiner.
[2]The natural indirect effect, natural direct effect, and total effect have been converted to the risk ratio scale using the method described by [111] for interpretation purposes as e-values are calculated on the risk ratio scale.
[3]E-value: minimum strength of association on the risk ratio scale that an unmeasured confounder would have to have with both the mediator and the outcome, given a set of covariates, to explain away the effect (VanderWeele and Ding 2017).
[4]Delis-Kaplan Executive Function System (D-KEFS) scaled score
[5]Wide Range Assessment of Memory and Learning, 2nd Edition (WRAML2) scaled score

In secondary analyses, we examined the association of cord blood Mn concentrations with measures of inhibition, working memory, cognitive flexibility, and problem-solving by first including a Mn-sex interaction term in each model followed by separate models for each sex. There were no statistically significant interactions between Mn and sex. The associations between prenatal Mn and the executive functions trended negative among both sexes with a few exceptions which were quite imprecise (Supplemental Figure 5.1; Supplemental Table 5.1). In sex-stratified mediation models, the total effect of a two-fold increase in prenatal Mn concentration was a 0.68-point reduction on Sort Recognition scaled scores in males, while among females, it was a 0.45-point reduction (Supplemental Table 5.2). The combination of inhibition, working memory, and cognitive flexibility mediated 36% of the total effect of prenatal Mn on problem-solving among males and 56% among females. When each building block of executive function was assessed as an individual mediator, we found that working memory mediated the highest proportion of the total effect among males (42%), while cognitive flexibility mediated the highest proportion of the total effect among females (48%) (Supplemental Table 5.2).

Discussion

This study is among the first to provide evidence of an adverse association between a biomarker of prenatal exposure to Mn and a measure of higher-level executive function, specifically problem-solving, among adolescents. We observed negative associations between cord blood Mn and both scaled scores of the D-KEFS Sorting subtest (Table 5.2). The Sort Recognition task appeared more sensitive to Mn exposure than the Free Sort task. In previous studies, prenatal exposure to Mn has been adversely associated with inhibition and working memory in early childhood and adolescence.[29,38] Within the NBC, we have previously found

adverse associations between cord blood Mn and psychometric tests of inhibition, working memory, and cognitive flexibility.[96,99,104] The current findings provide further evidence that prenatal exposures to Mn at levels similar to the general population in the United States and Canada[75,112] may have adverse impacts on higher-level executive function during adolescence. As there is not a well-established industrial source of Mn exposure located near the NBC study communities, participants may have been exposed via common sources of Mn such as diet or ingestion of Mn-contaminated water.[23,82] Of note, we did not find evidence of an adverse association between prenatal Mn exposure and the D-KEFS Tower subtest, a non-verbal problem-solving task. This is consistent with other studies, including those conducted within the NBC, that have found verbal tasks to be especially susceptible to Mn-related decrements.[79,96,97,99,104]

In subsequent mediation analyses, we found that 44% of the total association between Mn and problem-solving was mediated by a combination of inhibition, working memory, and cognitive flexibility (Table 5.5). We also noted a significant NIE through this particular joint mediator pathway and a non-significant NDE representing all other pathways from Mn to problem-solving. These results point to the potentially important role that the building blocks of executive function play in mediating the association between Mn and higher-level executive function skills. It is also supported by neuropsychiatric literature that has previously found these core skills to be employed in problem-solving tasks.[1,100] Given that the types of cognitive processes and skills necessary to complete inhibition, working memory, and cognitive flexibility tasks may overlap,[1] we conducted individual mediation analyses of each of the mediators of interest to better understand which pathway is most disrupted by prenatal Mn exposure. We found inhibition alone mediated 14% of the total effect, cognitive flexibility mediated 23% of the total effect, and working memory mediated 37% of the total effect. The NIE of working memory was the largest of the three measures

and the only individual mediator that reached statistical significance, pointing to the likely importance of working memory in mediating the association between prenatal Mn and problem-solving skills. These results are supported by previous findings that as children get older, they engage working memory and cognitive flexibility more than inhibition in solving complex problems.[3]

Although we did not find evidence of Mn-sex interactions, other studies have found evidence of sexual dimorphism in Mn-related associations with executive function and certain behavioral skills.[29,105,106] It is possible that we were underpowered to detect these interactions. In subsequent sex-stratified mediation analyses, the combination of inhibition, working memory, and cognitive flexibility mediated 36% and 56% of the total effect of prenatal Mn on problem-solving among males and females, respectively. Similar to the overall population, working memory mediated the highest proportion of the total effect among males. However, among females, cognitive flexibility mediated the highest proportion of the total effect. This is likely due to differences in the strength of the Mn-Trail-making: Number-Letter Switching associations that we observed in sex-stratified analyses (Supplemental Figure 5.1; Table 5.1). Specifically, we found that a doubling of prenatal Mn was negatively associated with Trail-making among females (difference= -0.73 points; 95% CI: -1.51, 0.04), but not males (difference= -0.02 points; 95% CI: -0.80, 0.76). Our results provide some evidence that the main pathways that are disrupted in the association between prenatal Mn and problem-solving may differ between males and females.

NBC study participants completed psychometric tests of multiple levels of executive function allowing us to investigate the association between cord blood Mn and problem-solving skills and to identify potential mediators on the pathway of this association. However, there were some limitations. As is common in cohort studies, some participants were lost to follow-up. In previous

analyses of this group of NBC adolescents, inverse probability weighting (IPW) had been applied and results assessing chemical associations with core executive functions (inhibition, working memory and cognitive flexibility) were similar to unweighted analyses confirming that retention bias was unlikely.[96,99,104] In the current analyses, we confirmed that IPW did not significantly alter the association of Mn with problem-solving skills (Supplemental Table 5.3) before concluding that an unweighted analysis was appropriate. Another limitation in this study is the use of cord blood Mn as a biomarker of Mn exposure. There is no consensus about which biological specimen is the most reliable biomarker of Mn exposure though there is evidence that cord blood Mn is a valid measure of fetal exposure and cord blood Mn concentrations are better-correlated with other biomarkers of prenatal Mn exposure than maternal biomarkers.[91] In addition, Mn in cord blood was measured using ICP-MS, a method which detects ions based on their mass to charge ratio.[88] One isotope of Mn has a similar mass to two isotopes of iron which may result in iron contributing to the Mn signal among participants with high iron levels.[88] The laboratory in which these analyses were conducted reported that, although there was some inflation of Mn concentrations, they did not impact the relative Mn levels of participants in this study.

Some limitations pertain specifically to the mediation analyses. First, we identified which measures of each executive function were to be used in this analysis by focusing on measures that had the strongest association with the exposure and outcome. This may result in an increase in Type 1 errors as well as potentially an incomplete description of important features of each executive function. However, only those mediators that were significantly associated with the exposure and the outcome could be considered to satisfy the requirements of a potential mediator.[107] In addition, in the case of inhibition and working memory, previous findings suggesting that verbal skills (and executive functions assessed with verbal tasks) may be more

sensitive to Mn exposure than non-verbal skills or tasks[78,79,96,99,104] supported the empiric choices that were made. In addition, reducing the frequency to type I errors may increase the frequency of type II errors and was therefore not done in this study.[87] Second, both the mediators and outcome assessed were measured at the same time, however it has been established that inhibition, working memory, and cognitive flexibility begin their development in early childhood and function as the building blocks of problem-solving skills throughout development,[1,3] thereby satisfying the temporal ordering requirements of a causal mediation analysis.[107] Finally, the data collected in this study were not originally intended to be used in a mediation analysis, therefore there is potential for unmeasured mediator-outcome confounding. To account for this, we conducted a sensitivity analysis in which we calculated e-values to examine the strength of the association that the unmeasured confounder would have to have with both the mediator(s) and the outcome to invalidate the results of our main mediation analysis. We found that the e-values for the NIEs ranged from 0.73-0.84 and the e-values for the NDEs ranged from 0.62-0.69, providing reassurance that an unmeasured confounder would have to have a strong association with both the mediator and the outcome to invalidate the results of the mediation analyses.

In conclusion, we found evidence of an adverse association of prenatal Mn exposure with a higher-level executive function, specifically problem-solving, among adolescents from a socio-demographically diverse prospective cohort study. The problem-solving tasks that included a verbal component were more sensitive to Mn exposures than problem-solving skills assessed with non-verbal tasks. In addition, we found that the combination of inhibition, working memory, and cognitive flexibility mediated over 40% of the total effect of Mn on problem-solving. In analyses of individual mediators, working memory appeared to mediate the largest proportion of the effect compared to inhibition and cognitive flexibility. This is the first study to not only estimate the

association between prenatal exposures to Mn and problem-solving skills among adolescents, but to also identify neuropsychological mechanisms that act as potential mediators of this association. To fully characterize this association, future studies should assess the potential impact of prenatal Mn on higher-level executive functions among other groups of adolescents. In addition, other potential pathways that may mediate this association should be explored.

CHAPTER 6:

Conclusion

The purpose of this dissertation was to identify modifiable environmental risk factors associated with decrements in executive functions, determine which executive function skills were most sensitive to chemical impacts, and to characterize how the complex relationships between executive functions may play a role in these associations. The findings from the first three chapters provided suggestive evidence that joint exposure to a prevalent chemical mixture was adversely associated with performance on verbal, but not non-verbal, inhibition, working memory, and cognitive flexibility tasks. In each of these studies, after accounting for multiple exposures and other covariates, Mn was more consistently adverse than the remaining chemicals in the mixture. We found little evidence of effect modification by sex, though we found some evidence of effect modification by social disadvantage, particularly in associations of organochlorines with working memory and cognitive flexibility. In a subsequent analysis focused on Mn and higher order-executive functions, we found adverse associations of Mn with certain problem-solving tasks. We also found that the three core executive functions, particularly working memory, are neuropsychological mechanisms that partially mediate the Mn-problem-solving association.

This dissertation had some limitations. First, as is common in prospective cohort studies, there was missing data resulting from a combination of loss to follow-up and missing covariate or exposure data among those adolescents who completed psychometric testing of executive function. In Chapters 1 through 3, we accounted for potential selection bias due to this missingness using inverse probability weighting (IPW) and found that, in most cases, results were similar to unweighted analyses. This provided evidence that bias due to missingness in our findings was minimal. We had limited power to assess associations with biomarkers of prenatal MeHg and As

exposure because of greater missingness for these exposures as compared to other chemicals. Limited power may have also impacted our ability to observe evidence of interactions between chemicals, as well as interactions with potential effect modifiers such as sex and social disadvantage. Because of co-exposure to beneficial nutrients and contaminants, seafood consumption may be an important confounder of the association of chemicals such as PCBs and MeHg with cognitive outcomes,[84] therefore we adjusted for maternal seafood consumption in models using a food frequency questionnaire (FFQ) to estimate intake. However, FFQs are prone to measurement error,[113] therefore using this instrument to account for the nutritional benefits of foods that are also sources of chemical exposure may not have been adequate and residual confounding could occur. In particular, residual negative confounding by diet may have resulted in underestimates of the impacts of PCBs and MeHg on executive functions. We did not adjust for multiple comparisons in this study as reducing the frequency of type I errors may come at the cost of increasing type II errors.[87] This may have, however, resulted in some spurious associations among our findings. Finally, there are two important limitations in the use of cord blood Mn as a biomarker of Mn exposure. First, it is not clear which biological matrix is the most valid biomarker of Mn exposure.[89,90] However, there is some evidence that cord blood Mn is a useful measure of fetal exposure and that it is better-correlated with third-trimester dentin Mn levels than maternal biomarkers,[89,91] thereby supporting the use of cord blood Mn in these analyses. Additionally, in this study, cord blood Mn concentrations were detected by ICP-MS, which uses the mass to charge ratio to detect ions.[88] One isotope of Mn has a similar mass to two isotopes of iron, which may result in overestimates of Mn concentrations in blood where iron levels are high.[88] However, quality assurance and quality control methods used by the laboratory in which these analyses were

conducted showed that inflation of Mn levels was similar across all observations, therefore relative Mn concentrations were unlikely to be affected in our study.

This dissertation also had a number of strengths. First, the New Bedford Cohort (NBC) has a prospective study design, which allowed us to establish temporality and minimize bias associated with other types of study designs. Participants in the NBC also had biomarkers of prenatal exposures to multiple neurotoxic organochlorines and metals that have been found to be associated with executive functions in previous studies. At the adolescent (15-year) follow-up, detailed psychometric tests of both core and higher-level executive functions were administered. Adolescence is the optimal time to not only measure distinct executive functions, but also to discern decrements. In addition, having collected data on multiple levels of executive function, we were able to test the hypothesis that the core executive functions may mediate the association of prenatal exposure to Mn and the higher order executive function of problem-solving. Finally, comprehensive health, sociodemographic, dietary, and lifestyle data was collected at birth and at follow-up visits allowing us to account for important confounders, predictors of the outcomes, and effect modifiers.

In Chapters 1 through 3, the main analysis involved standard parametric regression, while Bayesian kernel machine regression (BKMR) was used as an exploratory tool. The benefit of using both statistical approaches was that, while the standard method improved the interpretability of the results and allowed us to compare our findings with other studies, BKMR allowed us to explore non-linear associations, interactions, and the association of the overall mixture with executive functions while accounting for certain limitations of standard approaches such as multicollinearity and high dimensionality. In addition, BKMR confirmed the appropriateness of a traditional analysis in this study. In Chapter 4, we used a causal mediation approach, which had several

benefits over traditional mediation methods. Traditional mediations methods are limited in that they cannot accommodate exposure-mediator interactions and often ignore mediator-outcome confounding. Causal methods allowed for exposure-mediator interaction, though this was not present in our study. They also accommodated sensitivity analyses to ensure that findings do not change in case the stated assumptions are violated. These steps ensured the robustness of our mediation results.

Reproducibility is key to fully describing the contribution of prevalent prenatal environmental exposure mixtures on adolescent executive function development, therefore future studies should examine the associations analyzed in this dissertation in other adolescent populations. Larger studies with more power are also needed to assess interactions between chemicals as well as the potential modification of chemical risk by social disparities. In conclusion, this dissertation contributed to the literature implicating prenatal Mn exposure as a potential modifiable environmental risk factor that may adversely impact cognition. Our findings are among the first to find adverse associations of Mn with both core and higher-order executive functions, specifically in adolescence, a critical time period for executive function development. Besides Mn, there were no other consistently adverse associations of the remaining chemicals analyzed with executive functions, yet we still observed adverse joint associations of the chemical mixtures. This points to the importance of assessing multiple exposures simultaneously, rather than one-at-a-time. Finally, we found suggestive evidence of effect modification by social disadvantage in our analyses, particularly with organochlorine chemicals, supporting the need to consider social factors as potential sources of increased susceptibility to neurodevelopmental risks associated with environmental chemical exposures.

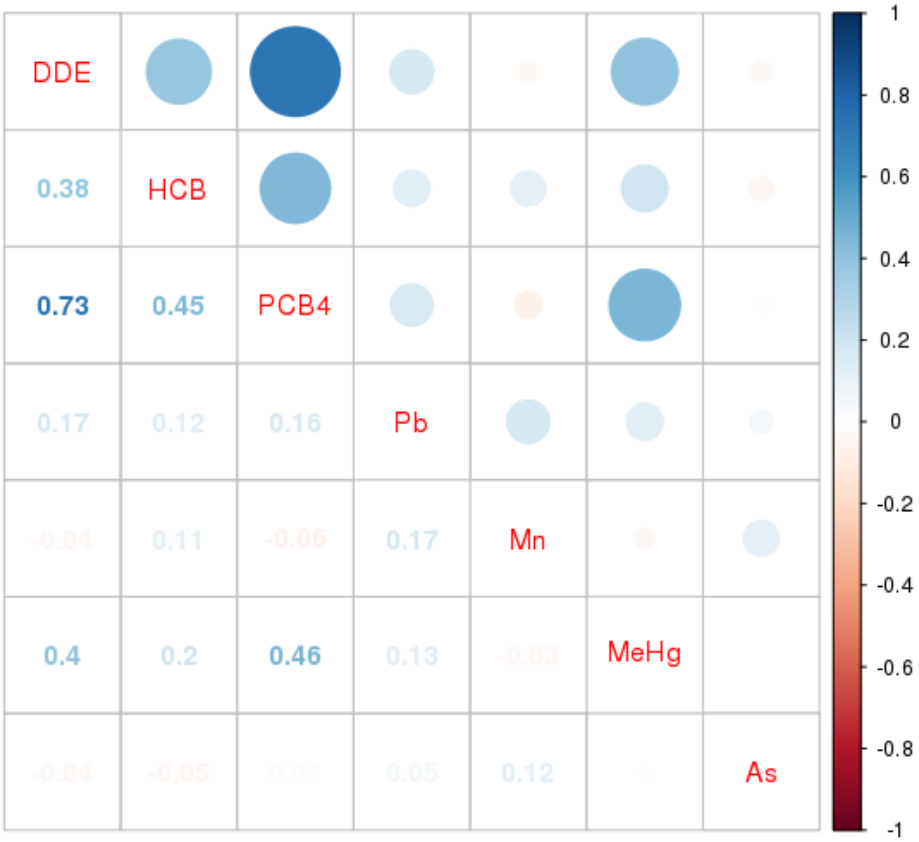
LIST OF SUPPLEMENTAL FIGURES

quantiles and Delis-Kaplan Executive Function System (D-KEFS) cognitive flexibility scaled scores, among adolescents in the secondary analysis group. In each plot, all remaining exposures are assigned their median value.

Supplemental Figure 4.3 Joint association between chemical mixture composed of DDE, HCB, ΣPCB_4, Pb, Mn, MeHg, and As (estimates and 95% credible intervals) and the Delis-Kaplan Executive Function System (D-KEFS) cognitive flexibility scaled scores, comparing chemical mixture levels at various percentiles compared to their median levels, among adolescents in the secondary analysis group.

Supplemental Figures from Chapter 2

Supplemental Figure 2.1 Spearman correlations between exposures in *Set 2*[1].

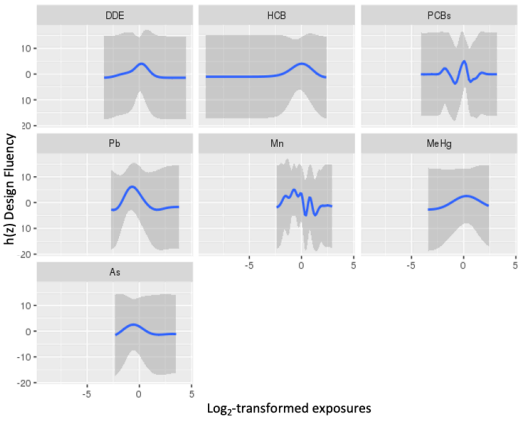


[1]*Set 2*: complete outcome, covariate and exposure data for PCBs, DDE, HCB, Pb, Mn, MeHg, and As n=235

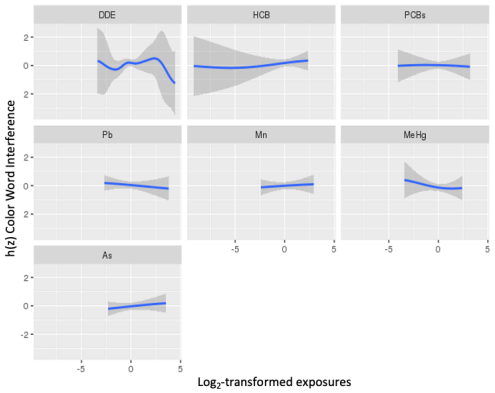
Abbreviations: DDE: dichlorodiphenyldichloroethylene; HCB: hexachlorobenzene; ΣPCB_4: sum of 4 PCB congeners (118, 138, 153, 180); Pb: lead; Mn: manganese; MeHg: methylmercury; As: arsenic.

Supplemental Figure 2.2 Estimated univariate exposure-response functions and 95% credible intervals[1] between each of the 7 exposures in *Set 2*[2] and the latent continuous outcome representing the binary inhibition outcomes[3]: Design Fluency[4] and Color-Word Interference[5], where all remaining exposures are assigned to their median value among adolescents in the secondary analysis group.

Design Fluency
Best (n=87) vs. Poor (n=148) performance



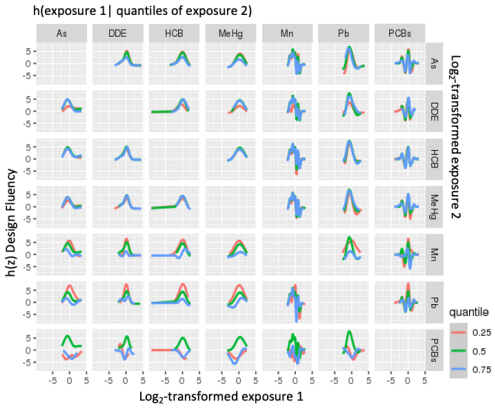
Color-Word Interference
Best (n=88) vs. Poor (n=147) performance



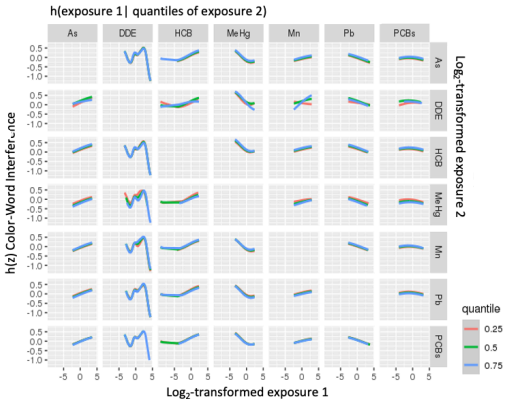
[1]Exposures have been log2-transformed and models have been adjusted for child race, sex, age at exam, and HOME score; maternal marital status at child's birth, IQ, seafood consumption during pregnancy, and smoking during pregnancy; maternal and paternal education and annual household income at child's birth; and study examiner.
[2]*Set 2*: complete outcome, covariate and exposure data for PCBs, DDE, HCB, Pb, Mn, MeHg, and As, n=235.
[3]h(z) can be interpreted as the association between the chemical mixture and a latent continuous variable which represents being in the poor Design Fluency or Color-Word Interference performance group. When h(z) > 0, the probability of being in the poor performance group is equal to 1, and 0 otherwise.
[4]Reference is best performance group (total correct raw scores > median and total errors raw score < median) compared to remaining participants (poor performance group).
[5]Reference is best performance group (total completion time raw score < median and total errors raw score < median) compared to remaining participants (poor performance group).
Abbreviations: DDE: dichlorodiphenyldichloroethylene; HCB: hexachlorobenzene; PCBs: Sum of 4 PCB congeners (118, 138, 153, 180); Pb: lead; Mn: manganese, MeHg: methylmercury, As: arsenic.

Supplemental Figure 2.3 Bivariate exposure-response functions[1] between one of the 7 exposures in *Set 2*[2] where a second exposure is fixed at various quantiles and the latent continuous variable representing the binary inhibition outcomes:[3] Design Fluency[4] and Color-Word Interference[5], while the remaining exposures are assigned to their median value among adolescents in the secondary analysis group.

Best (n=87) vs. Poor (n=148) performance



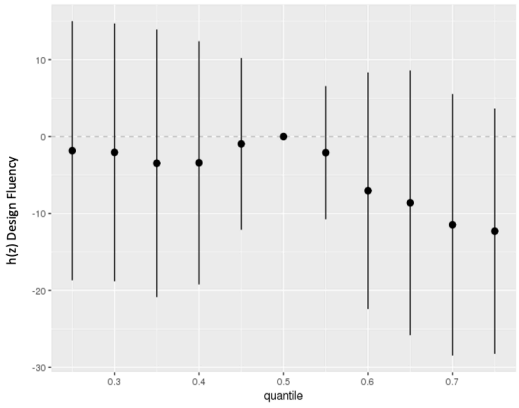
Best (n=88) vs. Poor (n=147) performance



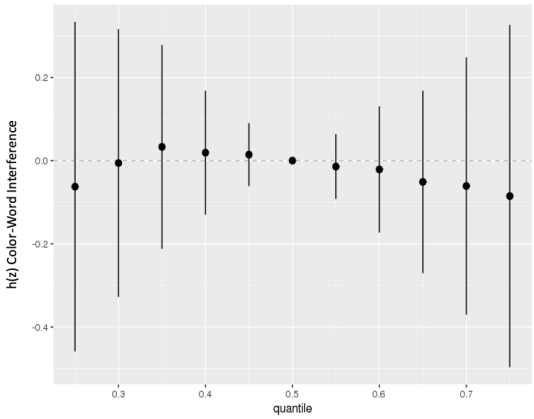
[1]Exposures have been log2-transformed and models have been adjusted for child race, sex, age at exam, and HOME score; maternal marital status at child's birth, IQ, seafood consumption during pregnancy, and smoking during pregnancy; maternal and paternal education and annual household income at child's birth; and study examiner.
[2]*Set 2*: complete outcome, covariate and exposure data for PCBs, DDE, HCB, Pb, Mn, MeHg, and As, n=373
[3]h(z) can be interpreted as the association between the chemical mixture and a latent continuous variable which represents being in the poor Design Fluency or Color-Word Interference performance group. When h(z) > 0, the probability of being in the poor performance group is equal to 1, and 0 otherwise.
[4]Reference is best performance group (total correct raw scores > median and total errors raw score < median) compared to remaining participants (poor performance group).
[5]Reference is best performance group (total completion time raw score < median and total errors raw score < median) compared to remaining participants (poor performance group).
Abbreviations: DDE: dichlorodiphenyldichloroethylene; HCB: hexachlorobenzene; PCBs: Sum of 4 PCB congeners (118, 138, 153, 180); Pb: lead; Mn: manganese; MeHg: methylmercury; As: arsenic.

Supplemental Figure 2.4 Joint association (estimates and 95% credible intervals[1]) of the seven chemical mixture[2] on the latent continuous outcome representing the binary inhibition outcomes:[3] Design Fluency[4] and Color-Word Interference[5], comparing chemical mixture levels at various percentiles compared to their median levels among adolescents in the secondary analysis group.

Best (n=87) vs. Poor (n=148) performance



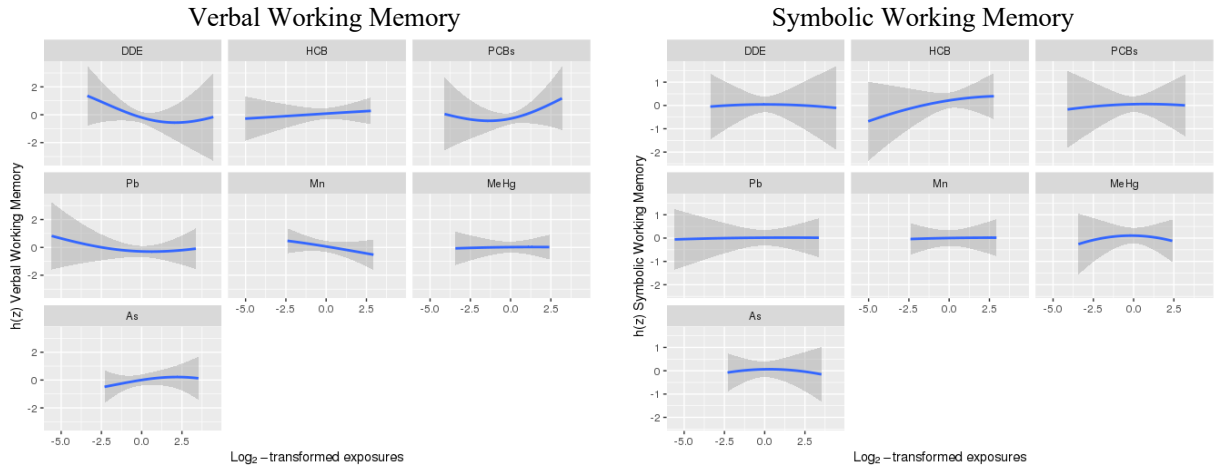
. Best (n=88) vs. Poor (n=147) performance



[1]Exposures have been log2-transformed and models have been adjusted for child race, sex, age at exam, and HOME score; maternal marital status at child's birth, IQ, seafood consumption during pregnancy, and smoking during pregnancy; maternal and paternal education and annual household income at child's birth; and study examiner.
[2]*Set 1*: complete outcome, covariate and exposure data for PCBs, DDE, HCB, Pb and Mn, n=373
[3]h(z) can be interpreted as the association between the chemical mixture and a latent continuous variable which represents being in the poor Design Fluency or Color-Word Interference performance group. When h(z) > 0, the probability of being in the poor performance group is equal to 1, and 0 otherwise.
[4]Reference is best performance group (total correct raw scores > median and total errors raw score < median) compared to remaining participants (poor performance group).
[5]Reference is best performance group (total completion time raw score < median and total errors raw score < median) compared to remaining participants (poor performance group).

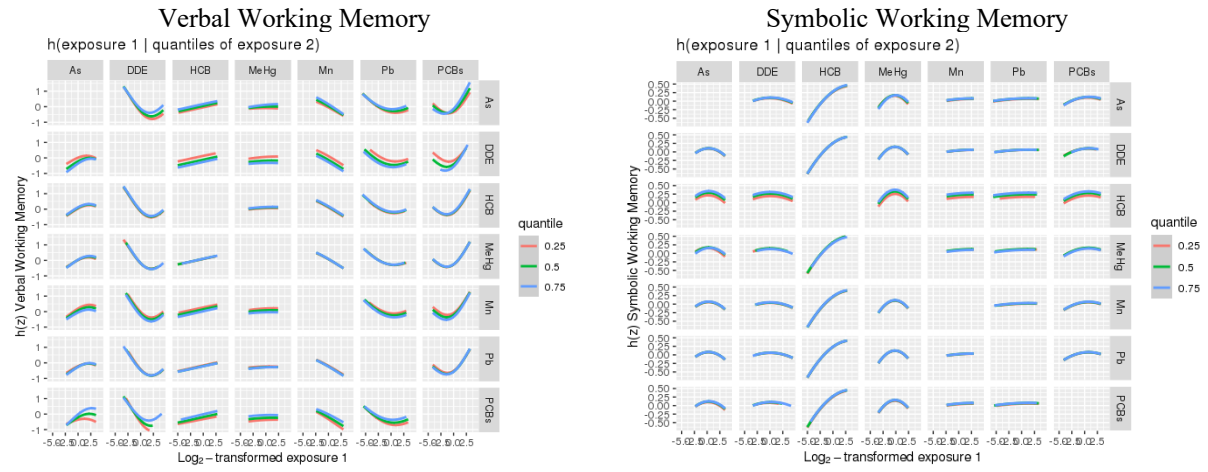
Supplemental Figures from Chapter 3

Supplemental Figure 3.1 Estimated exposure-response functions and 95% credible intervals[1] associating DDE, HCB, PCBs, Pb, Mn, MeHg, and As with the Wide Range Assessment of Memory and Learning 2nd Edition working memory scaled scores, where all of the remaining exposures are assigned to their median value, among adolescents in the secondary analysis group[2].



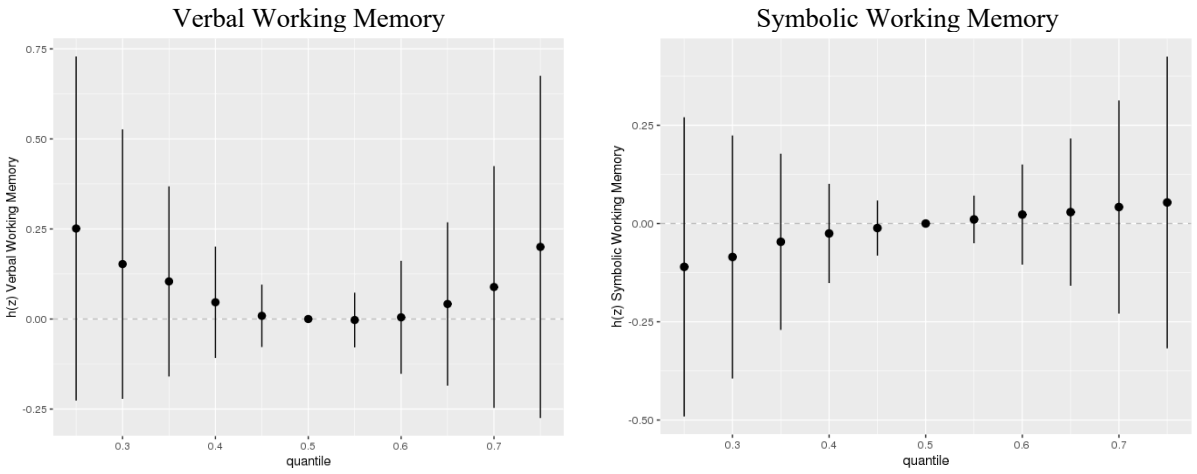
[1]Exposures have been log2-transformed and models have been adjusted for all listed exposures, child race, sex, age at exam, and HOME score; maternal marital status at child's birth, IQ, seafood consumption during pregnancy, and smoking during pregnancy; maternal and paternal education and annual household income at child's birth; study examiner.
[2]Secondary analysis group: complete working memory outcome, covariate and exposure data for DDE, HCB, PCBs, Pb, Mn, MeHg, and As, n=235.
Abbreviations: DDE: dichlorodiphenyldichloroethylene; HCB: hexachlorobenzene; PCBs: Sum of 4 PCB congeners (118, 138, 153, 180); Pb: lead; Mn: manganese; MeHg: methylmercury; As: arsenic.

Supplemental Figure 3.2 Bivariate exposure-response functions[1] associating each of the 7 secondary exposures (DDE, HCB, PCBs, Pb, Mn, MeHg, As) and a second exposure fixed at various quantiles with the Wide Range Assessment of Memory and Learning 2nd Edition working memory scaled scores, while the remaining exposures are assigned to their median value, among adolescents in the secondary analysis group[2].



[1]Exposures have been log2-transformed and models have been adjusted for all listed exposures, child race, sex, age at exam, and HOME score; maternal marital status at child's birth, IQ, seafood consumption during pregnancy, and smoking during pregnancy; maternal and paternal education and annual household income at child's birth; study examiner.
2Secondary analysis group: complete working memory outcome, covariate and exposure data for DDE, HCB, PCBs, Pb, Mn, MeHg, and As, n=235.
Abbreviations: DDE: dichlorodiphenyldichloroethylene; HCB: hexachlorobenzene; ΣPCB_4: Sum of 4 PCB congeners (118, 138, 153, 180); Pb: lead; Mn: manganese; MeHg: methylmercury; As: arsenic.

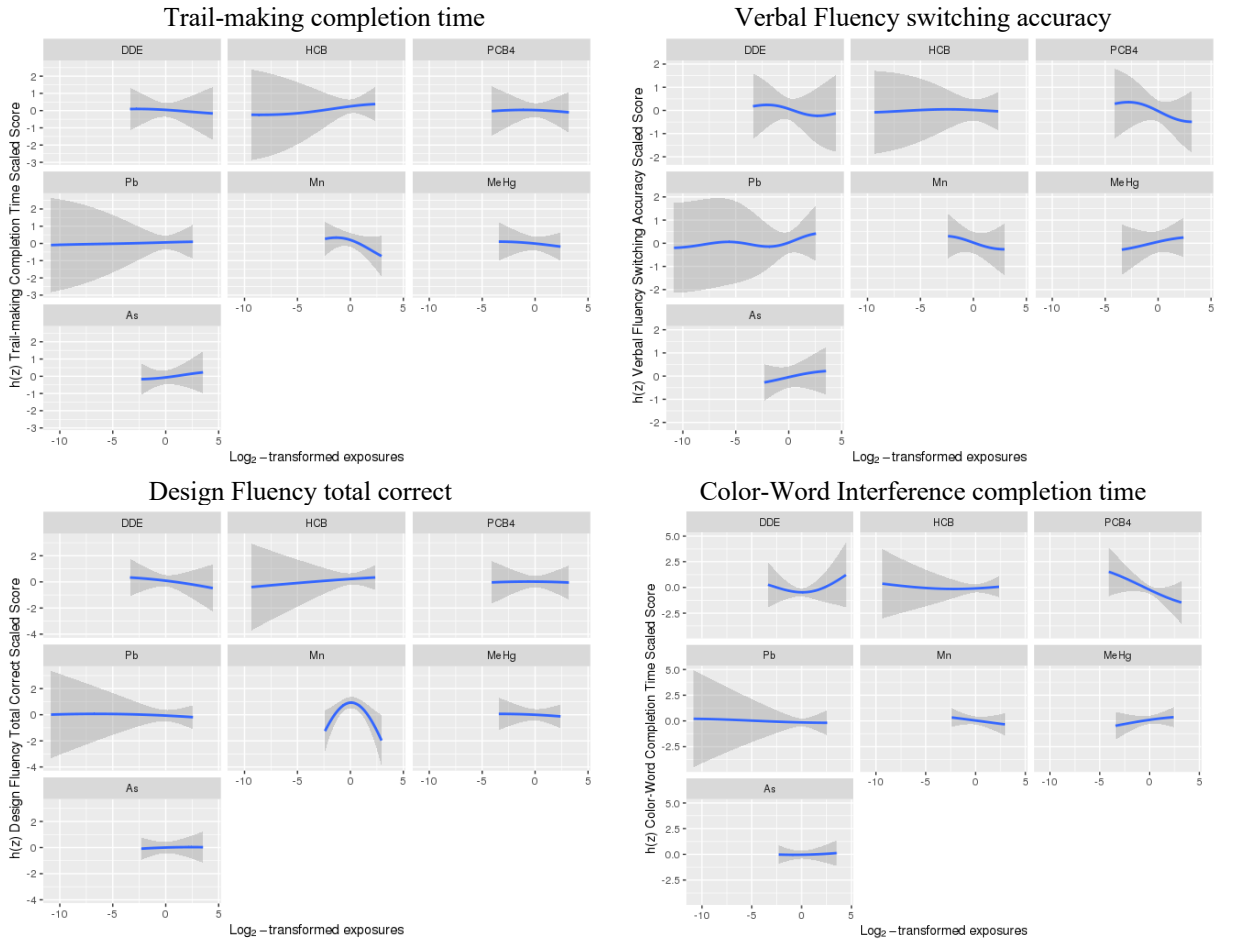
Supplemental Figure 3.3 Joint association of the chemical mixture composed of DDE, HCB, PCBs, Pb, Mn, MeHg, and As (estimates and 95% credible intervals[1]) with the Wide Range Assessment of Memory and Learning 2nd Edition working memory scaled scores, comparing chemical mixture levels at various percentiles compared to their median levels, among adolescents in the secondary analysis group[2].



[1]Exposures have been log2-transformed and models have been adjusted for all listed exposures, child race, sex, age at exam, and HOME score; maternal marital status at child's birth, IQ, seafood consumption during pregnancy, and smoking during pregnancy; maternal and paternal education and annual household income at child's birth; study examiner.
[2]Secondary analysis group: complete working memory outcome, covariate and exposure data for DDE, HCB, PCBs, Pb, Mn, MeHg, and As, n=235.
Abbreviations: DDE: dichlorodiphenyldichloroethylene; HCB: hexachlorobenzene; ΣPCB_4: Sum of 4 PCB congeners (118, 138, 153, 180); Pb: lead; Mn: manganese; MeHg: methylmercury; As: arsenic.

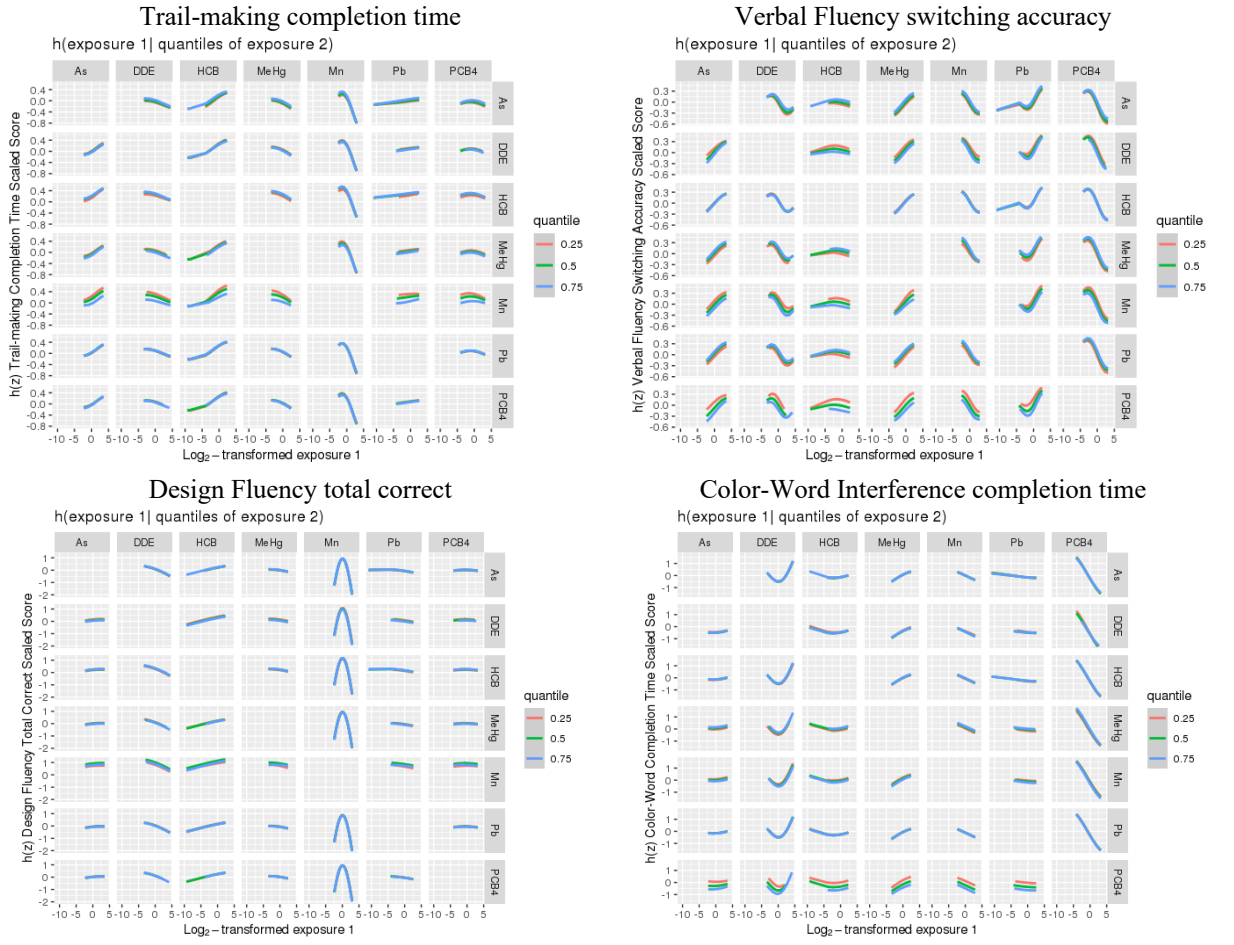
Supplemental Figures from Chapter 4

Supplemental Figure 4.1 Estimated covariate-adjusted exposure-response functions and 95% credible intervals[1] between DDE, HCB, ΣPCB$_4$, Pb, Mn, MeHg, and As and Delis Kaplan Executive Function System (D-KEFS) cognitive flexibility scaled scores, among New Bedford Cohort adolescents in the secondary analysis group[2]. In each plot, all remaining exposures are assigned to their median value.



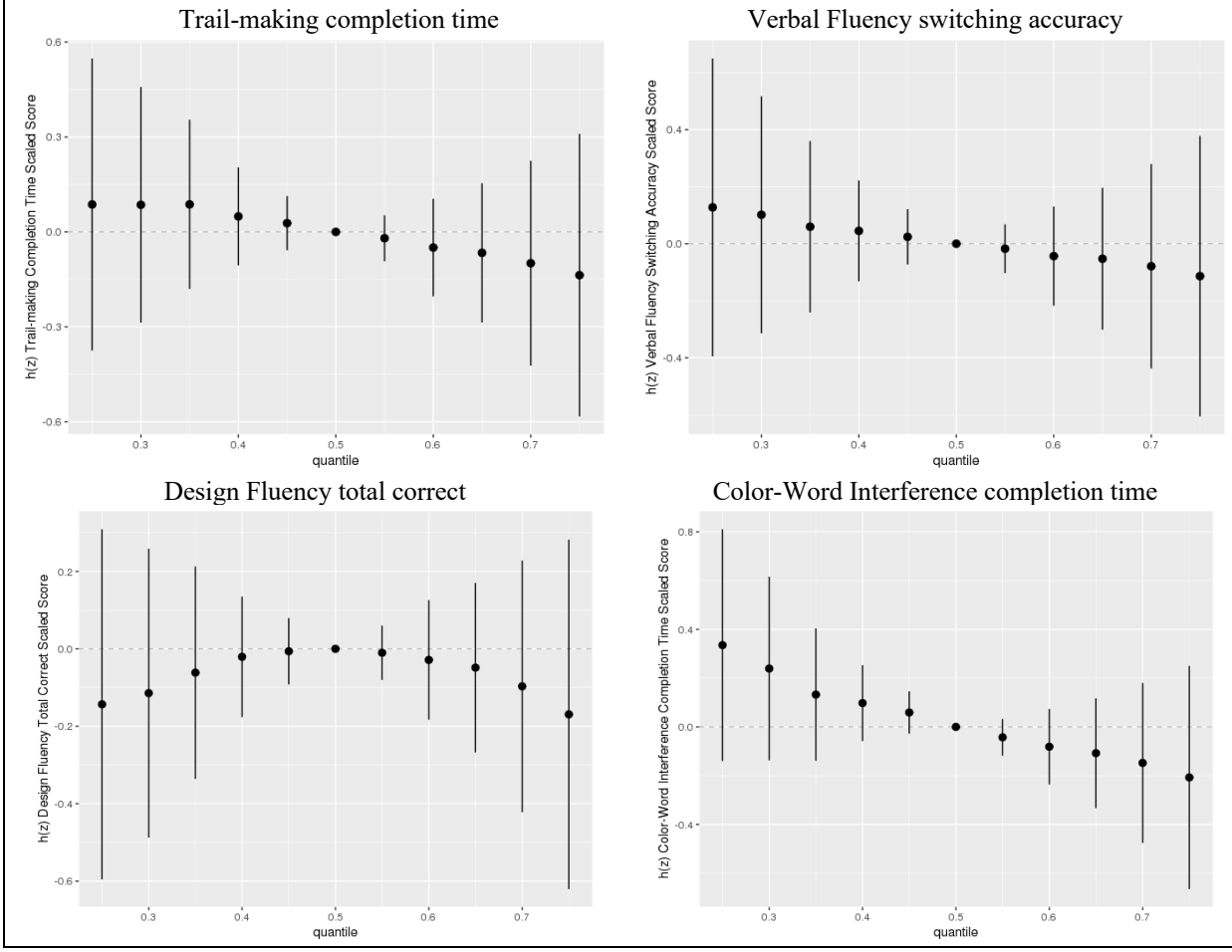
[1]Exposures have been log2-transformed and models have been adjusted for child race, sex, age at exam, year of birth, and HOME score; maternal marital status at child's birth, IQ, seafood consumption during pregnancy, and smoking during pregnancy; maternal and paternal education and annual household income at child's birth; and study examiner.
[2]Secondary analysis group: complete outcome, covariate and prenatal exposure biomarker data for DDE, HCB, ΣPCB$_4$, Pb, Mn, MeHg, and As, n=235.
Abbreviations: DDE: dichlorodiphenyldichloroethylene; HCB: hexachlorobenzene; ΣPCB$_4$: Sum of 4 PCB congeners (118, 138, 153, 180); Pb: lead; Mn: manganese; MeHg: methylmercury; As: arsenic

Supplemental Figure 4.2 Covariate-adjusted exposure-response functions[1] between one of 7 exposures (DDE, HCB, ΣPCB$_4$, Pb, Mn, MeHg, As) where a second exposure is fixed at various quantiles and Delis-Kaplan Executive Function System (D-KEFS) cognitive flexibility scaled scores, among New Bedford cohort adolescents in the secondary analysis group[2]. In each plot, all remaining exposures are assigned their median value.



[1]Exposures have been log2-transformed and models have been adjusted for child race, sex, age at exam, year of birth, and HOME score; maternal marital status at child's birth, IQ, seafood consumption during pregnancy, and smoking during pregnancy; maternal and paternal education and annual household income at child's birth; and study examiner.
[2]Secondary analysis group: complete outcome, covariate and prenatal exposure biomarker data for DDE, HCB, ΣPCB$_4$, Pb, Mn, MeHg, and As, n=235.
Abbreviations: DDE: dichlorodiphenyldichloroethylene; HCB: hexachlorobenzene; ΣPCB$_4$: Sum of 4 PCB congeners (118, 138, 153, 180); Pb: lead; Mn: manganese; MeHg: methylmercury; As: arsenic.

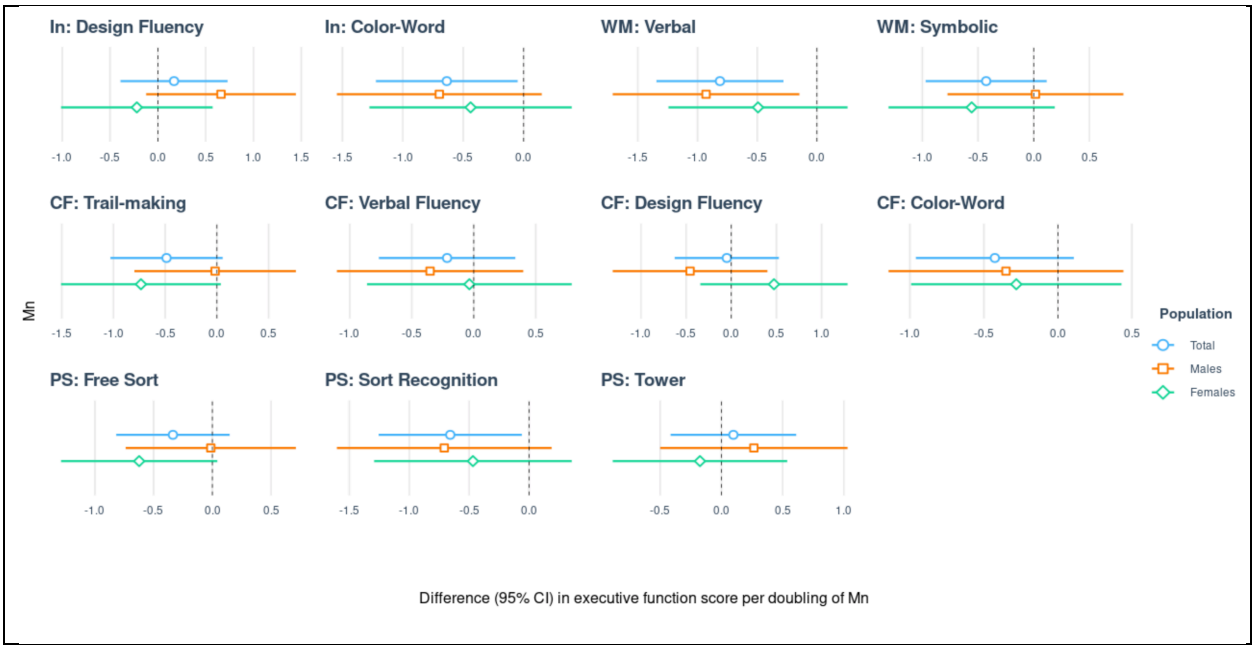
Supplemental Figure 4.3 Joint association between chemical mixture composed of DDE, HCB, ΣPCB_4, Pb, Mn, MeHg, and As (estimates and 95% credible intervals[1]) and the Delis-Kaplan Executive Function System (D-KEFS) cognitive flexibility scaled scores, comparing chemical mixture levels at various percentiles compared to their median levels, among New Bedford Cohort adolescents in the secondary analysis group[2].



[1]Exposures have been log2-transformed and models have been adjusted for child race, sex, age at exam, year of birth, and HOME score; maternal marital status at child's birth, IQ, seafood consumption during pregnancy, and smoking during pregnancy; maternal and paternal education and annual household income at child's birth; and study examiner.
[2]Secondary analysis group: complete outcome, covariate and prenatal exposure biomarker data for DDE, HCB, ΣPCB_4, Pb, Mn, MeHg, and As, n=235.
Abbreviations: DDE: dichlorodiphenyldichloroethylene; HCB: hexachlorobenzene; ΣPCB_4: Sum of 4 PCB congeners (118, 138, 153, 180); Pb: lead; Mn: manganese; MeHg: methylmercury; As: arsenic.

Supplemental Figures from Chapter 5

Supplemental Table 5.1 Sex-stratified results of multivariable linear regression analyses of difference (95% confidence intervals)[1] in inhibition, working memory, cognitive flexibility, and problem-solving scaled scores per doubling of cord blood manganese concentrations among New Bedford Cohort adolescents.



[1]Manganese exposure has been log2-transformed and models have been adjusted for child race, sex, age at exam, HOME score; maternal marital status at child's birth, IQ, seafood consumption during pregnancy, smoking during pregnancy; maternal and paternal education and annual household income at child's birth; study examiner.

Abbreviations:
In: Inhibition scaled scores[2]
WM: Working memory scaled scores[3]
CF: Cognitive flexibility scaled scores[2]
PS: Problem-solving scaled scores[2]

[2]Delis-Kaplan Executive Function System (D-KEFS) scaled score
[3]Wide Range Assessment of Memory and Learning, 2nd Edition (WRAML2) scaled score

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Supplemental Table 3.3 Inverse probability weighted results of multivariable linear regression analyses (difference in points associated with a twofold increase in exposure and 95% CI) assessing the relation of prenatal exposure to a five-chemical mixture with Wide Range Assessment of Memory and Learning, 2nd Edition working memory scaled scores among adolescents in the main analysis group.

Supplemental Table 3.4 Inverse probability weighted sex-stratified results of multivariable linear regression analyses (difference in points associated with a twofold increase in exposure and 95% CI) assessing the relation of prenatal exposure to a five-chemical mixture with Wide Range Assessment of Memory and Learning, 2nd Edition working memory scaled scores among adolescents in the main analysis group.

Supplemental Table 3.5 Inverse probability weighted prenatal social disadvantage index (PNSDI)-stratified results of multivariable linear regression analyses (difference in points associated with a twofold increase in exposure and 95% CI) assessing the relation of prenatal exposure to a five-chemical mixture with Wide Range Assessment of Memory and Learning, 2nd Edition working memory scaled scores among adolescents in the main analysis group.

Supplemental Table 3.6 Characteristics of all New Bedford Cohort participants who were evaluated as adolescents and included in the secondary analysis and those who were excluded.

Supplemental Table 3.7 Complete-case results of multivariable linear regression analyses (difference in points associated with a twofold increase in exposure and 95% CI) assessing the relation of prenatal exposure to a seven-chemical mixture with Wide Range Assessment of Memory and Learning, 2nd Edition working memory scaled scores among adolescents in the secondary analysis group.

Supplemental Table 3.8 Inverse probability weighted results of multivariable linear regression analyses (difference in points associated with a twofold increase in exposure and 95% CI) assessing the relation of prenatal exposure to a seven-chemical mixture with Wide Range Assessment of Memory and Learning, 2nd Edition working memory scaled scores among adolescents in the secondary analysis group.

Supplemental Table 4.1 Inverse-probability weighted results of multivariable linear regression analyses (difference in points associated with a twofold increase in exposure and 95% CI) assessing the relation of prenatal exposure to a five-chemical mixture with Delis-Kaplan Executive Function System (D-KEFS) cognitive flexibility scaled scores among adolescents in the main analysis group.

Supplemental Table 4.2 Sex-stratified complete-case results of multivariable linear regression analyses (difference in points associated with a twofold increase in exposure and 95% CI) assessing the relation of prenatal exposure to a five-chemical mixture with Delis-Kaplan Executive Function System (D-KEFS) cognitive flexibility scaled scores among adolescents in the main analysis group.

Supplemental Table 4.3 Sex-stratified inverse probability weighted results of multivariable linear regression analyses (difference in points associated with a twofold increase in exposure and 95%

CI) assessing the relation of prenatal exposure to a five-chemical mixture with Delis-Kaplan Executive Function System (D-KEFS) cognitive flexibility scaled scores among New Bedford Cohort adolescents in the main analysis group.

Supplemental Table 4.4 Sex-stratified inverse probability weighted results of multivariable linear regression analyses (difference in points associated with a twofold increase in exposure and 95% CI) assessing the relation of prenatal exposure to a five-chemical mixture with Delis-Kaplan Executive Function System (D-KEFS) cognitive flexibility scaled scores among adolescents in the main analysis group.

Supplemental Table 4.5 Prenatal social disadvantage index (PNSDI)-stratified inverse probability weighted results of multivariable linear regression analyses (difference in points associated with a twofold increase in exposure and 95% CI) assessing the relation of prenatal exposure to a five-chemical mixture with Delis-Kaplan Executive Function System (D-KEFS) cognitive flexibility scaled scores among adolescents in the main analysis group.

Supplemental Table 4.6 Inverse probability weighted results of negative binomial regression analyses (RR (rate ratio) and 95% CI) assessing the relation of prenatal exposure to a five-chemical mixture with Delis-Kaplan Executive Function System (D-KEFS) cognitive flexibility error raw scores among adolescents in the main analysis group.

Supplemental Table 4.7 Inverse probability weighted results of logistic regression analyses (odds ratio (OR) and 95% CI) assessing the relation of prenatal exposure to a five-chemical mixture with Delis-Kaplan Executive Function System (D-KEFS) Trail-making and Color-Word Interference performance among adolescents in the main analysis group.

Supplemental Table 4.8 Characteristics of New Bedford Cohort participants who were evaluated as adolescents and included in the secondary analysis group, and those who were excluded from the secondary analysis group.

Supplemental Table 4.9 Complete-case results of multivariable linear regression analyses (difference in points associated with a twofold increase in exposure and 95% CI) assessing the relation of prenatal exposure to a seven-chemical mixture with Delis-Kaplan Executive Function System (D-KEFS) cognitive flexibility scaled scores among adolescents in the secondary analysis group.

Supplemental Table 4.10 Inverse-probability weighted results of multivariable linear regression analyses (difference in points associated with a twofold increase in exposure and 95% CI) assessing the relation of prenatal exposure to a seven-chemical mixture with Delis-Kaplan Executive Function System (D-KEFS) cognitive flexibility scaled scores among adolescents in the secondary analysis group.

Supplemental Table 5.1 Sex-stratified results of multivariable linear regression analyses of difference (95% confidence intervals) in inhibition, working memory, cognitive flexibility, and problem-solving scaled scores per doubling of cord blood manganese concentrations among New Bedford Cohort adolescents.

Supplemental Table 5.2 Results of analyses assessing potential mediation of the association between cord blood manganese and problem-solving skills by measures of inhibition, working memory and cognitive flexibility among New Bedford Cohort adolescents stratified by sex. Problem-solving is measured by the Sorting: Sort Recognition description scaled score of the Delis Kaplan Executive Function System.

Supplemental Table 5.3 Inverse-probability weighted (IPW) results of multivariable linear regression analyses of difference (95% confidence intervals) in problem-solving scaled scores per doubling of cord blood manganese concentrations among 379 New Bedford Cohort adolescents.

Supplemental Tables from Chapter 2

Supplemental Table 2.1 Characteristics of New Bedford Cohort participants who were included in the secondary analysis group[1] and those who were excluded from the secondary analysis group.

Descriptive Characteristic	Secondary analysis group n =235			Excluded group, n= 553			
Inhibition Measures[3]	n(%)	Mean (SD)	Range	n(%)	Mean (SD)	Range	p-value[2]
Design Fluency raw scores							
Total number correct	235	10.2 (3.0)	1-19	293	9.5 (3.4)	0-23	0.01*
Total number set loss designs	235	0.3 (0.9)	0-9	293	0.5 (1.8)	0-22	0.1
Overall Design Fluency performance							
Best performance	87 (37.0)			82 (28.0)			0.03*
Poor performance	148 (63.0)			211 (72.0)			
Color-Word Interference							
Completion time (seconds)		52.5 (12.3)	29-103	292	55.0 (13.5)	34-132	0.02*
Total number errors		2.1 (2.2)	0-11	292	2.5 (2.5)	0-19	0.02*
Overall Color-Word Interference performance							
Best performance	88 (37.4)			66 (22.5)			<0.001
Poor performance	147 (62.6)			226 (77.1)			
Missing	0			1 (0.3)			
Exposure Measures[4]							
Cord serum DDE (ng/g)	235	0.6 (1.4)	0.02-14.9	516	0.4 (0.7)	0-10.2	0.04*
Cord serum HCB (ng/g)	235	0.03 (0.02)	0-0.1	516	0.03 (0.04)	0-0.7	0.2
Cord serum ΣPCB_4 (ng/g)	235	0.3 (0.3)	0.01-2.3	516	0.2 (0.3)	0.01-4.4	0.2
Cord blood Pb (µg/dL)	235	1.4 (0.9)	0-9.4	513	1.6 (1.5)	0-17.4	0.02*
Cord blood Mn (µg/dL)	235	4.3 (1.6)	1.7-11.2	473	4.2 (1.9)	0.2-22.1	0.8
Maternal hair total Hg (µg/g)	235	0.6 (0.6)	0.03-3.1	276	0.6 (0.7)	0.03-9.2	0.3
Maternal toenail As (µg/g)	235	0.1 (0.1)	0.02-0.8	181	0.1 (0.1)	0.02-1.0	0.5
Covariate Measures[5]							
Child Characteristics							
Race/Ethnicity							<0.001*
Non-Hispanic White	186 (79.1)			345 (62.4)			
Hispanic	16 (6.8)			73 (13.2)			
Other	33 (14.0)			133 (24.1)			
Missing	0			2 (0.4)			
Sex							0.3
Male	114 (48.5)			294 (53.2)			
Female	121 (51.5)			259 (46.8)			

Supplemental Table 2.1 (Continued)

Age at Exam	235	15.5 (0.6)	14.4-17.7	155	15.6 (0.7)	14.0-17.9	0.5
Home Score	235	44.4 (.0)	27-56	256	42.8 (6.5)	21-56	0.003*
Maternal Characteristics							
Marital status at birth							<0.001*
Not married	74 (31.5)			257 (46.5)			
Married	161 (68.5)			241 (43.6)			
Missing	0			55 (9.9)			
Maternal IQ	235	100.6 (9.7)	67-124	400	96.3 (10.5)	57-126	<0.001*
Seafood during pregnancy (serv/day)	235	0.5 (0.6)	0-5.3	398	0.6 (0.7)	0-6	0.5
Smoking during pregnancy							0.3
No	171 (72.8)			311 (56.2)			
Yes	64 (27.2)			140 (25.3)			
Missing	0			102 (18.4)			
Maternal education							<0.001*
≤ High School	108 (46.0)			313 (56.6)			
> High School	127 (54.0)			183 (33.1)			
Missing	0			57 (10.3)			
Household Characteristics at Birth							
Paternal Education							0.01*
≤ High School	152 (64.7)			360 (65.1)			
> High School	83 (35.3)			125 (22.6)			
Missing	0			68 (12.3)			
Annual Household Income							<0.001*
< $20,000	62 (26.4)			203 (36.7)			
≥ $20,000	173 (73.6)			286 (51.7)			
Missing	0			64 (11.6)			
Examination Characteristics							
Examiner							0.3
1	171 (72.8)			227 (77.5)			
2	64 (27.2)			66 (22.5)			

[1]Secondary analytic group: complete outcome, covariate and exposure data for DDE, HCB, ΣPCB_4, Pb, Mn, MeHg, As, n=235. [2]P-values represent results comparing characteristics between participants included in *Set 2* and those excluded from *Set 2* using t-tests, Wilcoxon rank sum tests, and chi-square tests. [3]NBC participants with missing inhibition measures: Design Fluency total correct n=260, total set loss designs n=260; Color-Word Interference completion time n=261, total errors n=261. [4]NBC participants with missing exposure measures: DDE n=37; HCB n=37; ΣPCB_4 n=37; Pb n= 40; Mn n=80; Hg n= 277; As n=372. [5]NBC participants with missing covariate measures: age at exam n=260; HOME score n= 297; maternal IQ n=153; seafood during pregnancy n=155. *p<0.05. Abbreviations: DDE: dichlorodiphenyldichloroethylene; HCB: hexachlorobenzene; ΣPCB_4: Sum of 4 PCB congeners (118, 138, 153, 180); Pb: lead; Mn: manganese; MeHg: methylmercury; As: arsenic.

Supplemental Table 2.2 Inverse probability weighted results of multivariable logistic regression [odds ratio (OR) and 95% CI][1] assessing the relation of prenatal exposure to a five-chemical mixture with Delis Kaplan Executive Function System (D-KEFS) Design Fluency[2] and Color-Word Interference[3] performance among adolescents in the main analysis group.

Exposure	**Performance**	**Design Fluency OR (95% CI)**	**Color Word OR (95% CI)**
	Best	*n=129*	*n=117*
	Poor	*n=244*	*n=256*
Log_2 DDE		0.85 (0.67, 1.08)	0.75 (0.55, 1.02)
Log_2 DDE^2		-	0.91 (0.84, 0.98)*
Log_2 HCB		1.03 (0.83, 1.28)	1.15 (0.91, 1.44)
Log_2 ΣPCB_4		1.16 (0.89, 1.51)	1.19 (0.90, 1.59)
Log_2 Pb		1.03 (0.83, 1.27)	0.76 (0.57, 0.97)*
Log_2 Mn		1.07 (0.67, 1.73)	1.81 (1.09, 3.05)*

[1]Exposures have been log2-transformed and models have been adjusted for child race, sex, age at exam, and HOME score; maternal marital status at child's birth, IQ, seafood consumption during pregnancy, and smoking during pregnancy; maternal and paternal education and annual household income at child's birth; and study examiner.
[2]Reference is best performance group (total correct raw scores > median and total errors raw score < median) compared to remaining participants (poor performance group).
[3]Reference is best performance group (total completion time raw score < median and total errors raw score < median) compared to remaining participants (poor performance group).
*$p<0.05$
Abbreviations: DDE: dichlorodiphenyldichloroethylene; HCB: hexachlorobenzene; ΣPCB_4: Sum of 4 PCB congeners (118, 138, 153, 180); Pb: lead; Mn: manganese.

Supplemental Table 2.3 Sex-stratified inverse probability weighted results of multivariable logistic regression analyses [odds ratio (OR) and 95% CI][1] assessing the relation of prenatal exposure to a five-chemical mixture with Delis-Kaplan Executive Function System (D-KEFS) Design Fluency[2] and Color-Word Interference[3] performance among adolescents in the main analysis group.

		Males	Females		Males	Females	
Exposure	**Performance**	**Design Fluency OR (95% CI)**	**Design Fluency OR (95% CI)**	**p for interaction**	**Color Word OR (95% CI)**	**Color Word OR (95% CI)**	**p for interaction**
	Best	*n=60*	*n=69*		*n=47*	*n=70*	
	Poor	*n=119*	*n=125*		*n=132*	*n=124*	
Log_2 DDE		0.71 (0.50, 1.00)	0.89 (0.58, 1.34)	0.4	0.84 (0.50, 1.54)	0.75 (0.46, 1.17)	0.6
Log_2 DDE^2		-	-	-	0.94 (0.83, 1.09)	0.88 (0.77, 1.00)	0.3
Log_2 HCB		1.13 (0.86, 1.53)	0.71 (0.45, 1.10)	0.1	1.06 (0.71, 1.43)	1.39 (0.91, 2.17)	0.5
Log_2 ΣPCB_4		1.53 (1.06, 2.26)*	1.12 (0.69, 1.87)	0.2	1.48 (0.96, 2.35)	0.97 (0.62, 1.54)	0.2
Log_2 Pb		0.93 (0.61, 1.42)	1.05 (0.80, 1.39)	0.5	0.66 (0.39, 1.09)	0.76 (0.53, 1.01)	0.5
Log_2 Mn		0.97 (0.49, 1.92)	1.40 (0.64, 3.13)	0.5	1.50 (0.72, 3.26)	2.47 (1.14, 5.67)*	0.8

[1]Exposures have been log2-transformed and models have been adjusted for child race, sex, age at exam, and HOME score; maternal marital status at child's birth, IQ, seafood consumption during pregnancy, and smoking during pregnancy; maternal and paternal education and annual household income at child's birth; and study examiner.
[2]Reference is best performance group (total correct raw scores > median and total errors raw score < median) compared to remaining participants (poor performance group).
[3]Reference is best performance group (total completion time raw score < median and total errors raw score < median) compared to remaining participants (poor performance group).
*$p<0.05$
Abbreviations: DDE: dichlorodiphenyldichloroethylene; HCB: hexachlorobenzene; ΣPCB_4: Sum of 4 PCB congeners (118, 138, 153, 180); Pb: lead; Mn: manganese.

Supplemental Table 2.4 Inverse probability weighted results of multivariable linear regression (difference and 95% CI)[1] and negative binomial regression [rate ratio (RR) and 95% CI][1] assessing the relation of prenatal exposure to a five-chemical mixture with Delis Kaplan Executive Function System (D-KEFS) Design Fluency and Color-Word Interference scores among 373 adolescents in the main analysis group.

Exposure	Design Fluency Total Correct Difference (95% CI)	Design Fluency Set Loss Designs[2] RR (95% CI)	Color-Word Completion Time (seconds) Difference (95% CI)	Color- Word Total Errors[2] RR (95% CI)
Log_2 DDE	0.13 (-0.22, 0.48)	0.85 (0.65, 1.10)	-0.50 (-2.27, 1.28)	0.93 (0.82, 1.06)
Log_2 DDE^2	-	-	-0.22 (-0.69, 0.26)	1.00 (0.97, 1.04)
Log_2 HCB	0.03 (-0.35, 0.29)	1.02 (0.81, 1.30)	-0.24 (-1.57, 1.10)	1.09 (0.98, 1.23)
Log_2 ΣPCB_4	-0.28 (-0.66, 0.10)	0.94 (0.72, 1.21)	0.99 (-0.61, 2.59)	1.10 (0.98, 1.24)
Log_2 Pb	-0.03 (-0.34, 0.27)	1.09 (0.86, 1.43)	-0.48 (-1.78, 0.83)	0.94 (0.85, 1.03)
Log_2 Mn	0.03 (-0.65, 0.71)	0.97 (0.61, 1.55)	3.71 (0.85, 6.57)*	1.08 (0.87, 1.33)

[1]Exposures have been log2-transformed and models have been adjusted for child race, sex, age at exam, and HOME score; maternal marital status at child's birth, IQ, seafood consumption during pregnancy, and smoking during pregnancy; maternal and paternal education and annual household income at child's birth; and study examiner.
[2]Raw scores
*$p<0.05$
Abbreviations: DDE: dichlorodiphenyldichloroethylene; HCB: hexachlorobenzene; ΣPCB_4: Sum of 4 PCB congeners (118, 138, 153, 180); Pb: lead; Mn: manganese.

Supplemental Table 2.5 Complete-case results of multivariable logistic regression [odds ratio (OR) and 95% CI][1] assessing the relation of prenatal exposure to a seven-chemical mixture with Delis Kaplan Executive Function System (D-KEFS) Design Fluency[2] and Color Word Interference[3] performance among adolescents in the secondary analysis group.

Exposure	Performance	Design Fluency OR (95% CI)	Color Word OR (95% CI)
	Best	*n=87*	*n=88*
	Poor	*n=148*	*n=147*
Log_2 DDE		0.90 (0.64, 1.29)	1.08 (0.75, 1.55)
Log_2 HCB		0.92 (0.66, 1.21)	1.01 (0.73, 1.33)
Log_2 ΣPCB$_4$		1.13 (0.77, 1.66)	1.19 (0.81, 1.79)
Log_2 Pb		1.06 (0.81, 1.41)	0.76 (0.53, 1.04)
Log_2 Mn		0.91 (0.50, 1.68)	1.73 (0.94, 3.24)
Log_2 MeHg		1.21 (0.89, 1.65)	0.70 (0.51, 0.95)*
Log_2 As		1.10 (0.82, 1.48)	1.20 (0.89, 1.63)

[1]Exposures have been log2-transformed and models have been adjusted for child race, sex, age at exam, and HOME score; maternal marital status at child's birth, IQ, seafood consumption during pregnancy, and smoking during pregnancy; maternal and paternal education and annual household income at child's birth; and study examiner.
[2]Reference is best performance group (total correct raw scores > median and total errors raw score < median) compared to remaining participants (poor performance group).
[3]Reference is best performance group (total completion time raw score < median and total errors raw score < median) compared to remaining participants (poor performance group).
*$p<0.05$
Abbreviations: DDE: dichlorodiphenyldichloroethylene; HCB: hexachlorobenzene; ΣPCB$_4$: Sum of 4 PCB congeners (118, 138, 153, 180); Pb: lead; Mn: manganese; MeHg: methylmercury; As: arsenic.

Supplemental Table 2.6 Inverse probability weighted results of multivariable logistic regression [odds ratio (OR) and 95% CI][1] assessing the relation of prenatal exposure to a seven-chemical mixture with Delis Kaplan Executive Function System (D-KEFS) Design Fluency[2] and Color Word Interference[3] performance among adolescents in the secondary analysis group.

Exposure	Performance	Design Fluency OR (95% CI)	Color Word OR (95% CI)
		IPW	
	Best	*n=87*	*n=88*
	Poor	*n=148*	*n=147*
Log_2 DDE		0.88 (0.62, 1.24)	1.07 (0.74, 1.54)
Log_2 HCB		0.99 (0.74, 1.27)	1.01 (0.73, 1.31)
Log_2 ΣPCB_4		1.14 (0.78, 1.67)	1.18 (0.80, 1.79)
Log_2 Pb		1.05 (0.82, 1.38)	0.74 (0.50, 1.02)
Log_2 Mn		0.97 (0.53, 1.80)	1.85 (0.99, 3.53)
Log_2 MeHg		1.21 (0.88, 1.66)	0.69 (0.50, 0.95)*
Log_2 As		1.09 (0.81, 1.48)	1.17 (0.86, 1.60)

[1]Exposures have been log2-transformed and models have been adjusted for child race, sex, age at exam, and HOME score; maternal marital status at child's birth, IQ, seafood consumption during pregnancy, and smoking during pregnancy; maternal and paternal education and annual household income at child's birth; and study examiner.
[2]Reference is best performance group (total correct raw scores > median and total errors raw score < median) compared to remaining participants (poor performance group).
[3]Reference is best performance group (total completion time raw score < median and total errors raw score < median) compared to remaining participants (poor performance group).
*$p<0.05$
Abbreviations: DDE: dichlorodiphenyldichloroethylene; HCB: hexachlorobenzene; ΣPCB_4: Sum of 4 PCB congeners (118, 138, 153, 180); Pb: lead; Mn: manganese; MeHg: methylmercury; As: arsenic.

Supplemental Tables from Chapter 3

Supplemental Table 3.1 Sex-stratified results of multivariable linear regression analyses (difference in points associated with a twofold increase in exposure and 95% CI)[1] assessing the relation of prenatal exposure to a five-chemical mixture with Wide Range Assessment of Memory and Learning, 2nd Edition working memory scaled scores among adolescents in the main analysis group[2].

	Verbal Working Memory			Symbolic Working Memory			Working Memory Sum Index		
	Males Difference (95% CI)	Females Difference (95% CI)	p[3]	Males Difference (95% CI)	Females Difference (95% CI)	p[3]	Males Difference (95% CI)	Females Difference (95% CI)	p[3]
Log_2 DDE	-0.30 (-0.73, 0.12)	-0.32 (-0.74, 0.10)	1	-0.11 (-0.55, 0.32)	-0.15 (-0.58, 0.27)	0.8	-1.15 (-3.25, 0.94)	-1.29 (-3.26, 0.68)	0.9
Log_2 HCB	0.06 (-0.41, 0.52)	0.15 (-0.28, 0.58)	0.6	0.09 (-0.38, 0.57)	0.48 (0.04, 0.91)*	0.1	0.46 (-1.82, 2.75)	1.72 (-0.29, 3.73)	0.3
Log_2 ΣPCB_4	0.40 (-0.07, 0.88)	0.31 (-0.15, 0.76)	0.9	0.17 (-0.31, 0.65)	0.05 (-0.41, 0.51)	0.9	1.56 (-0.76, 3.87)	1.00 (-1.13, 3.14)	1
Log_2 Pb	0.14 (-0.40, 0.68)	-0.27 (-0.61, 0.08)	0.2	-0.06 (-0.61, 0.49)	-0.14 (-0.49, 0.21)	0.9	0.24 (-2.41, 2.89)	-1.15 (-2.76, 0.46)	0.5
Log_2 Mn	-0.88 (-1.70, -0.07)*	-0.38 (-1.14, 0.39)	0.6	0.03 (-0.80, 0.86)	-0.57 (-1.34, 0.19)	0.3	-2.45 (-6.44, 1.54)	-2.47 (-6.02, 1.09)	0.9

[1]Exposures have been log2-transformed and models have been adjusted for all listed exposures, child race, sex, age at exam, and HOME score; maternal marital status at child's birth, IQ, seafood consumption during pregnancy, and smoking during pregnancy; maternal and paternal education and annual household income at child's birth; study examiner.
[2]Main analysis group: complete working memory outcome, covariate and exposure data for PCBs, DDE, HCB, Pb and Mn. Total n=373; Males n= 179; Females n=194.
[3]P-value for chemical-sex interaction term included in multivariable linear regression model.
*$p<0.05$
Abbreviations: DDE: dichlorodiphenyldichloroethylene; HCB: hexachlorobenzene; ΣPCB_4: Sum of 4 PCB congeners (118, 138, 153, 180); Pb: lead; Mn: manganese

Supplemental Table 3.2 Prenatal social disadvantage index (PNSDI)[1]-stratified results of multivariable linear regression analyses (difference in points associated with a twofold increase in exposure and 95% CI)[2] assessing the relation of prenatal exposure to a five-chemical mixture with Wide Range Assessment of Memory and Learning, 2nd Edition working memory scaled scores among adolescents in the main analysis group[3].

	Verbal Working Memory			Symbolic Working Memory			Working Memory Sum Index		
	PNSDI < 3 Difference (95% CI)	PNSDI ≥ 3 Difference (95% CI)	p[4]	PNSDI < 3 Difference (95% CI)	PNSDI ≥ 3 Difference (95% CI)	p[4]	PNSDI < 3 Difference (95% CI)	PNSDI ≥ 3 Difference (95% CI)	p[4]
Log_2 DDE	-0.04 (-0.37, 0.30)	-0.60 (-1.16, -0.03)*	0.08	0.02 (-0.32, 0.37)	-0.21 (-0.78, 0.36)	0.48	-0.04 (-1.66, 1.57)	-2.22 (-4.88, 0.44)	0.16
Log_2 HCB	0.32 (-0.05, 0.70)	-0.30 (-0.85, 0.26)	0.13	0.41 (0.02, 0.80)*	-0.12 (-0.68, 0.44)	0.19	2.03 (0.22, 3.84)*	-1.13 (-3.74, 1.49)	0.10
Log_2 ΣPCB_4	0.10 (-0.27, 0.47)	0.73 (0.11, 1.35)*	0.15	-0.09 (-0.47, 0.30)	0.40 (-0.24, 1.03)	0.30	0.06 (-1.73, 1.85)	3.10 (0.15, 6.05)*	0.16
Log_2 Pb	-0.31 (-0.67, 0.06)	0.11 (-0.43, 0.64)	0.31	-0.06 (-0.44, 0.32)	-0.21 (-0.75, 0.33)	0.61	-1.01 (-2.78, 0.77)	-0.32 (-2.85, 2.20)	0.82
Log_2 Mn	-1.00 (-1.66, -0.35)*	-0.41 (-1.41, 0.58)	0.27	-0.80 (-1.48, -0.11)*	0.19 (-0.82, 1.19)	0.14	-4.91 (-8.11, -1.70)*	-0.51 (-5.22, 4.19)	0.13

[1]Prenatal social disadvantage index (PNSDI) was constructed as the sum of five adverse social or economic exposures at the time of the child's birth where presence of each risk factor was assigned a value of 1, absence a value of 0: mother unmarried, mother's education as high school graduate or less, father's education as high school graduate or less, annual household income less than $20,000, and mother's age at birth less than 20 years.
[2]Exposures have been log2-transformed and models have been adjusted for all listed exposures, child race, sex, age at exam, and HOME score; maternal marital status at child's birth, IQ, seafood consumption during pregnancy, and smoking during pregnancy; maternal and paternal education and annual household income at child's birth; study examiner.
[3]Main analysis group: complete working memory outcome, covariate and exposure data for PCBs, DDE, HCB, Pb and Mn. Total n=373; SDI < 3 n= 241; SDI ≥ 3 n=132.
[4]P-value for chemical-PNSDI interaction term included in multivariable linear regression model.
*p<0.05
Abbreviations: DDE: dichlorodiphenyldichloroethylene; HCB: hexachlorobenzene; ΣPCB_4: Sum of 4 PCB congeners (118, 138, 153, 180); Pb: lead; Mn: manganese.

Supplemental Table 3.3 Inverse probability weighted results of multivariable linear regression analyses (difference in points associated with a twofold increase in exposure and 95% CI)[1] assessing the relation of prenatal exposure to a five-chemical mixture with Wide Range Assessment of Memory and Learning, 2nd Edition working memory scaled scores among adolescents in the main analysis group[2].

Exposure	Verbal Working Memory Difference (95% CI)	Symbolic Working Memory Difference (95% CI)	Working Memory Sum Index Difference (95% CI)
Log_2 DDE	-0.28 (-0.56, 0.00)	-0.10 (-0.39, 0.19)	-1.04 (-2.39, 0.32)
Log_2 HCB	0.09 (-0.22, 0.39)	0.18 (-0.13, 0.50)	0.77 (-0.70, 2.24)
Log_2 ΣPCB_4	0.37 (0.06, 0.68)*	0.14 (-0.18, 0.46)	1.40 (-0.09, 2.88)
Log_2 Pb	-0.08 (-0.37, 0.21)	-0.08 (-0.38, 0.22)	-0.44 (-1.85, 0.97)
Log_2 Mn	-0.74 (-1.29, -0.20)*	-0.40 (-0.96, 0.16)	-3.09 (-5.72, -0.46)*

[1]Exposures have been log2-transformed and models have been adjusted for all listed exposures, child race, sex, age at exam, and HOME score; maternal marital status at child's birth, IQ, seafood consumption during pregnancy, and smoking during pregnancy; maternal and paternal education and annual household income at child's birth; study examiner.
[2]Main analysis group: complete working memory outcome, covariate and exposure data for PCBs, DDE, HCB, Pb and Mn, n=373.
*$p<0.05$
Abbreviations: DDE: dichlorodiphenyldichloroethylene; HCB: hexachlorobenzene; ΣPCB_4: Sum of 4 PCB congeners (118, 138, 153, 180); Pb: lead; Mn: manganese.

Supplemental Table 3.4 Inverse probability weighted sex-stratified results of multivariable linear regression analyses (difference in points associated with a twofold increase in exposure and 95% CI)[1] assessing the relation of prenatal exposure to a five-chemical mixture with Wide Range Assessment of Memory and Learning, 2nd Edition working memory scaled scores among adolescents in the main analysis group[2].

	Verbal Working Memory			Symbolic Working Memory			Working Memory Sum Index		
	Males Difference (95% CI)	Females Difference (95% CI)	p[3]	Males Difference (95% CI)	Females Difference (95% CI)	p[3]	Males Difference (95% CI)	Females Difference (95% CI)	p[3]
Log_2 DDE	-0.34 (-0.77, 0.08)	-0.36 (-0.78, 0.06)	0.98	-0.13 (-0.55, 0.30)	-0.15 (-0.58, 0.27)	0.71	-1.31 (-3.36, 0.75)	-1.40 (-3.35, 0.55)	0.81
Log_2 HCB	0.00 (-0.46, 0.47)	0.14 (-0.29, 0.57)	0.47	0.04 (-0.43, 0.50)	0.42 (-0.02, 0.86)	0.11	0.17 (-2.08, 2.42)	1.54 (-0.47, 3.54)	0.20
Log_2 ΣPCB_4	0.45 (-0.02, 0.92)	0.33 (-0.12, 0.78)	0.72	0.27 (-0.20, 0.74)	0.03 (-0.42, 0.49)	0.73	1.96 (-0.31, 4.24)	1.03 (-1.06, 3.12)	0.71
Log_2 Pb	0.16 (-0.38, 0.71)	-0.24 (-0.59, 0.10)	0.25	-0.06 (-0.60, 0.48)	-0.14 (-0.49, 0.21)	0.85	0.31 (-2.32, 2.93)	-1.08 (-2.68, 0.52)	0.58
Log_2 Mn	-0.85* (-1.67, -0.03)*	-0.38 (-1.14, 0.37)	0.71	0.16 (-0.66, 0.98)	-0.62 (-1.39, 0.14)	0.13	-2.00 (-5.97, 1.97)	-2.60 (-6.12, 0.92)	0.57

[1]Exposures have been log2-transformed and models have been adjusted for all listed exposures, child race, sex, age at exam, and HOME score; maternal marital status at child's birth, IQ, seafood consumption during pregnancy, and smoking during pregnancy; maternal and paternal education and annual household income at child's birth; study examiner.
[2]Main analysis group: complete working memory outcome, covariate and exposure data for PCBs, DDE, HCB, Pb and Mn. Total n=373; Males n=179 ; Females n=194.
[3]P-value of chemical-sex interaction term included in multivariable linear regression model.
*p<0.05
Abbreviations: DDE: dichlorodiphenyldichloroethylene; HCB: hexachlorobenzene; ΣPCB_4: Sum of 4 PCB congeners (118, 138, 153, 180); Pb: lead; Mn: manganese

Supplemental Table 3.5 Inverse probability weighted prenatal social disadvantage index (PNSDI)[1]-stratified results of multivariable linear regression analyses (difference in points associated with a twofold increase in exposure and 95% CI)[2] assessing the relation of prenatal exposure to a five-chemical mixture with Wide Range Assessment of Memory and Learning, 2nd Edition working memory scaled scores among adolescents in the main analysis group[3].

	Verbal Working Memory			Symbolic Working Memory			Working Memory Sum Index		
	PNSDI < 3 Difference (95% CI)	PNSDI ≥ 3 Difference (95% CI)	p[4]	PNSDI < 3 Difference (95% CI)	PNSDI ≥ 3 Difference (95% CI)	p[4]	PNSDI < 3 Difference (95% CI)	PNSDI ≥ 3 Difference (95% CI)	p[4]
Log_2 DDE	-0.06 (-0.38, 0.26)	-0.61 (-1.17, -0.04)*	0.08	0.01 (-0.32, 0.34)	-0.18 (-0.75, 0.40)	0.58	-0.14 (-1.69, 1.42)	-2.17 (-4.85, 0.51)	0.19
Log_2 HCB	0.33 (-0.05, 0.70)	-0.26 (-0.80, 0.28)	0.11	0.38 (-0.01, 0.77)	-0.13 (-0.68, 0.43)	0.18	1.98 (0.16, 3.80)*	-1.02 (-3.59, 1.55)	0.09
Log_2 ΣPCB_4	0.13 (-0.22, 0.49)	0.72 (0.11, 1.33)*	0.19	-0.03 (-0.41, 0.34)	0.33 (-0.30, 0.95)	0.52	0.29 (-1.46, 2.03)	2.89 (-0.01, 5.79)	0.26
Log_2 Pb	-0.30 (-0.67, 0.06)	0.11 (-0.43, 0.66)	0.28	-0.05 (-0.43, 0.33)	-0.19 (-0.75, 0.36)	0.61	-0.97 (-2.75, 0.81)	-0.25 (-2.83, 2.32)	0.79
Log_2 Mn	-1.02 (-1.68, -0.36)*	-0.43 (-1.41, 0.55)	0.30	-0.81 (-1.50, -0.12)*	0.18 (-0.82, 1.19)	0.12	-4.98 (-8.19, -1.77)*	-0.56 (-5.23, 4.11)	0.13

[1]Prenatal social disadvantage index (PNSDI) was constructed as the sum of five adverse social or economic exposures at the time of the child's birth where presence of each risk factor was assigned a value of 1, absence a value of 0: mother unmarried, mother's education as high school graduate or less, father's education as high school graduate or less, annual household income less than $20,000, and mother's age at birth less than 20 years.

[2]Exposures have been log2-transformed and models have been adjusted for all listed exposures, child race, sex, age at exam, and HOME score; maternal marital status at child's birth, IQ, seafood consumption during pregnancy, and smoking during pregnancy; maternal and paternal education and annual household income at child's birth; study examiner.

[3]Main analysis group: complete working memory outcome, covariate and exposure data for PCBs, DDE, HCB, Pb and Mn. Total n=373; SDI < 3 n= 241; SDI ≥ 3 n=132.

[4]P-value of chemical-sex interaction term included in multivariable linear regression model.

*p<0.05

Abbreviations: DDE: dichlorodiphenyldichloroethylene; HCB: hexachlorobenzene; ΣPCB_4: Sum of 4 PCB congeners (118, 138, 153, 180); Pb: lead; Mn: manganese.

Supplemental Table 3.6 Characteristics of all New Bedford Cohort participants who were evaluated as adolescents and included in the secondary analysis[1] and those who were excluded.

Descriptive Characteristic	Secondary analysis group n =235			Excluded group			
Working Memory Measures[3]	n(%)	Mean (SD)	Range	n(%)	Mean ± SD	Range	p-value[2]
Verbal Working Memory	235	9.3 ± 2.5	1-14	293	8.5 ± 2.9	1-17	<0.001*
Symbolic Working Memory	235	10.1 ± 2.6	0-5	292	9.3 ± 2.8	1-19	<0.001*
Working Memory Index	235	97.9 ± 12.1		292	93.6 ± 13.8	57-142	< 0.001*
Exposure Measures[4]							
Cord serum DDE (ng/g)	235	0.6 (1.4)	0.02-14.9	516	0.4 (0.7)	0-10.2	0.04*
Cord serum HCB (ng/g)	235	0.03 (0.02)	0-0.1	516	0.03 (0.04)	0-0.7	0.2
Cord serum ΣPCB_4 (ng/g)	235	0.3 (0.3)	0.01-2.3	516	0.2 (0.3)	0.01-4.4	0.2
Cord blood Pb (µg/dL)	235	1.4 (0.9)	0-9.4	513	1.6 (1.5)	0-17.4	0.01*
Cord blood Mn (µg/dL)	235	4.3 (1.6)	1.7-11.2	473	4.2 (1.9)	0.2-22.1	0.8
Maternal hair total Hg (µg/g)	235	0.6 (0.6)	0.03-3.1	276	0.6 (0.7)	0.03-9.2	0.3
Maternal toenail As (µg/g)	235	0.1 (0.1)	0.02-0.8	181	0.1 (0.1)	0.02-1.0	0.5
Covariate Measures[5]							
Child Characteristics							
Race/Ethnicity							<0.001*
Non-Hispanic White	186 (79.1)			345 (62.4)			
Hispanic	16 (6.8)			73 (13.2)			
Other	33 (14.0)			133 (24.1)			
Missing	0			2 (0.4)			
Sex							0.3
Male	114 (48.5)			294 (53.2)			
Female	121 (51.5)			259 (46.8)			
Age at Exam	235	15.5 (0.6)	14.4-17.7	155	15.6 (0.7)	14.0-17.9	0.5
Home Score	235	44.4 (.0)	27-56	256	42.8 (6.5)	21-56	0.003*
Year of Birth							0.03*
1993-1994	76 (32.3)			183 (33.1)			
1995-1996	104 (44.3)			196 (35.4)			
1997-1998	55 (23.4)			174 (31.5)			
Maternal Characteristics							
Marital status at birth							<0.001*
Not married	74 (31.5)			257 (46.5)			
Married	161 (68.5)			241 (43.6)			
Missing	0			55 (9.9)			

Supplemental Table 3.6 (Continued)

Maternal IQ	235	100.6 (9.7)	67-124	400	96.3 (10.5)	57-126	<0.001*
Seafood during pregnancy (serv/day)	235	0.5 (0.6)	0-5.3	398	0.6 (0.7)	0-6	0.5
Smoking during pregnancy							0.3
No	171 (72.8)			311 (56.2)			
Yes	64 (27.2)			140 (25.3)			
Missing	0			102 (18.4)			
Maternal education							<0.001*
≤ High School	108 (46.0)			313 (56.6)			
> High School	127 (54.0)			183 (33.1)			
Missing	0			57 (10.3)			
Household Characteristics at Birth							
Paternal Education							0.01*
≤ High School	152 (64.7)			360 (65.1)			
> High School	83 (35.3)			125 (22.6)			
Missing	0			68 (12.3)			
Annual Household Income							<0.001*
< $20,000	62 (26.4)			203 (36.7)			
≥ $20,000	173 (73.6)			286 (51.7)			
Missing	0			64 (11.6)			
Examination Characteristics							
Examiner							0.3
1	171 (72.8)			227 (77.5)			
2	64 (27.2)			66 (22.5)			

[1]Secondary analysis group: complete working memory outcome, covariate and exposure data for DDE, HCB, PCBs, Pb Mn, MeHg, and As, n=235. [2]P-values represent results comparing characteristics between participants included in the secondary analysis group and those excluded from the secondary analysis group using t-tests and chi-square tests. [3]NBC participants with missing working memory measures: Verbal Working Memory n=260; Symbolic Working Memory n=261; Working Memory Index n=261. [4]NBC participants with missing exposure measures: DDE n=37; HCB n=37; ΣPCB_4 n=37; Pb n= 40; Mn n=80; Hg n=277; As n=372. [5]NBC participants with missing covariate measures: age at exam n=260; HOME score n= 297; maternal IQ n=153; seafood during pregnancy n= 155. *p<0.05.

Abbreviations: DDE: dichlorodiphenyldichloroethylene; HCB: hexachlorobenzene; ΣPCB_4: Sum of 4 PCB congeners (118, 138, 153, 180); Pb: lead; Mn: manganese; MeHg: methylmercury; As: arsenic.

Supplemental Table 3.7 Complete-case results of multivariable linear regression analyses (difference in points associated with a twofold increase in exposure and 95% CI)[1] assessing the relation of prenatal exposure to a seven-chemical mixture with Wide Range Assessment of Memory and Learning, 2nd Edition working memory scaled scores among adolescents in the secondary analysis group[2].

Exposure	Verbal Working Memory Difference (95% CI)	Symbolic Working Memory Difference (95% CI)	Working Memory Sum Index Difference (95% CI)
Log_2 DDE	-0.09 (-0.47, 0.30)	-0.02 (-0.42, 0.37)	-0.35 (-2.19, 1.50)
Log_2 HCB	0.12 (-0.25, 0.49)	0.17 (-0.21, 0.56)	0.85 (-0.93, 2.63)
Log_2 ΣPCB_4	0.10 (-0.33, 0.53)	0.00 (-0.44, 0.45)	0.29 (-1.79, 2.38)
Log_2 Pb	-0.16 (-0.55, 0.23)	-0.03 (-0.43, 0.37)	-0.52 (-2.39, 1.35)
Log_2 Mn	-0.51 (-1.18, 0.16)	0.01 (-0.68, 0.70)	-1.47 (-4.69, 1.75)
Log_2 MeHg	0.06 (-0.28, 0.40)	0.02 (-0.33, 0.37)	0.27 (-1.35, 1.89)
Log_2 As	0.10 (-0.23, 0.43)	-0.02 (-0.36, 0.32)	0.27 (-1.30, 1.84)

[1]Exposures have been log2-transformed and models have been adjusted for all listed exposures, child race, sex, age at exam, and HOME score; maternal marital status at child's birth, IQ, seafood consumption during pregnancy, and smoking during pregnancy; maternal and paternal education and annual household income at child's birth; study examiner.
[2]Secondary analysis group: complete working memory outcome, covariate and exposure data for DDE, HCB, PCBs, Pb, Mn, MeHg, and As, n=235.
*$p<0.05$
Abbreviations: DDE: dichlorodiphenyldichloroethylene; HCB: hexachlorobenzene; ΣPCB_4: Sum of 4 PCB congeners (118, 138, 153, 180); Pb: lead; Mn: manganese; MeHg: methylmercury; As: arsenic.

Supplemental Table 3.8 Inverse probability weighted results of multivariable linear regression analyses (difference in points associated with a twofold increase in exposure and 95% CI)[1] assessing the relation of prenatal exposure to a five-chemical mixture with Wide Range Assessment of Memory and Learning, 2nd Edition working memory scaled scores among adolescents in the secondary analysis group[2].

Exposure	Verbal Working Memory Difference (95% CI)	Symbolic Working Memory Difference (95% CI)	Working Memory Sum Index Difference (95% CI)
Log_2 DDE	-0.12 (-0.50, 0.26)	-0.05 (-0.43, 0.33)	-0.50 (-2.31, 1.31)
Log_2 HCB	0.04 (-0.32, 0.41)	0.12 (-0.25, 0.48)	0.47 (-1.26, 2.20)
Log_2 ΣPCB_4	0.19 (-0.25, 0.63)	0.09 (-0.35, 0.53)	0.77 (-1.32, 2.86)
Log_2 Pb	-0.22 (-0.59, 0.15)	-0.04 (-0.42, 0.33)	-0.70 (-2.48, 1.08)
Log_2 Mn	-0.30 (-0.99, 0.39)	0.30 (-0.39, 0.99)	-0.09 (-3.36, 3.17)
Log_2 MeHg	0.16 (-0.20, 0.51)	0.06 (-0.29, 0.41)	0.63 (-1.04, 2.31)
Log_2 As	0.09 (-0.26, 0.44)	-0.05 (-0.40, 0.29)	0.15 (-1.49, 1.79)

[1]Exposures have been log2-transformed and models have been adjusted for all listed exposures, child race, sex, age at exam, and HOME score; maternal marital status at child's birth, IQ, seafood consumption during pregnancy, and smoking during pregnancy; maternal and paternal education and annual household income at child's birth; study examiner.
[2]Secondary analysis group: complete working memory outcome, covariate and exposure data for DDE, HCB, PCBs, Pb, Mn, MeHg, and As, n=235.
*$p<0.05$
Abbreviations: DDE: dichlorodiphenyldichloroethylene; HCB: hexachlorobenzene; ΣPCB_4: Sum of 4 PCB congeners (118, 138, 153, 180); Pb: lead; Mn: manganese; MeHg: methylmercury; As: arsenic.

Supplemental Tables from Chapter 4

Supplemental Table 4.1 Inverse-probability weighted results of multivariable linear regression analyses (difference in points associated with a twofold increase in exposure and 95% CI)[1] assessing the relation of prenatal exposure to a five-chemical mixture with Delis-Kaplan Executive Function System (D-KEFS) cognitive flexibility scaled scores among New Bedford Cohort adolescents in the main analysis group[2].

Exposure	Trail-making completion time (seconds) Difference (95% CI)	Verbal Fluency switching accuracy Difference (95% CI)	Design Fluency total correct Difference (95% CI)	Color-Word Interference completion time (seconds) Difference (95% CI)
Log_2 DDE	-0.23 (-0.52, 0.06)	-0.09 (-0.38, 0.20)	0.00 (-0.31, 0.30)	-0.08 (-0.34, 0.18)
Log_2 HCB	0.07 (-0.19, 0.33)	-0.06 (-0.33, 0.20)	1.15 (0.28, 2.03)*	0.10 (-0.14, 0.33)
Log_2 HCB^2	-	-	0.07 (0.02, 0.12)*	-
Log_2 ΣPCB_4	0.07 (-0.24, 0.39)	0.02 (-0.29, 0.34)	-0.11 (-0.45, 0.22)	0.18 (-0.10, 0.47)
Log_2 Pb	0.12 (-0.14, 0.38)	0.29 (0.03, 0.55)*	0.07 (-0.20, 0.35)	0.17 (-0.06, 0.40)
Log_2 Mn	-0.60 (-1.16, -0.04)*	-0.31 (-0.88, 0.25)	-0.22 (-0.81, 0.37)	-0.20 (-0.70, 0.30)

[1]Exposures have been log2-transformed and models have been adjusted for child race, sex, age at exam, year of birth, and HOME score; maternal marital status at child's birth, IQ, seafood consumption during pregnancy, and smoking during pregnancy; maternal and paternal education and annual household income at child's birth; and study examiner.
[2]Main analysis group: complete outcome, covariate and prenatal exposure biomarker data for DDE, HCB, ΣPCB_4, Pb and Mn, n=373.
*$p<0.05$
Abbreviations: DDE: dichlorodiphenyldichloroethylene; HCB: hexachlorobenzene; ΣPCB_4: Sum of 4 PCB congeners (118, 138, 153, 180); Pb: lead; Mn: manganese.

Supplemental Table 4.2 Sex-stratified complete-case results of multivariable linear regression analyses (difference in points associated with a twofold increase in exposure and 95% CI)[1] assessing the relation of prenatal exposure to a five-chemical mixture with Delis-Kaplan Executive Function System (D-KEFS) cognitive flexibility scaled scores among adolescents in the main analysis group[2].

	Trail-making completion time Difference (95% CI)			Verbal Fluency switching accuracy Difference (95% CI)		
	Males	Females	p for interaction	Males	Females	p for interaction
Log_2 DDE	-0.30 (-0.72, 0.12)	-0.14 (-0.58, 0.30)	0.6	-0.25 (-0.66, 0.15)	0.02 (-0.45, 0.50)	0.3
Log_2 HCB	0.07 (-0.30, 0.43)	0.14 (-0.31, 0.59)	0.6	-0.17 (-0.52, 0.18)	-0.06 (-0.55, 0.43)	1.0
Log_2 ΣPCB_4	0.19 (-0.28, 0.65)	-0.16 (-0.64, 0.32)	0.6	0.09 (-0.36, 0.54)	-0.05 (-0.56, 0.47)	0.4
Log_2 Pb	0.44 (-0.10, 0.97)	0.00 (-0.29, 0.29)	0.07	0.64 (0.12, 1.16)*	0.11 (-0.21, 0.43)	0.2
Log_2 Mn	-0.14 (-0.94, 0.66)	-0.80 (-1.60, -0.01)	0.3	-0.48 (-1.26, 0.29)	-0.07 (-0.94, 0.79)	0.6

	Design Fluency total correct Difference (95% CI)			Color-Word Interference completion time Difference (95% CI)		
	Males	Females	p for interaction	Males	Females	p for interaction
Log_2 DDE	0.16 (-0.31, 0.64)	-0.09 (-0.54, 0.36)	0.5	0.10 (-0.33, 0.52)	0.10 (-0.31, 0.51)	1.0
Log_2 HCB	0.39 (-1.03, 1.81)	4.19 (1.48, 6.90)*	0.02*	-0.06 (-0.42, 0.30)	0.07 (-0.35, 0.49)	0.5
Log_2 HCB^2	0.04 (-0.04, 0.11)	0.30 (0.07, 0.53)*	0.04*	-	-	-
Log_2 ΣPCB_4	-0.07 (-0.59, 0.46)	-0.32 (-0.82, 0.17)	0.7	-0.36 (-0.83, 0.10)	-0.07 (-0.52, 0.37)	0.2
Log_2 Pb	0.30 (-0.30, 0.90)	-0.08 (-0.38, 0.22)	0.2	0.03 (-0.51, 0.57)	-0.05 (-0.32, 0.22)	0.7
Log_2 Mn	-0.49 (-1.39, 0.40)	0.44 (-0.38, 1.26)	0.1	-0.53 (-1.34, 0.27)	-0.30 (-1.04, 0.44)	0.8

[1]Exposures have been log2-transformed and models have been adjusted for child race, sex, age at exam, year of birth, and HOME score; maternal marital status at child's birth, IQ, seafood consumption during pregnancy, and smoking during pregnancy; maternal and paternal education and annual household income at child's birth; and study examiner.
[2]Main analysis group: complete outcome, covariate and prenatal exposure biomarker data for DDE, HCB, ΣPCB_4, Pb and Mn. Total n=373; Males n= 179; Females n=194.
*$p<0.05$
Abbreviations: DDE: dichlorodiphenyldichloroethylene; HCB: hexachlorobenzene; ΣPCB_4: Sum of 4 PCB congeners (118, 138, 153, 180); Pb: lead; Mn: manganese

Supplemental Table 4.3 Prenatal social disadvantage index (PNSDI)[1]-stratified complete-case results of multivariable linear regression analyses (difference in points associated with a twofold increase in exposure and 95% CI)[2] assessing the relation of prenatal exposure to a five-chemical mixture with Delis-Kaplan Executive Function System (D-KEFS) cognitive flexibility scaled scores among adolescents in the main analysis group[3].

	Trail-making completion time Difference (95% CI)			Verbal Fluency switching accuracy Difference (95% CI)		
	PNSDI < 3	PNSDI ≥ 3	p for interaction	PNSDI < 3	PNSDI ≥ 3	p for interaction
Log_2 DDE	-0.13 (-0.46, 0.20)	-0.37 (-0.98, 0.24)	0.3	-0.12 (-0.48, 0.24)	-0.16 (-0.72, 0.40)	0.9
Log_2 HCB	0.23 (-0.14, 0.60)	-0.07 (-0.51, 0.36)	0.2	0.07 (-0.34, 0.47)	-0.17 (-0.58, 0.23)	0.4
Log_2 ΣPCB_4	-0.10 (-0.47, 0.26)	0.40 (-0.28, 1.07)	0.3	0.09 (-0.30, 0.49)	0.12 (-0.50, 0.74)	0.9
Log_2 Pb	-0.01 (-0.30, 0.28)	0.08 (-0.49, 0.66)	0.7	0.20 (-0.12, 0.51)	0.26 (-0.28, 0.79)	0.6
Log_2 Mn	-0.64 (-1.31, 0.02)	-0.38 (-1.46, 0.69)	0.9	-0.29 (-1.02, 0.43)	-0.24 (-1.23, 0.76)	0.7

	Design Fluency total correct Difference (95% CI)			Color-Word Interference completion time Difference (95% CI)		
	PNSDI < 3	PNSDI ≥ 3	p for interaction	PNSDI < 3	PNSDI ≥ 3	p for interaction
Log_2 DDE	0.02 (-0.38, 0.42)	-0.19 (-0.72, 0.34)	0.4	0.21 (-0.12, 0.53)	-0.08 (-0.65, 0.49)	0.2
Log_2 HCB	3.05 (0.58, 5.53)*	-0.24 (-1.65, 1.17)	0.04*	0.13 (-0.24, 0.49)	-0.09 (-0.50, 0.32)	0.3
Log_2 HCB^2	0.20 (0.00, 0.41)*	0.00 (-0.07, 0.08)	0.09	-	-	-
Log_2 ΣPCB_4	-0.32 (-0.75, 0.12)	0.40 (-0.18, 0.99)	0.07	-0.36 (-0.71, 0.00)	0.21 (-0.42, 0.83)	0.2
Log_2 Pb	-0.05 (-0.40, 0.29)	0.13 (-0.38, 0.63)	0.5	-0.13 (-0.41, 0.15)	0.20 (-0.34, 0.74)	0.3
Log_2 Mn	-0.12 (-0.92, 0.68)	-0.29 (-1.22, 0.65)	0.7	-0.50 (-1.16, 0.15)	-0.51 (-1.51, 0.49)	0.9

[1]Prenatal social disadvantage index (PNSDI) was constructed as the sum of five adverse social or economic exposures at the time of the child's birth where presence of each risk factor was assigned a value of 1, absence a value of 0: mother unmarried, mother's education as high school graduate or less, father's education as high school graduate or less, annual household income less than $20,000, and mother's age at birth less than 20 years.

[2]Exposures have been log2-transformed and models have been adjusted for child race, sex, age at exam, year of birth, and HOME score; maternal marital status at child's birth, IQ, seafood consumption during pregnancy, and smoking during pregnancy; maternal and paternal education and annual household income at child's birth; and study examiner.

[3]Main analysis group: complete outcome, covariate and prenatal exposure biomarker data for DDE, HCB, ΣPCB_4, Pb and Mn. Total n=373; PNSDI < 3 n= 241; PNSDI ≥ 3 n=132.

*p<0.05

Abbreviations: DDE: dichlorodiphenyldichloroethylene; HCB: hexachlorobenzene; ΣPCB_4: Sum of 4 PCB congeners (118, 138, 153, 180); Pb: lead; Mn: manganese.

Supplemental Table 4.4 Sex-stratified inverse probability weighted results of multivariable linear regression analyses (difference in points associated with a twofold increase in exposure and 95% CI)[1] assessing the relation of prenatal exposure to a five-chemical mixture with Delis-Kaplan Executive Function System (D-KEFS) cognitive flexibility scaled scores among New Bedford Cohort adolescents in the main analysis group[2].

	Trail-making completion time Difference (95 % CI)			Verbal Fluency switching accuracy Difference (95% CI)		
	Males	Females	p for interaction	Males	Females	p for interaction
Log_2 DDE	-0.29 (-0.71, 0.14)	-0.14 (-0.58, 0.30)	0.6	-0.26 (-0.66, 0.14)	0.07 (-0.40, 0.54)	0.2
Log_2 HCB	0.04 (-0.31, 0.38)	0.14 (-0.31, 0.59)	0.6	-0.19 (-0.52, 0.14)	-0.09 (-0.58, 0.39)	0.9
Log_2 ΣPCB_4	0.20 (-0.27, 0.66)	-0.16 (-0.64, 0.32)	0.7	0.08 (-0.37, 0.52)	-0.10 (-0.60, 0.41)	0.3
Log_2 Pb	0.48 (-0.07, 1.02)	0.00 (-0.29, 0.29)	0.08	0.72 (0.20, 1.24)*	0.12 (-0.19, 0.43)	0.1
Log_2 Mn	-0.13 (-0.95, 0.69)	-0.80 (-1.60, -0.01)	0.3	-0.59 (-1.37, 0.19)	0.02 (-0.84, 0.87)	0.4

	Design Fluency total correct Difference (95% CI)			Color-Word Interference completion time Difference (95% CI)		
	Males	Females	p for interaction	Males	Females	p for interaction
Log_2 DDE	0.17 (-0.30, 0.65)	-0.03 (-0.47, 0.41)	0.5	0.04 (-0.38, 0.46)	0.09 (-0.33, 0.50)	0.8
Log_2 HCB	0.15 (-1.24, 1.55)*	3.83 (1.11, 6.55)*	0.03*	-0.09 (-0.43, 0.26)	0.06 (-0.36, 0.49)	0.4
Log_2 HCB^2	0.03 (-0.05, 0.10)*	0.27 (0.03, 0.50)*	0.09	-	-	-
Log_2 ΣPCB_4	-0.03 (-0.55, 0.49)	-0.38 ((-0.86, 0.10)	0.5	-0.35 (-0.82, 0.11)	0.00 (-0.44, 0.45)	0.1
Log_2 Pb	0.33 (-0.26, 0.93)	-0.06 (-0.35, 0.23)	0.2	0.09 (-0.45, 0.63)	-0.03 (-0.30, 0.25)	0.7
Log_2 Mn	-0.62 (-1.53, 0.28)	0.49 (-0.31, 1.29)	0.06	-0.55 (-1.36, 0.26)	-0.44 (-1.19, 0.32)	1.0

[1]Exposures have been log2-transformed and models have been adjusted for child race, sex, age at exam, year of birth, and HOME score; maternal marital status at child's birth, IQ, seafood consumption during pregnancy, and smoking during pregnancy; maternal and paternal education and annual household income at child's birth; and study examiner.
[2]Main analysis group: complete outcome, covariate and prenatal exposure biomarker data for DDE, HCB, ΣPCB_4, Pb and Mn. Total n=373; Males n= 179; Females n=194.
*$p<0.05$
Abbreviations: DDE: dichlorodiphenyldichloroethylene; HCB: hexachlorobenzene; ΣPCB_4: Sum of 4 PCB congeners (118, 138, 153, 180); Pb: lead; Mn: manganese

Supplemental Table 4.5 Prenatal social disadvantage index (PNSDI)[1]-stratified inverse probability weighted results of multivariable linear regression analyses (difference in points associated with a twofold increase in exposure and 95% CI)[2] assessing the relation of prenatal exposure to a five-chemical mixture with Delis-Kaplan Executive Function System (D-KEFS) cognitive flexibility scaled scores among New Bedford Cohort adolescents in the main analysis group[3].

	Trail-making completion time Difference (95% CI)			Verbal Fluency switching accuracy Difference (95% CI)		
	PNSDI < 3	PNSDI ≥ 3	p for interaction	PNSDI < 3	PNSDI ≥ 3	p for interaction
Log_2 DDE	-0.10 (-0.42, 0.23)	-0.42 (-1.04, 0.19)	0.2	-0.10 (-0.45, 0.25)	-0.16 (-0.73, 0.41)	0.8
Log_2 HCB	0.23 (-0.15, 0.61)	-0.06 (-0.47, 0.36)	0.2	0.07 (-0.34, 0.47)	-0.18 (-0.57, 0.20)	0.3
Log_2 ΣPCB_4	-0.09 (-0.45, 0.27)	0.43 (-0.22, 1.09)	0.2	0.08 (-0.31, 0.47)	0.06 (-0.55, 0.67)	0.8
Log_2 Pb	0.01 (-0.27, 0.30)	0.06 (-0.53, 0.65)	0.8	0.21 (-0.10, 0.52)	0.34 (-0.21, 0.89)	0.4
Log_2 Mn	-0.72 (-1.39, -0.04)*	-0.30 (-1.37, 0.76)	0.7	-0.33 (-1.06, 0.39)	-0.23 (-1.22, 0.76)	0.7

	Design Fluency total correct Difference (95% CI)			Color-Word Interference completion time Difference (95% CI)		
	PNSDI < 3	PNSDI ≥ 3	p for interaction	PNSDI < 3	PNSDI ≥ 3	p for interaction
Log_2 DDE	0.06 (-0.32, 0.44)	-0.20 (-0.74, 0.35)	0.3	0.22 (-0.09, 0.53)	-0.16 (-0.75, 0.42)	0.2
Log_2 HCB	2.87 (0.39, 5.35)*	-0.37 (-1.80, 1.05)	0.05*	0.12 (-0.25, 0.48)	-0.07 (-0.47, 0.32)	0.3
Log_2 HCB^2	0.19 (-0.01, 0.39)	0.00 (-0.08, 0.07)	0.1	-	-	-
Log_2 ΣPCB_4	-0.33 (-0.75, 0.10)	0.38 (-0.21, 0.97)	0.06	-0.35 (-0.69, 0.00)	0.23 (-0.40, 0.85)	0.2
Log_2 Pb	-0.04 (-0.38, 0.30)	0.18 (-0.34, 0.71)	0.5	-0.12 (-0.39, 0.16)	0.23 (-0.33, 0.79)	0.3
Log_2 Mn	-0.22 (-1.02, 0.57)	-0.35 (-1.29, 0.60)	0.7	-0.60 (-1.25, 0.06)	-0.47 (-1.49, 0.55)	0.8

[1]Prenatal social disadvantage index (PNSDI) was constructed as the sum of five adverse social or economic exposures at the time of the child's birth where presence of each risk factor was assigned a value of 1, absence a value of 0: mother unmarried, mother's education as high school graduate or less, father's education as high school graduate or less, annual household income less than $20,000, and mother's age at birth less than 20 years.
[2]Exposures have been log2-transformed and models have been adjusted for child race, sex, age at exam, year of birth, and HOME score; maternal marital status at child's birth, IQ, seafood consumption during pregnancy, and smoking during pregnancy; maternal and paternal education and annual household income at child's birth; and study examiner.
[3]Main analysis group: complete outcome, covariate and prenatal exposure biomarker data for DDE, HCB, ΣPCB_4, Pb and Mn. Total n=373; PNSDI < 3 n= 241; PNSDI ≥ 3 n=132.
*p<0.05
Abbreviations: DDE: dichlorodiphenyldichloroethylene; HCB: hexachlorobenzene; ΣPCB_4: Sum of 4 PCB congeners (118, 138, 153, 180); Pb: lead; Mn: manganese

Supplemental Table 4.6 Inverse probability weighted results of negative binomial regression analyses (RR (rate ratio) and 95% CI)[1] assessing the relation of prenatal exposure to a five-chemical mixture with Delis-Kaplan Executive Function System (D-KEFS) cognitive flexibility error raw scores among New Bedford Cohort adolescents in the main analysis group[2].

Exposure	Trail-making total errors RR (95% CI)	Verbal Fluency total errors RR (95% CI)	Design Fluency total errors RR (95% CI)	Color-Word Interference total errors RR (95% CI)
Log_2 DDE	1.00 (0.88, 1.13)	1.24 (1.06, 1.46)*	0.99 (0.89, 1.12)	0.97 (0.89, 1.06)
Log_2 HCB	1.02 (0.90, 1.16)	0.90 (0.81, 0.99)	1.07 (0.95, 1.21)	1.04 (0.96, 1.14)
Log_2 ΣPCB_4	1.01 (0.88, 1.16)	0.94 (0.79, 1.12)	0.98 (0.87, 1.12)	1.06 (0.96, 1.17)
Log_2 Pb	0.97 (0.88, 1.08)	1.14 (0.97, 1.35)	1.03 (0.91, 1.15)	1.10 (1.00, 1.21)*
Log_2 Mn	1.28 (1.00, 1.64)	0.79 (0.59, 1.07)	0.82 (0.66, 1.04)	0.96 (0.80, 1.14)

[1]Exposures have been log2-transformed and models have been adjusted for child race, sex, age at exam, year of birth, and HOME score; maternal marital status at child's birth, IQ, seafood consumption during pregnancy, and smoking during pregnancy; maternal and paternal education and annual household income at child's birth; and study examiner.
[2]Main analysis group: complete outcome, covariate and prenatal exposure biomarker data for DDE, HCB, ΣPCB_4, Pb and Mn, n=373.
*$p<0.05$
Abbreviations: DDE: dichlorodiphenyldichloroethylene; HCB: hexachlorobenzene; ΣPCB_4: Sum of 4 PCB congeners (118, 138, 153, 180); Pb: lead; Mn: manganese.

Supplemental Table 4.7 Inverse probability weighted results of logistic regression analyses (odds ratio (OR) and 95% CI)[1] assessing the relation of prenatal exposure to a five-chemical mixture with Delis-Kaplan Executive Function System (D-KEFS) Trail-making and Color-Word Interference performance[2] among New Bedford Cohort adolescents in the main analysis group[3].

Exposure	Trail-making Overall Performance OR (95% CI)	Color-Word Interference Overall Performance OR (95% CI)
Log_2 DDE	1.13 (0.86, 1.49)	0.91 (0.64, 1.28)
Log_2 HCB	0.95 (0.72, 1.19)	1.15 (0.88, 1.50)
Log_2 ΣPCB_4	0.94 (0.72, 1.26)	1.46 (1.03, 2.13)*
Log_2 Pb	1.05 (0.82, 1.34)	1.34 (1.00, 1.88)
Log_2 Mn	1.56 (0.93, 2.69)	1.61 (0.87, 3.07)

[1]Exposures have been log2-transformed and models have been adjusted for child race, sex, age at exam, year of birth, and HOME score; maternal marital status at child's birth, IQ, seafood consumption during pregnancy, and smoking during pregnancy; maternal and paternal education and annual household income at child's birth; and study examiner.
[2]Performance takes into account both completion time and total errors raw scores. Those in the best performance group include anyone with < median level completion time and < median level total errors with the remaining observations in the poor performance group.
[2]Main analysis group: complete outcome, covariate and prenatal exposure biomarker data for DDE, HCB, ΣPCB_4, Pb and Mn, n=373.
*$p<0.05$
Abbreviations: DDE: dichlorodiphenyldichloroethylene; HCB: hexachlorobenzene; ΣPCB_4: Sum of 4 PCB congeners (118, 138, 153, 180); Pb: lead; Mn: manganese

Supplemental Table 4.8 Characteristics of New Bedford Cohort participants who were evaluated as adolescents and included in the secondary analysis group[1], and those who were excluded from the secondary analysis group.

Descriptive Characteristic	Secondary analysis group n =235			Excluded group			
Cognitive Flexibility Measures[3]	n(%)	Mean ± SD	Range	n(%)	Mean ± SD	Range	p-value[2]
Trail-making							
Completion time scaled score	235	9.8 (2.7)	1-14	293	9.1 (2.8)	1-14	0.005*
Total errors	235	0.8 (1.1)	0-5	292	1 (1.3)	0-13	0.03*
Overall Trail-making performance							
Best performance	83 (35.3)			75 (25.6)			0.02*
Poor performance	152 (64.7)			217 (74.1)			
Missing	0			1 (0.3)			
Verbal Fluency scores							
Total switching accuracy scaled score	235	9.2 (2.8)	3-17	293	9.1 (2.8)	1-17	0.6
Total errors	235	0.8 (1.2)	0-7	293	0.9 (1.2)	0-7	0.4
Design Fluency							
Total correct scaled score	235	10.0 (2.8)	2-18	293	9.7 (2.7)	2-17	0.2
Total errors	235	2.5 (3.0)	0-22	293	2.7 (3.0)	0-20	0.2
Color-Word Interference							
Completion time scaled score		10 (2.6)	1-15	292	9.8 (2.6)	1-15	0.2
Total errors		2.6 (2.5)	0-19	292	2.8 (2.4)	0-11	0.2
Overall Color-Word Interference performance							
Best performance	58 (24.7)			59 (20.1)			1.0
Poor performance	177 (75.3)			233 (79.5)			
Missing	0			1 (0.3)			
Exposure Measures[4]							
Cord serum DDE (ng/g)	235	0.6 (1.4)	0.02-14.9	516	0.4 (0.7)	0-10.2	0.04*
Cord serum HCB (ng/g)	235	0.03 (0.02)	0-0.1	516	0.03 (0.04)	0-0.7	0.2
Cord serum ΣPCB_4 (ng/g)	235	0.3 (0.3)	0.01-2.3	516	0.2 (0.3)	0.01-4.4	0.2
Cord blood Pb (µg/dL)	235	1.4 (0.9)	0-9.4	513	1.6 (1.5)	0-17.4	0.01*
Cord blood Mn (µg/dL)	235	4.3 (1.6)	1.7-11.2	473	4.2 (1.9)	0.2-22.1	0.8
Maternal hair total Hg (µg/g)	235	0.6 (0.6)	0.03-3.1	276	0.6 (0.7)	0.03-9.2	0.3
Maternal toenail As (µg/g)	235	0.1 (0.1)	0.02-0.8	181	0.1 (0.1)	0.02-1.0	0.5
Covariate Measures[5]							
Child Characteristics							
Race/Ethnicity							<0.001*
Non-Hispanic White	186 (79.1)			345 (62.4)			

Supplemental Table 4.6 (Continued)

Hispanic	16 (6.8)			73 (13.2)			
Other	33 (14.0)			133 (24.1)			
Missing	0			2 (0.4)			
Sex							0.3
Male	114 (48.5)			294 (53.2)			
Female	121 (51.5)			259 (46.8)			
Age at Exam	235	15.5 (0.6)	14.4-17.7	155	15.6 (0.7)	14.0-17.9	0.5
Home Score	235	44.4 (.0)	27-56	256	42.8 (6.5)	21-56	0.003*
Year of Birth							0.03*
1993-1994	76 (32.3)			183 (33.1)			
1995-1996	104 (44.3)			196 (35.4)			
1997-1998	55 (23.4)			174 (31.5)			
Maternal Characteristics							
Marital status at birth							<0.001*
Not married	74 (31.5)			257 (46.5)			
Married	161 (68.5)			241 (43.6)			
Missing	0			55 (9.9)			
Maternal IQ	235	100.6 (9.7)	67-124	400	96.3 (10.5)	57-126	<0.001*
Seafood during pregnancy (serv/day)	235	0.5 (0.6)	0-5.3	398	0.6 (0.7)	0-6	0.5
Smoking during pregnancy							0.3
No	171 (72.8)			311 (56.2)			
Yes	64 (27.2)			140 (25.3)			
Missing	0			102 (18.4)			
Maternal education							<0.001*
≤ High School	108 (46.0)			313 (56.6)			
> High School	127 (54.0)			183 (33.1)			
Missing	0			57 (10.3)			
Household Characteristics at Birth							
Paternal Education							0.01*
≤ High School	152 (64.7)			360 (65.1)			
> High School	83 (35.3)			125 (22.6)			
Missing	0			68 (12.3)			
Annual Household Income							<0.001*
< $20,000	62 (26.4)			203 (36.7)			
≥ $20,000	173 (73.6)			286 (51.7)			
Missing	0			64 (11.6)			
Examination Characteristics							
Examiner							0.3

Supplemental Table 4.6 (Continued)

1	171 (72.8)	227 (77.5)	
2	64 (27.2)	66 (22.5)	

[1]Secondary analysis group: complete outcome, covariate and prenatal exposure biomarker data for DDE, HCB, ΣPCB_4, Pb Mn, MeHg, and As, n=235.[2]P-values represent results comparing characteristics between participants included in the secondary analysis group and those excluded from the secondary analysis group using t-tests, chi-square, and Wilcoxon rank sum tests. P-values reflect comparisons between groups with non-missing data. [3]NBC participants with missing cognitive flexibility measures: Trail-making completion time n=260, total errors n = 261; Verbal Fluency total switching accuracy n = 260, total errors n= 260; Design Fluency total correct n= 260, total errors n=260; Color-Word Interference completion time n = 261, total errors n=261. [4]NBC participants with missing exposure measures: DDE n=37; HCB n=37; ΣPCB_4 n=37; Pb n= 40; Mn n=80; Hg n=277; As n=372. [5]NBC participants with missing covariate measures: age at exam n=260; HOME score n= 297; maternal IQ n=153; seafood during pregnancy n= 155. *$p<0.05$.
Abbreviations: DDE: dichlorodiphenyldichloroethylene; HCB: hexachlorobenzene; ΣPCB_4: Sum of 4 PCB congeners (118, 138, 153, 180); Pb: lead; Mn: manganese; MeHg: methylmercury; As: arsenic.

Supplemental Table 4.9 Complete-case results of multivariable linear regression analyses (difference in points associated with a twofold increase in exposure and 95% CI)[1] assessing the relation of prenatal exposure to a seven-chemical mixture with Delis-Kaplan Executive Function System (D-KEFS) cognitive flexibility scaled scores among New Bedford Cohort adolescents in the secondary analysis group[2].

Exposure	Trail-making completion time Difference (95% CI)	Verbal Fluency switching accuracy Difference (95% CI)	Design Fluency total correct Difference (95% CI)	Color-Word Interference completion time Difference (95% CI)
Log_2 DDE	-0.03 (-0.44, 0.37)	0.05 (-0.37, 0.48)	-0.11 (-0.54, 0.33)	0.21 (-0.19, 0.62)
Log_2 HCB	0.11 (-0.21, 0.43)	0.02 (-0.31, 0.35)	0.07 (-0.27, 0.41)	0.02 (-0.30, 0.34)
Log_2 ΣPCB_4	-0.05 (-0.50, 0.41)	-0.33 (-0.80, 0.15)	0.00 (-0.49, 0.49)	-0.51 (-0.96, -0.05)*
Log_2 Pb	0.01 (-0.30, 0.31)	0.23 (-0.09, 0.55)	-0.09 (-0.42, 0.24)	-0.04 (-0.35, 0.27)
Log_2 Mn	-0.52 (-1.23, 0.18)	-0.48 (-1.22, 0.26)	6.67 (2.25, 11.09)*	-0.37 (-1.08, 0.33)
Log_2 Mn^2	-	-	-1.64 (-2.69, -0.59)*	-
Log_2 MeHg	-0.09 (-0.45, 0.27)	0.21 (-0.16, 0.58)	-0.04 (-0.42, 0.34)	0.21 (-0.15, 0.56)
Log_2 As	0.10 (-0.24, 0.45)	0.17 (-0.19, 0.53)	0.04 (-0.33, 0.41)	0.03 (-0.31, 0.38)

[1]Exposures have been log2-transformed and models have been adjusted for child race, sex, age at exam, year of birth, and HOME score; maternal marital status at child's birth, IQ, seafood consumption during pregnancy, and smoking during pregnancy; maternal and paternal education and annual household income at child's birth; and study examiner.
[2]Secondary analysis group: complete outcome, covariate and prenatal exposure biomarker data for DDE, HCB, ΣPCB_4, Pb, Mn, MeHg, and As, n=235.
*$p<0.05$
Abbreviations: DDE: dichlorodiphenyldichloroethylene; HCB: hexachlorobenzene; ΣPCB_4: Sum of 4 PCB congeners (118, 138, 153, 180); Pb: lead; Mn: manganese; MeHg: methylmercury; As: arsenic.

Supplemental Table 4.10 Inverse-probability weighted results of multivariable linear regression analyses (difference in points associated with a twofold increase in exposure and 95% CI)[1] assessing the relation of prenatal exposure to a seven-chemical mixture with Delis-Kaplan Executive Function System (D-KEFS) cognitive flexibility scaled scores among New Bedford Cohort adolescents in the secondary analysis group[2].

Exposure	Trail-making completion time Difference (95% CI)	Verbal Fluency switching accuracy Difference (95% CI)	Design Fluency total correct Difference (95% CI)	Color-Word Interference completion time Difference (95% CI)
Log_2 DDE	-0.12 (-0.53, 0.30)	0.06 (-0.36, 0.47)	-0.17 (-0.60, 0.25)	0.07 (-0.32, 0.47)
Log_2 HCB	0.08 (-0.22, 0.39)	-0.05 (-0.35, 0.26)	0.01 (-0.31, 0.32)	0.04 (-0.25, 0.33)
Log_2 ΣPCB_4	0.02 (-0.45, 0.50)	-0.43 (-0.90, 0.05)	0.07 (-0.42, 0.55)	-0.01 (-0.46, 0.44)
Log_2 Pb	-0.02 (-0.34, 0.31)	0.29 (-0.04, 0.62)	-0.05 (-0.38, 0.28)	0.10 (-0.20, 0.41)
Log_2 Mn	-0.39 (-1.13, 0.36)	-0.36 (-1.11, 0.39)	6.16 (1.70, 10.61)*	0.01 (-0.69, 0.72)
Log_2 Mn^2	-	-	-1.53 (-2.58, -0.49)*	-
Log_2 MeHg	-0.04 (-0.42, 0.35)	0.26 (-0.13, 0.65)	-0.11 (-0.51, 0.28)	0.13 (-0.24, 0.49)
Log_2 As	0.09 (-0.29, 0.46)	0.18 (-0.20, 0.56)	0.12 (-0.26, 0.50)	0.29 (-0.07, 0.64)

[1] Exposures have been log2-transformed and models have been adjusted for child race, sex, age at exam, year of birth, and HOME score; maternal marital status at child's birth, IQ, seafood consumption during pregnancy, and smoking during pregnancy; maternal and paternal education and annual household income at child's birth; and study examiner.

[2]Secondary analysis group: complete outcome, covariate and prenatal exposure biomarker data for DDE, HCB, ΣPCB_4, Pb, Mn, MeHg, and As, n=235.

*$p<0.05$

Abbreviations: DDE: dichlorodiphenyldichloroethylene; HCB: hexachlorobenzene; ΣPCB_4: Sum of 4 PCB congeners (118, 138, 153, 180); Pb: lead; Mn: manganese; MeHg: methylmercury; As: arsenic.

Supplemental Tables from Chapter 5

Supplemental Table 5.1 Sex-stratified results of multivariable linear regression analyses of difference (95% confidence intervals)[1] in inhibition, working memory, cognitive flexibility, and problem-solving scaled scores per doubling of cord blood manganese concentrations among New Bedford Cohort adolescents.

Executive Function Outcome Measures	Overall (n=379) Difference (95% CI)	Males (n=181) Difference (95% CI)	Females (n=198) Difference (95% CI)	p for interaction
Inhibition				
Design Fluency: Empty Dots Only[2]	0.17 (-0.39, 0.73)	0.66 (-0.12, 1.44)*	-0.22 (-1.01, 0.57)	0.1
Color-Word Interference: Inhibition[2]	-0.64 (-1.22, -0.05)**	-0.70 (-1.55, 0.15)	-0.44 (-1.28, 0.40)	0.6
Working Memory				
Verbal Working Memory[3]	-0.81 (-1.34, -0.28)**	-0.93 (-1.71, -0.15)**	-0.49 (-1.24, 0.26)	0.7
Symbolic Working Memory[3]	-0.43 (-0.97, 0.12)	0.02 (-0.77, 0.81)	-0.56 (-1.30, 0.19)	0.3
Cognitive Flexibility				
Trail-making: Number-Letter Switching[2]	-0.49 (-1.03, 0.06)*	-0.02 (-0.80, 0.76)	-0.73 (-1.51, 0.04)*	0.2
Verbal Fluency: Category Switching[2]	-0.22 (-0.76, 0.33)	-0.35 (-1.10, 0.40)	-0.04 (-0.86, 0.79)	0.6
Design Fluency: Filled & Empty Dots Switching[2]	-0.05 (-0.63, 0.53)	-0.46 (-1.31, 0.40)	0.47 (-0.34, 1.29)	0.1
Color-Word Interference Inhibition/Switching[2]	-0.43 (-0.96, 0.11)	-0.35 (-1.14, 0.44)	-0.28 (-0.99, 0.43)	0.9
Problem-solving:				
Sorting: Free Sort[2]	-0.34 (-0.82, 0.15)	-0.01 (-0.74, 0.71)	-0.62 (-1.29, 0.04)*	0.2
Sorting: Sort Recognition[2]	-0.66 (-1.26, -0.06)**	-0.71 (-1.60, 0.19)	-0.47 (-1.29, 0.36)	0.8
Tower: Total Achievement[2]	0.10 (-0.41, 0.61)	0.27 (-0.50, 1.03)	-0.17 (-0.89, 0.54)	0.7

[1]Manganese exposure has been log2-transformed and models have been adjusted for child race, sex, age at exam, HOME score; maternal marital status at child's birth, IQ, seafood consumption during pregnancy, smoking during pregnancy; maternal and paternal education and annual household income at child's birth; study examiner.
[2]Delis-Kaplan Executive Function System (D-KEFS) scaled score
[3]Wide Range Assessment of Memory and Learning, 2nd Edition (WRAML2) scaled score
**p<0.05*p<0.10

Supplemental Table 5.2 Results of analyses assessing potential mediation of the association between cord blood manganese and problem-solving skills by measures of inhibition, working memory and cognitive flexibility among New Bedford Cohort adolescents stratified by sex[1]. Problem-solving is measured by the Sorting: Sort Recognition description scaled score of the Delis Kaplan Executive Function System.

Mediator	Natural Indirect Effect (95% CI)[1]	Natural Direct Effect (95% CI)[1]	Total Effect[1] (95% CI)	Proportion Mediated
Males (n=181)				
Joint mediation by inhibition, working memory, and cognitive flexibility[2,3]	-0.25 (-0.60, 0.11)	-0.43 (-1.31, 0.52)	-0.68 (-1.50, 0.34)	0.36
Inhibition				
Color-Word Interference: Inhibition[2]	-0.13 (-0.42, 0.04)	-0.54 (-1.43, 0.45)	-0.68 (-1.51, 0.32)	0.20
Working Memory				
Verbal Working Memory[3]	-0.28 (-0.59, -0.03)**	-0.40 (-1.20, 0.50)	-0.68 (-1.51, 0.32)	0.42
Cognitive Flexibility				
Trail-making: Number Letter Switching[2]	-0.002 (-0.21, 0.24)	-0.68 (-1.52, 0.33)	-0.68 (-1.51, 0.32)	0.003
Females (n=198)				
Joint mediation by inhibition, working memory, and cognitive flexibility[2,3]	-0.25 (-0.60, -0.01)**	-0.20 (-1.00, 0.64)	-0.45 (-1.29, 0.37)	0.56
Inhibition				
Color-Word Interference: Inhibition[2]	-0.03 (-0.19, 0.05)	-0.42 (-1.27, 0.44)	-0.45 (-1.29, 0.37)	0.07
Working Memory				
Verbal Working Memory[3]	-0.12 (-0.31, 0.04)	-0.33 (-1.20, 0.48)	-0.45 (-1.29, 0.37)	0.27
Cognitive Flexibility				
Trail-making: Number Letter Switching[2]	-0.21 (-0.56, 0.01)*	-0.24 (-1.02, 0.57)	-0.45 (-1.29, 0.37)	0.48

[1]Manganese exposure has been log2-transformed and models have been adjusted for child race, sex, age at exam, HOME score; maternal marital status at child's birth, IQ, seafood consumption during pregnancy, smoking during pregnancy; maternal and paternal education and annual household income at child's birth; study examiner.
[2]Delis-Kaplan Executive Function System (D-KEFS) scaled score
[3]Wide Range Assessment of Memory and Learning, 2nd Edition (WRAML2) scaled score
**p<0.05*p<0.10

Supplemental Table 5.3 Inverse-probability weighted (IPW) results of multivariable linear regression analyses of difference (95% confidence intervals)[1] in problem-solving scaled scores per doubling of cord blood manganese concentrations among 379 New Bedford Cohort adolescents.

Problem-solving Measure (scaled scores)	Difference (95% CI)
Sorting: Free Sort[2]	-0.26 (-0.74, 0.22)
Sorting: Sort Recognition[2]	-0.59 (-1.18, 0.00)*
Tower: Total Achievement[2]	0.16 (-0.35, 0.67)

[1]Manganese exposure has been log2-transformed and models have been adjusted for child race, sex, age at exam, HOME score; maternal marital status at child's birth, IQ, seafood consumption during pregnancy, smoking during pregnancy; maternal and paternal education and annual household income at child's birth; study examiner.
[2]Delis-Kaplan Executive Function System (D-KEFS) scaled score
**p<0.05*p<0.10

References

1. Diamond A. Executive Functions. *Annu Rev Psychol*. 2013;64:135-168. doi:10.1146/annurev-psych-113011-143750
2. Lehto JE, Juujärvi P, Kooistra L, Pulkkinen L. Dimensions of executive functioning: Evidence from children. *Br J Dev Psychol*. 2003. doi:10.1348/026151003321164627
3. Best JR, Miller PH, Jones LL. Executive functions after age 5: Changes and correlates. *Dev Rev*. 2009. doi:10.1016/j.dr.2009.05.002
4. García-Madruga JA, Gómez-Veiga I, Vila J. Executive functions and the improvement of thinking abilities: The intervention in reading comprehension. *Front Psychol*. 2016. doi:10.3389/fpsyg.2016.00058
5. Blakemore SJ, Choudhury S. Development of the adolescent brain: Implications for executive function and social cognition. *J Child Psychol Psychiatry Allied Discip*. 2006. doi:10.1111/j.1469-7610.2006.01611.x
6. Blair C, Razza RP. Relating effortful control, executive function, and false belief understanding to emerging math and literacy ability in kindergarten. *Child Dev*. 2007;78(2):647-663. doi:10.1111/j.1467-8624.2007.01019.x
7. Bailey CE. Cognitive accuracy and intelligent executive function in the brain and in business. *Ann N Y Acad Sci*. 2007;1118:122-141. doi:10.1196/annals.1412.011
8. Baler RD, Volkow ND. Drug addiction: the neurobiology of disrupted self-control. *Trends Mol Med*. 2006;12(12):559-566. doi:10.1016/j.molmed.2006.10.005
9. Diamond A. Attention-deficit disorder (attention-deficit/hyperactivity disorder without hyperactivity): A neurobiologically and behaviorally distinct disorder from attention-deficit/hyperactivity disorder (with hyperactivity). *Dev Psychopathol*. 2005;17(3):807-825. doi:10.1017/S0954579405050388
10. Fairchild G, van Goozen SHM, Stollery SJ, et al. Decision Making and Executive Function in Male Adolescents with Early-Onset or Adolescence-Onset Conduct Disorder and Control Subjects. *Biol Psychiatry*. 2009;66(2):162-168. doi:10.1016/j.biopsych.2009.02.024
11. Taylor Tavares J V., Clark L, Cannon DM, Erickson K, Drevets WC, Sahakian BJ. Distinct Profiles of Neurocognitive Function in Unmedicated Unipolar Depression and Bipolar II Depression. *Biol Psychiatry*. 2007;62(8):917-924. doi:10.1016/j.biopsych.2007.05.034
12. Penadés R, Catalán R, Rubia K, Andrés S, Salamero M, Gastó C. Impaired response inhibition in obsessive compulsive disorder. *Eur Psychiatry*. 2007;22(6):404-410. doi:10.1016/j.eurpsy.2006.05.001
13. Barch DM. The Cognitive Neuroscience of Schizophrenia. *Annu Rev Clin Psychol*. 2005;1(1):321-353. doi:10.1146/annurev.clinpsy.1.102803.143959
14. Glahn DC, Bearden CE, Cakir S, et al. Differential working memory impairment in bipolar disorder and schizophrenia: Effects of lifetime history of psychosis. *Bipolar Disord*. 2006;8(2):117-123. doi:10.1111/j.1399-5618.2006.00296.x
15. Lee J. Working Memory Impairments in Schizophrenia: A Meta-Analysis. *Artic J Abnorm Psychol*. 2005. doi:10.1037/0021-843X.114.4.599
16. Kasper LJ, Alderson RM, Hudec KL. Moderators of working memory deficits in children with attention-deficit/hyperactivity disorder (ADHD): A meta-analytic review. *Clin Psychol Rev*. 2012;32(7):605-617. doi:10.1016/j.cpr.2012.07.001

17. Habib A, Harris L, Pollick F, Melville C. A meta-analysis of working memory in individuals with autism spectrum disorders. *PLoS One*. 2019;14(4). doi:10.1371/journal.pone.0216198
18. Grandjean P, Landrigan P. Developmental neurotoxicity of industrial chemicals. *Lancet*. 2006. doi:10.1016/S0140-6736(06)69665-7
19. Needham LL, Grandjean P, Heinzow B, et al. Partition of environmental chemicals between maternal and fetal blood and tissues. *Environ Sci Technol*. 2011. doi:10.1021/es1019614
20. Rice D, Barone S. Critical periods of vulnerability for the developing nervous system: Evidence from humans and animal models. *Environ Health Perspect*. 2000. doi:10.1289/ehp.00108s3511
21. Korrick SA, Sagiv SK. Polychlorinated biphenyls, organochlorine pesticides and neurodevelopment. *Curr Opin Pediatr*. 2008. doi:10.1097/MOP.0b013e3282f6a4e9
22. Grandjean P, Landrigan PJ. Neurobehavioural effects of developmental toxicity. *Lancet Neurol*. 2014;13(3):330-338. doi:10.1016/S1474-4422(13)70278-3
23. Horning KJ, Caito SW, Tipps KG, Bowman AB, Aschner M. Manganese is Essential for Neuronal Health. *Annu Rev Nutr*. 2015. doi:10.1146/annurev-nutr-071714-034419
24. Sanders AP, Claus Henn B, Wright RO. Perinatal and Childhood Exposure to Cadmium, Manganese, and Metal Mixtures and Effects on Cognition and Behavior: A Review of Recent Literature. *Curr Environ Heal reports*. 2015;2(3):284-294. doi:10.1007/s40572-015-0058-8
25. Carlin DJ, Rider C V., Woychik R, Birnbaum LS. Unraveling the health effects of environmental mixtures: An NIEHS priority. *Environ Health Perspect*. 2013. doi:10.1289/ehp.1206182
26. Carpenter DO, Arcaro K, Spink DC. Understanding the human health effects of chemical mixtures. *Environ Health Perspect*. 2002. doi:10.1289/ehp.02110s125
27. Jacobson JL, Jacobson SW. Prenatal exposure to polychlorinated biphenyls and attention at school age. *J Pediatr*. 2003;143(6):780-788. doi:10.1067/S0022-3476(03)00577-8
28. Stewart P, Fitzgerald S, Reihman J, et al. Prenatal PCB exposure, the corpus callosum, and response inhibition. *Environ Health Perspect*. 2003;111(13):1670-1677. doi:10.1289/ehp.6173
29. Anglen Bauer J, Henn BC, Austin C, et al. Manganese in teeth and neurobehavior: sex-specific windows of susceptibility HHS Public Access. *Env Int*. 2017;108:299-308. doi:10.1016/j.envint.2017.08.013
30. Jacobson JL, Muckle G, Ayotte P, Dewailly É, Jacobson SW. Relation of prenatal methylmercury exposure from environmental sources to childhood IQ. *Environ Health Perspect*. 2015;123(8):827-833. doi:10.1289/ehp.1408554
31. Stewart P, Reihman J, Gump B, Lonky E, Darvill T, Pagano J. Response inhibition at 8 and 9 1/2 years of age in children prenatally exposed to PCBs. *Neurotoxicol Teratol*. 2005;27(6):771-780. doi:10.1016/j.ntt.2005.07.003
32. Sagiv SK, Thurston SW, Bellinger DC, Altshul LM, Korrick SA. Neuropsychological measures of attention and impulse control among 8-year-old children exposed prenatally to organochlorines. *Environ Health Perspect*. 2012;120(6):904-909. doi:10.1289/ehp.1104372
33. Ribas-Fitó N, Torrent M, Carrizo D, et al. In Utero Exposure to Background Concentrations of DDT and Cognitive Functioning among Preschoolers. *Am J Epidemiol*.

2006;164:955-962. doi:10.1093/aje/kwj299
34. Gaspar FW, Harley KG, Kogut K, et al. Prenatal DDT and DDE exposure and child IQ in the CHAMACOS cohort. *Environ Int*. 2015;85:206-212. doi:10.1016/j.envint.2015.09.004
35. Kyriklaki A, Vafeiadi M, Kampouri M, et al. Prenatal exposure to persistent organic pollutants in association with offspring neuropsychological development at 4 years of age: The Rhea mother-child cohort, Crete, Greece. *Environ Int*. 2016;97:204-211. doi:10.1016/j.envint.2016.09.012
36. Forns J, Torrent M, Garcia-Esteban R, et al. Prenatal exposure to polychlorinated biphenyls and child neuropsychological development in 4-year-olds: An analysis per congener and specific cognitive domain. *Sci Total Environ*. 2012;432:338-343. doi:10.1016/j.scitotenv.2012.06.012
37. Fruh V, Rifas-Shiman SL, Amarasiriwardena C, et al. Prenatal lead exposure and childhood executive function and behavioral difficulties in project viva. *Neurotoxicology*. 2019;75:105-115. doi:10.1016/j.neuro.2019.09.006
38. Ericson JE, Crinella FM, Clarke-Stewart KA, Allhusen VD, Chan T, Robertson RT. Prenatal manganese levels linked to childhood behavioral disinhibition. *Neurotoxicol Teratol*. 2007;29(2):181-187. doi:10.1016/j.ntt.2006.09.020
39. Debes F, Budtz-Jørgensen E, Weihe P, White RF, Grandjean P. Impact of prenatal methylmercury exposure on neurobehavioral function at age 14 years. *Neurotoxicol Teratol*. 2006;28(5):536-547. doi:10.1016/j.ntt.2006.02.005
40. Rosado JL, Ronquillo D, Kordas K, et al. Arsenic exposure and cognitive performance in Mexican Schoolchildren. *Environ Health Perspect*. 2007;115(9):1371-1375. doi:10.1289/ehp.9961
41. Wasserman GA, Liu X, LoIacono NJ, et al. A cross-sectional study of well water arsenic and child IQ in Maine schoolchildren. *Environ Heal A Glob Access Sci Source*. 2014;13(1):23. doi:10.1186/1476-069X-13-23
42. Wasserman GA, Liu X, Parvez F, et al. A cross-sectional study of water arsenic exposure and intellectual function in adolescence in Araihazar, Bangladesh. *Environ Int*. 2018;118:304-313. doi:10.1016/j.envint.2018.05.037
43. Tsai SY, Chou HY, The HW, Chen CM, Chen CJ. The effects of chronic arsenic exposure from drinking water on the neurobehavioral development in adolescence. In: *NeuroToxicology*. Vol 24. Elsevier; 2003:747-753. doi:10.1016/S0161-813X(03)00029-9
44. Boucher O, Burden MJ, Muckle G, et al. Response inhibition and error monitoring during a visual Go/No-Go task in Inuit children exposed to lead, polychlorinated biphenyls, and methylmercury. *Environ Health Perspect*. 2012;120(4):608-615. doi:10.1289/ehp.1103828
45. Yorifuji T, Debes F, Weihe P, Grandjean P. Prenatal exposure to lead and cognitive deficit in 7- and 14-year-old children in the presence of concomitant exposure to similar molar concentration of methylmercury. *Neurotoxicol Teratol*. 2011;33(2):205-211. doi:10.1016/j.ntt.2010.09.004
46. Lucchini RG, Guazzetti S, Renzetti S, et al. Neurocognitive impact of metal exposure and social stressors among schoolchildren in Taranto, Italy. *Environ Heal A Glob Access Sci Source*. 2019;18(1):67. doi:10.1186/s12940-019-0505-3
47. Wasserman GA, Liu X, Parvez F, et al. Arsenic and manganese exposure and children's intellectual function. *Neurotoxicology*. 2011;32(4):450-457. doi:10.1016/j.neuro.2011.03.009

48. Bobb JF, Valeri L, Claus Henn B, et al. Bayesian kernel machine regression for estimating the health effects of multi-pollutant mixtures. *Biostatistics*. 2014. doi:10.1093/biostatistics/kxu058
49. Delis D, Kaplan E, Kramer J. *Delis-Kaplan Executive Function System*. Harcourt Assessment, Inc.; 1994.
50. Sheslow D, Adams W. Wide Range Assessment of Memory and Learning, Second Edition. https://www.pearsonclinical.com/psychology/products/100001702/wide-range-assessment-of-memory-and-learning-second-edition-wraml2.html. Published 2003. Accessed November 7, 2017.
51. Barg G, Daleiro M, Queirolo EI, et al. Association of low lead levels with behavioral problems and executive function deficits in schoolers from Montevideo, Uruguay. *Int J Environ Res Public Health*. 2018;15(12). doi:10.3390/ijerph15122735
52. Behforooz B, Newman J, Gallo M V., Schell LM. PCBs and measures of attention and impulsivity on a continuous performance task of young adults. *Neurotoxicol Teratol*. 2017;64:29-36. doi:10.1016/j.ntt.2017.08.004
53. Dumontheil I. Adolescent brain development. *Curr Opin Behav Sceinces*. 2016;10:39-44. http://eprints.bbk.ac.uk/15037/1/15037.pdf. Accessed May 1, 2020.
54. EWG. Body Burden: The Pollution in Newborns | EWG. https://www.ewg.org/research/body-burden-pollution-newborns. Published 2005. Accessed May 11, 2020.
55. Centers for Disease Control and Prevention. *Fourth National Report on Human Exposure to Environmental Chemicals: Updated Tables, January 2019, Volume One*.; 2019. https://www.cdc.gov/exposurereport/pdf/FourthReport_UpdatedTables_Volume1_Jan2019-508.pdf.
56. Longnecker MP, Wolff MS, Gladen BC, et al. Comparison of polychlorinated biphenyl levels across studies of human neurodevelopment. *Environ Health Perspect*. 2003;111(1):65-70. doi:10.1289/ehp.5463
57. Letz R. *Neurobehavioral Evaluation System 2 (NES2) User's Manual*. version 4. Atlanta, Georgia: Neurobehavioral Systems; 1998.
58. van Wijngaarden E, Thurston SW, Myers GJ, et al. Methyl mercury exposure and neurodevelopmental outcomes in the Seychelles Child Development Study Main cohort at age 22 and 24 years. *Neurotoxicol Teratol*. 2017;59:35-42. doi:10.1016/j.ntt.2016.10.011
59. Forns J, Fort M, Casas M, et al. Exposure to metals during pregnancy and neuropsychological development at the age of 4 years. *Neurotoxicology*. 2014;40:16-22. doi:10.1016/j.neuro.2013.10.006
60. Choi AL, Levy JI, Dockery DW, et al. Does living near a Superfund site contribute to higher polychlorinated biphenyl (PCB) exposure? *Environ Health Perspect*. 2006;114(7):1092-1098. doi:10.1289/ehp.8827
61. Vieira VM, Fabian MP, Webster TF, Levy JI, Korrick SA. Spatial Variability in ADHD-Related Behaviors among Children Born to Mothers Residing Near the New Bedford Harbor Superfund Site. *Am J Epidemiol*. 2017;185(10):924-932. doi:10.1093/aje/kww208
62. Korrick SA, Altshul LM, Tolbert PE, Burse VW, Needham LL, Monson RR. Measurement of PCBs, DDE, and hexachlorobenzene in cord blood from infants born in towns adjacent to a PCB-contaminated waste site. *J Expo Anal Environ Epidemiol*. 2000;10(6 II SUPPL.):743-754. doi:10.1038/sj.jea.7500120
63. Sagiv SK, Thurston SW, Bellinger DC, Tolbert PE, Altshul LM, Korrick SA. Prenatal

organochlorine exposure and behaviors associated with attention deficit hyperactivity disorder in school-aged children. *Am J Epidemiol*. 2010;171(5):593-601. doi:10.1093/aje/kwp427
64. Orenstein STC, Thurston SW, Bellinger DC, et al. Prenatal organochlorine and methylmercury exposure and memory and learning in school-age children in communities near the new bedford harbor superfund site, Massachusetts. *Environ Health Perspect*. 2014;122(11):1253-1259. doi:10.1289/ehp.1307804
65. Myers GJ, Davidson PW. Prenatal Methylmercury Exposure and Children: Neurologic, Developmental, and Behavioral Research. *Environ Health Perspect*. 1998;106:841-847.
66. Oken E, Wright RO, Kleinman KP, et al. Maternal fish consumption, hair mercury, and infant cognition in a U.S. cohort. *Environ Health Perspect*. 2005;113(10):1376-1380. doi:10.1289/ehp.8041
67. Amaral AFS, Porta M, Silverman DT, et al. Pancreatic cancer risk and levels of trace elements. *Gut*. 2012;61(11):1583-1588. doi:10.1136/gutjnl-2011-301086
68. Caldwell B, Bradley R. *Home Observation for Measurement of the Environment*. Dorsey, New York; 1985.
69. Kaufman A, Kaufman N. *Kaufman Brief Intelligence Test*. Circle Pines, MN: American Guidance Service; 1990.
70. Bobb JF, Claus Henn B, Valeri L, Coull BA. Statistical software for analyzing the health effects of multiple concurrent exposures via Bayesian kernel machine regression. *Environ Heal A Glob Access Sci Source*. 2018;17(1):67. doi:10.1186/s12940-018-0413-y
71. R Core Team. R: The R Project for Statistical Computing. 2019. https://www.r-project.org/. Accessed May 12, 2020.
72. Bobb JF. bkmr: Bayesian Kernel Machine Regression. 2017. https://cran.r-project.org/web/packages/bkmr/index.html. Accessed May 12, 2020.
73. Hernán MA, Robins JM. How to adjust for selection bias. In: *Causal Inference: What If*. Boca Raton: Chapman & Hall/CRC; 2020:107-112.
74. Ettinger AS, Egan KB, Homa DM, Brown MJ. Blood lead levels in U.S. women of childbearing age, 1976–2016. *Environ Health Perspect*. 2020. doi:10.1289/EHP5925
75. Arbuckle TE, Liang CL, Morisset AS, et al. Maternal and fetal exposure to cadmium, lead, manganese and mercury: The MIREC study. *Chemosphere*. 2016. doi:10.1016/j.chemosphere.2016.08.023
76. Ettinger AS, Arbuckle TE, Fisher M, et al. Arsenic levels among pregnant women and newborns in Canada: Results from the Maternal-Infant Research on Environmental Chemicals (MIREC) cohort. *Environ Res*. 2017. doi:10.1016/j.envres.2016.11.008
77. McDowell MA, Dillon CF, Osterloh J, et al. Hair mercury levels in U.S. children and women of childbearing age: Reference range data from NHANES 1999-2000. *Environ Health Perspect*. 2004;112(11):1165-1171. doi:10.1289/ehp.7046
78. Carvalho CF, Menezes-Filho JA, Matos VP d., et al. Elevated airborne manganese and low executive function in school-aged children in Brazil. *Neurotoxicology*. 2014;45:301-308. doi:10.1016/j.neuro.2013.11.006
79. Torres-Agustín R, Rodríguez-Agudelo Y, Schilmann A, et al. Effect of environmental manganese exposure on verbal learning and memory in Mexican children. *Environ Res*. 2013. doi:10.1016/j.envres.2012.10.007
80. Wright RO, Amarasiriwardena C, Woolf AD, Jim R, Bellinger DC. Neuropsychological correlates of hair arsenic, manganese, and cadmium levels in school-age children residing

near a hazardous waste site. *Neurotoxicology*. 2006. doi:10.1016/j.neuro.2005.10.001
81. Erikson KM, Thompson K, Aschner J, Aschner M. Manganese neurotoxicity: A focus on the neonate. *Pharmacol Ther*. 2007. doi:10.1016/j.pharmthera.2006.09.002
82. Agency for Toxic Substances & Disease Registry (ATSDR). *Toxicological Profile for Manganese*.
83. Stewart PW, Sargent DM, Reihman J, et al. Response inhibition during Differential Reinforcement of Low Rates (DRL) schedules may be sensitive to low-level polychlorinated biphenyl, methylmercury, and lead exposure in children. *Environ Health Perspect*. 2006;114(12):1923-1929. doi:10.1289/ehp.9216
84. Choi AL, Cordier S, Weihe P, Grandjean P. Negative confounding in the evaluation of toxicity: The case of methylmercury in fish and seafood. *Crit Rev Toxicol*. 2008;38(10):877-893. doi:10.1080/10408440802273164
85. Stiles KM, Bellinger DC. Neuropsychological correlates of low-level lead exposure in school-age children: A prospective study. *Neurotoxicol Teratol*. 1993;15(1):27-35. doi:10.1016/0892-0362(93)90042-M
86. Menezes-Filho JA, de Carvalho-Vivas CF, Viana GFS, et al. Elevated manganese exposure and school-aged children's behavior: A gender-stratified analysis. *Neurotoxicology*. 2014;45:293-300. doi:10.1016/j.neuro.2013.09.006
87. Rothman KJ. No adjustments are needed for multiple comparisons. *Epidemiology*. 1990. doi:10.1097/00001648-199001000-00010
88. Wilschefski S, Baxter M. Inductively Coupled Plasma Mass Spectrometry: Introduction to Analytical Aspects. *Clin Biochem Rev*. 2019. doi:10.33176/aacb-19-00024
89. Coetzee DJ, McGovern PM, Rao R, Harnack LJ, Georgieff MK, Stepanov I. Measuring the impact of manganese exposure on children's neurodevelopment: Advances and research gaps in biomarker-based approaches. *Environ Heal A Glob Access Sci Source*. 2016. doi:10.1186/s12940-016-0174-4
90. Bauer JA, Devick KL, Bobb JF, et al. Associations of a metal mixture measured in multiple biomarkers with IQ: Evidence from italian adolescents living near ferroalloy industry. *Environ Health Perspect*. 2020. doi:10.1289/EHP6803
91. Gunier RB, Mora AM, Smith D, et al. Biomarkers of manganese exposure in pregnant women and children living in an agricultural community in California. *Environ Sci Technol*. 2014. doi:10.1021/es503866a
92. Crone EA, Wendelken C, Donohue S, Van Leijenhorst L, Bunge SA. Neurocognitive development of the ability to manipulate information in working memory. *Proc Natl Acad Sci U S A*. 2006;103(24):9315-9320. doi:10.1073/pnas.0510088103
93. Calderón J, Navarro ME, Jimenez-Capdeville ME, et al. Exposure to arsenic and lead and neuropsychological development in Mexican children. *Environ Res*. 2001;85(2):69-76. doi:10.1006/enrs.2000.4106
94. Nascimento S, Baierle M, Göethel G, et al. Associations among environmental exposure to manganese, neuropsychological performance, oxidative damage and kidney biomarkers in children. *Environ Res*. 2016;147:32-43. doi:10.1016/j.envres.2016.01.035
95. Sokol RJ, Rosen MG, Stojkov J, Chik L. Clinical application of high-risk scoring on an obstetric service. *Am J Obstet Gynecol*. 1977. doi:10.1016/0002-9378(77)90212-5
96. Oppenheimer A V., Bellinger DC, Coull BA, Weisskopf MA, Zemplenyi M, Korrick SA. Prenatal exposure to chemical mixtures and inhibition among adolescents in the New Bedford Cohort. 2021.

97. Carvalho CF de, Oulhote Y, Martorelli M, et al. Environmental manganese exposure and associations with memory, executive functions, and hyperactivity in Brazilian children. *Neurotoxicology*. 2018;69:253-259. doi:10.1016/j.neuro.2018.02.002
98. Davidson MC, Amso D, Anderson LC, Diamond A. Development of cognitive control and executive functions from 4 to 13 years: Evidence from manipulations of memory, inhibition, and task switching. *Neuropsychologia*. 2006. doi:10.1016/j.neuropsychologia.2006.02.006
99. Oppenheimer A V., Bellinger DC, Coull BA, Weisskopf MA, Korrick SA. Prenatal exposure to chemical mixtures and working memory among adolescents. 2021.
100. Best JR, Miller PH. A Developmental Perspective on Executive Function. *Child Dev*. 2010. doi:10.1111/j.1467-8624.2010.01499.x
101. Ferrer E, O'Hare ED, Bunge SA. Fluid reasoning and the developing brain. *Front Neurosci*. 2009. doi:10.3389/neuro.01.003.2009
102. Best JR, Miller PH, Naglieri JA. Relations between executive function and academic achievement from ages 5 to 17 in a large, representative national sample. *Learn Individ Differ*. 2011;21(4):327-336. doi:10.1016/j.lindif.2011.01.007
103. Krachler M, Rossipal E, Micetic-Turk D. Trace element transfer from the mother to the newborn - Investigations on triplets of colostrum, maternal and umbilical cord sera. *Eur J Clin Nutr*. 1999. doi:10.1038/sj.ejcn.1600781
104. Oppenheimer A V., Bellinger DC, Coull BA, Weisskopf MA, Korrick SA. Prenatal exposure to chemical mixtures and cognitive flexibility among adolescents. 2021.
105. Rahman SM, Kippler M, Tofail F, Bölte S, Hamadani JD, Vahter M. Manganese in drinking water and cognitive abilities and behavior at 10 years of age: A prospective cohort study. *Environ Health Perspect*. 2017. doi:10.1289/EHP631
106. Mora AM, Arora M, Harley KG, et al. Prenatal and postnatal manganese teeth levels and neurodevelopment at 7, 9, and 10.5years in the CHAMACOS cohort. *Environ Int*. 2015. doi:10.1016/j.envint.2015.07.009
107. Valeri L, VanderWeele TJ. Mediation analysis allowing for exposure-mediator interactions and causal interpretation: Theoretical assumptions and implementation with SAS and SPSS macros. *Psychol Methods*. 2013. doi:10.1037/a0031034
108. VanderWeele T, Vansteelandt S. Mediation analysis with multiple mediators. *Epidemiol Method*. 2013;2(1):95-115. doi:10.1515/em-2012-0010
109. Wang W, Zhang B. Assessing natural direct and indirect effects for a continuous exposure and a dichotomous outcome. *J Stat Theory Pract*. 2016;10(3):574-587. doi:10.1080/15598608.2016.1203843
110. Shi B, Choirat C, Valeri L. CMAverse: Causal Mediation Analysis. 2021.
111. VanderWeele TJ, Ding P. Sensitivity analysis in observational research: Introducing the E-Value. *Ann Intern Med*. 2017. doi:10.7326/M16-2607
112. Zota AR, Ettinger AS, Bouchard M, et al. Maternal blood manganese levels and infant birth weight. *Epidemiology*. 2009. doi:10.1097/EDE.0b013e31819b93c0
113. Freedman LS, Schatzkin A, Midthune D, Kipnis V. Dealing with dietary measurement error in nutritional cohort studies. *J Natl Cancer Inst*. 2011. doi:10.1093/jnci/djr189