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## Exposure to Metal Mixtures and Neuropsychological Functioning in Middle Childhood

**Cheryl R Stein,**

Hassenfeld Children's Hospital at NYU Langone, Department of Child and Adolescent Psychiatry, Child Study Center, One Park Avenue, 7<sup>th</sup> Floor, New York, NY 10016

**Haotian Wu,**

Department of Environmental Health Sciences, Mailman School of Public Health, Columbia University, 722 West 168<sup>th</sup> St, New York, NY, 10032

**David C Bellinger,**

Department of Neurology, Boston Children's Hospital, Farley Basement Box 127, 300 Longwood Ave, Boston MA 02115

**Donald R Smith,**

Department of Microbiology and Environmental Toxicology, University of California, 442 Physical Sciences Building, Santa Cruz, CA 95064

**Mary S Wolff,**

Department of Environmental Medicine & Public Health, Icahn School of Medicine at Mount Sinai, 17 East 102 Street, New York, NY 10029

**David A Savitz**

Department of Epidemiology, Brown University School of Public Health, 121 S. Main Street, Box G-S-121-2, Providence, RI 02912

### Abstract

Elevated exposure to multiple trace metals can be neurotoxic even at relatively low levels. These findings are primarily evident from adult occupational studies as well as in children exposed prenatally or in early childhood. Less research has focused on the neurodevelopmental impacts of exposure to metals among school-aged children.

We examined associations between exposure to a mixture of four metals (arsenic, cadmium, manganese, lead) measured in hair and markers of cognition, attention, and behavior among 222 6–12 year old children who participated in a 2009–2010 neurodevelopmental follow-up to the C8 Health Project. Using quantile-based g-computation we estimated the adjusted overall metal mixture effect  $\psi$  (95% CI) as the change in outcome per decile increase in all metals in the mixture.

Hair metal levels varied by metal, with cadmium being lowest (median 0.007, interquartile range (IQR) 0.013  $\mu\text{g/g}$ ) and lead the highest concentration (median 0.152, IQR 0.252  $\mu\text{g/g}$ ).

Children's cognitive skills and development, attention/impulsivity, and behavior were all close to

standardized population means. Each decile increase in all metals was associated with a Full Scale IQ reduction of 1.01 points (95% confidence interval (CI)  $-1.88, -0.15$ ) and Verbal IQ reduction of 1.11 points (95% CI  $-1.97, -0.25$ ), adjusted for child age, sex, secondhand smoke exposure, HOME score, maternal education, maternal IQ, and examiner. Maternal report of ADHD-like behaviors and executive functioning also showed adverse associations with the metal mixture.

Our findings suggest that similar to exposure during prenatal and early childhood periods, recent exposure to metals during middle childhood is associated with adverse neurodevelopmental consequences. Middle childhood may also be a developmental window of susceptibility to the negative consequences of exposure to environmental neurotoxicants.

## Keywords

Metal mixtures; School Age Population; Neuropsychological Tests; Child Behavior

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## 1. INTRODUCTION

Exposure to multiple trace metals can be neurotoxic even at low levels (Rodríguez-Barranco et al., 2013). Lead is harmful at any exposure level (2016; Lin-Fu, 1973; Prevention, 2012; Rabin, 1989; RUDDOCK, 1924). Manganese is an essential trace element, but toxic as exposure increases ((ATSDR), 2012). The neurotoxicity of arsenic (Vahidnia et al., 2007) and cadmium (Gonçalves et al., 2021) are also of well-established concern. These findings are primarily evident from adult occupational studies (Bowler et al., 2006; Lucchini et al., 1999; Roels et al., 1987), as well as in children exposed prenatally or in early childhood (Claus Henn et al., 2017; Freire et al., 2018; Leonhard et al., 2019; Muñoz-Rocha et al., 2018; Sanders et al., 2015).

Less is known about the neurodevelopmental impacts of exposure to metals among school-aged children, despite recognition of this age range as an important life stage to consider when assessing childhood exposures to environmental contaminants and adverse health outcomes (United States. Environmental Protection Agency. Risk Assessment Forum., 2005). This gap in knowledge is particularly concerning because studies have noted that childhood metal exposures are associated with adverse neurodevelopmental outcomes, sometimes even more strongly than prenatal or early life exposures (Lanphear et al., 2005; Mazumdar et al., 2011; Tellez-Rojo et al., 2006).

From a life stage exposure perspective, children 6–10 years start spending time in school environments and playing sports; beginning at ~11 years there is an increased rate of food consumption, increased risk of smoking, and more time away from home (United States. Environmental Protection Agency. Risk Assessment Forum., 2005). From a developmental perspective, middle childhood, colloquially referred to as 6–12 years of age, is a period of rapid neuro-adaptation (Mah and Ford-Jones, 2012). Synaptic pruning likely increases efficiency of processing in some brain regions (Giedd et al., 1999). Neurons responsible for cognition, language and social skills are consolidated (Szaflarski et al., 2006), and children gain cognitive control with an increasing ability to respond selectively to stimuli and can begin to process and understand material effectively (Carey, 2009). Emerging evidence

indicates that for some types of adverse health outcomes the strongest impacts may be tied to specific windows of heightened plasticity that occur later, rather than earlier, in childhood development (Andersen et al., 2008; Pechtel et al., 2014; Teicher et al., 2018). Middle childhood, considered a sensitive period because of the active role that environment and experiences play in brain development (Knudsen, 2004), may be a window of susceptibility for developmental neurotoxicants like metals.

Humans are exposed to metals through diet/ingestion, air and soil pollution, and from industrial sites (Landrigan et al., 2018), resulting in concomitant exposure to numerous metals. Interrogating the health effects of one metal at a time may miss interactions among metals (von Stackelberg et al., 2015), and importantly does not reflect real world experience of multiple exposures and exposure mixtures.

We examined cross-sectional associations between exposure to a mixture of four metals (arsenic, cadmium, manganese, lead) measured in hair and markers of cognition, attention, and behavior among 222 6–12 year old children who participated in a neurodevelopmental follow-up to the C8 Health Project. The C8 Health Project was a study in the Mid-Ohio Valley to examine the health effects of exposure to perfluorooctanoate (PFOA) through contaminated drinking water (Frisbee et al., 2009). This industrial region of the Mid-Ohio Valley also experienced elevated exposure to metals. The U.S. Agency for Toxic Substances and Disease Registry (ATSDR) Health Consultation Marietta Area Air Investigation (Services and Registry, 2009) reported that levels of arsenic, cadmium, and manganese in this region exceeded ATSDR and U.S. Environmental Protection Agency (EPA) health-based comparison values at all monitoring locations during their 2007 – 2008 investigation.

## 2. METHODS

### 2.1 Study Population

The C8 Health Project enrolled 69,030 people from 2005–2006 and has been described in detail previously (Frisbee et al., 2009). From 2009–2010 we conducted a follow-up to the C8 Health Project to assess neuropsychological functioning among 6–12 year old children (Stein et al., 2013, 2014); 322 children and their biological mothers (73% of the known eligible) participated in the follow-up study. During the mother's interview we requested contact information for the child's teacher and 188 (60% of requested) teachers completed surveys. There was no difference in C8 Health Project participants between those who enrolled and did not enroll in the neuropsychological follow-up study by age ( $p=0.79$ ), gender ( $p=0.32$ ), or maternal smoking ( $p=0.99$ ).

As part of the follow-up study, we collected hair samples for measurement of metals (Jursa et al., 2018). We collected two cm of hair closest to the scalp, which represents exposures from approximately two months prior to sampling (Smolders et al., 2009). Hair samples were collected concurrent to outcome assessments. From all children participating in the follow-up, hair metals data were available for 222 subjects (69% of follow-up study; Supplemental Figure 1). Subjects missing metals data occurred because the child or mother declined to allow the child's hair be sampled ( $n=3$ ), the child had insufficient hair to be sampled ( $n=55$ ), or the sampled hair was insufficient for analysis ( $n=41$ ). Compared to

all children enrolled in the neurodevelopment follow-up study, the sub-sample with metals data differed by gender ( $p < 0.001$ ; Supplemental Table 1) because girls were more likely to have sufficient hair for sampling and analysis. Other characteristics differing significantly between children with and without hair metals data were maternal education (lower proportion of college graduates among those without metals data;  $p = 0.03$ ), NEPSY Design Copying Total scaled score (worse scores among those without metals data;  $p = 0.02$ ), and the Clinical Confidence Index from Conners' Continuous Performance Test (less resemblance to ADHD canonical profile among those without metals data;  $p < 0.001$ ).

Mothers provided informed consent and children provided verbal assent. The Mount Sinai Program for the Protection of Human Subjects and the Battelle Centers for Public Health Research & Evaluation Institutional Review Board approved all study procedures; statistical analyses of de-identified data were categorized as exempt by the NYU Langone Institutional Review Board.

## 2.2 Exposure Assessment

Hair samples were collected proximal to the occipital lobe scalp and stored in zip top plastic bags at room temperature until analysis of a two cm segment of hair proximal to the scalp (Jursa et al., 2018). All cleaning and processing of hair samples was conducted in a HEPA filtered-air trace metal clean room using acid-cleaned labware and ultrapure trace metal grade reagents. Individual hair segments/samples weighing 5–30 mg each were cleaned of exogenous metal contamination as described previously (Eastman et al., 2013). Briefly, samples were placed in acid-cleaned 5 mL polypropylene syringe tubes and sonicated (20 minutes) in 0.5% Triton, rinsed five-times with ultrapure Milli-Q water, sonicated (10 minutes) in 1 N trace metal grade nitric acid (Fisher Scientific, Santa Clara, CA, USA), rinsed with 1 N nitric acid, rinsed five-times with Milli-Q water, and then dried at 65 C for 48 hours. Subsequently, samples were digested in 0.5 mL 15.7 N quartz-distilled nitric acid (Fisher Scientific, optima grade) at 80 C for 6 hours in a Class-100 HEPA filtered-air fume hood, and then diluted with 5 mL Milli-Q water. Typical analytical limits of detection (LOD) over five analytical runs were 0.0018, 0.0004, 0.0038, and 0.0077 ng/mL for arsenic, cadmium, lead, and manganese, respectively. For metal levels below the analytical LOD, the LOD was multiplied by 0.5 and adjusted using the sample dilution factor and sample weight of processed hair to derive a value for half the procedural detection limit; this value was used for statistical analyses. Standard reference material NIES 13 (human hair) was used to assess analytical accuracy; recoveries are means and standard deviations based on 17 replicates over five analytical runs. Arsenic: reference 0.10  $\mu\text{g/g}$ , recovered  $0.098 \pm 0.008 \mu\text{g/g}$  (98%). Cadmium: certified  $0.23 \pm 0.03 \mu\text{g/g}$ , recovered  $0.225 \pm 0.015 \mu\text{g/g}$  (98%). Lead: certified  $4.6 \pm 0.4 \mu\text{g/g}$ , recovered  $4.65 \pm 0.47 \mu\text{g/g}$  (101%). Manganese: reference 3.9  $\mu\text{g/g}$ , recovered  $2.92 \pm 0.42 \mu\text{g/g}$  (75%). Arsenic measurements were not speciated because of insufficient volume of hair and insufficient evidence demonstrating the utility of arsenic speciation in hair, in contrast to speciation in urine and blood.

## 2.3 Outcome Assessment

To collect information pertinent to clinically relevant behaviors, we selected a battery of tests that assess skills predictive of a child's ability to make adequate progress in school.

Outcome measures included direct assessment of the child and reports from mothers and teachers. Examined domains included cognitive skills and development; attention/impulsivity; and behavior. Eight examiners blinded to participants' exposure histories were trained and certified (by D.C.B.) to administer the child assessments.

**2.3.1 Cognitive Skills and Development**—The Wechsler Abbreviated Scale of Intelligence (WASI) consists of four subtests similar to subtests of the Wechsler Intelligence Scale for Children—Fourth Edition (Corporation, 1999). These two verbal (Vocabulary, Similarities) and two nonverbal (Block Design, Matrix Reasoning) subtests have the highest loadings on general intellectual function. The tests are normed and provide age-standardized scores for Full Scale Intelligence Quotient (IQ), Verbal IQ, and Performance IQ. IQ scores range from 50 to 160 with mean = 100 and standard deviation (SD) = 15; higher scores reflect better performance.

We used three subtests from the Wechsler Individual Achievement Test—II (WIAT) to assess academic skills (Corporation, 2002). Two subtests measure word attack and decoding skills (Word Attack, Pseudoword Decoding); we averaged the standardized scores from these two subtests to create a composite measure of reading fluency. The third subtest measures arithmetic capabilities (Numerical Operations). Age-standardized scores range from 40 to 160 with mean = 100 and SD = 15; higher scores reflect stronger academic skills.

The NEPSY-II is a neuropsychologically-based instrument designed to test specific brain-behavior relationships and identify markers of atypical cognitive development (Korkman et al., 2007). We focused on three domains: Language (Comprehension of Instructions, Word Generation), Memory and Learning (Narrative Memory), and Visual-spatial Processing (Design Copying). Visual-spatial processing involves the combination of fine motor skills and spatial abilities, plus requires executive functioning; impairment in this domain may impact ability to complete school work. We averaged the Semantic and Initial Letter scaled scores from the Word Generation subtest. Age-standardized scaled scores range from 1 to 19 with mean = 10 and SD = 3; higher scores indicate better performance.

**2.3.2 Attention/Impulsivity**—The Conners' Continuous Performance Test-II (CPT) is a computer-administered test that measures sustained attention and impulsivity (Conners, 2004). The child is instructed to push the spacebar every time a letter appears on the screen except when the letter is "X." The Clinical Confidence Index measures the extent to which the child's overall profile resembles the canonical profile of children with ADHD. The Clinical Confidence Index is a raw score ranging from 0 to 100; a higher score indicates greater resemblance to the canonical profile of children with ADHD. We also analyzed age-standardized T-scores for Omission Errors (no response after non-X), Commission Errors (response after X), and Hit Reaction Time (mean response time in milliseconds). T-scores have mean = 50 and SD = 10; lower scores indicate fewer ADHD-like behaviors.

Mother and teachers completed the Conners' ADHD DSM-IV Scales-Revised (CADS) to report on ADHD-like behaviors (Conners, 2001). The ADHD Index score is based on responses to 12 items that best discriminate children who have been diagnosed with ADHD from those who have not. There are also three DSM-IV Symptoms subscales: Inattentive,

Hyperactive-Impulsive, and Combined. Age-standardized T-scores have mean = 50 and SD = 10; lower scores indicate fewer ADHD-like behaviors.

**2.3.3 Behavior**—The Behavior Rating Inventory of Executive Function (BRIEF), completed by mothers and teachers, assesses executive function (Gioia, 2000). Executive function helps guide, direct, and manage cognition, emotion, and behavior. The BRIEF has eight clinical scales; we focused on three measures. The Global Executive Composite is a summary score that incorporates all eight clinical scales. The Behavioral Regulation Index focuses on inhibitory control – the ability to manage transitions and regulate emotions. The Metacognition Index reflects the ability to plan, organize, and monitor performance. To ensure validity, as recommended in the manual, we excluded mother (n=1) and teacher (n=1) reports with negativity scores greater than 6 or inconsistency scores greater than 8. The Negativity scale measures the extent to which the respondent answered selected BRIEF items in an unusually negative manner relative to the normed sample. Similarly, the inconsistency scale indicates the extent to which the respondent answered similar BRIEF items in an inconsistent manner. Age-standardized T-scores have mean = 50 and standard deviation (SD) = 10; higher scores represent greater difficulties with regard to executive functioning.

The Behavior Assessment System for Children-2 (BASC-2), completed by mothers and teachers, evaluates child personality, behavioral problems, and emotional disturbances (Reynolds and Kamphaus, 2004). We focus on the Behavioral Symptom Index and three composite scales: Adaptive Skills, Internalizing Problems, and Externalizing Problems. To ensure validity, as recommended in the manual, we excluded mother reports (n=6) with F-index (negativity scale) greater than 6, Pattern Response Index greater than 12, or Consistency Index greater than 17, as well as teacher reports (n=4) with F-index greater than 3, Pattern Response Index greater than 11, or Consistency Index greater than 15. The Pattern Response Index detects repeated and cyclical response patterns. Age-standardized T-scores have mean = 50 and SD = 10. For Adaptive Skills higher scores indicate better adaptive skills; for the Behavioral Symptom Index, Internalizing, and Externalizing Problems, lower scores indicate fewer problematic behaviors.

## 2.4 Covariate Assessment

We interviewed mothers to collect information to address confounding. The Home Observation for Measurement of the Environment—Short Form Mother Supplement (HOME) measures the quality and extent of stimulation in the home based on maternal report (Surveys). Maternal IQ was measured using the Wechsler Abbreviated Scale of Intelligence (Corporation, 1999). Mothers also completed an extensive interview to solicit data on factors that may have affected their child’s neurobehavioral development, such as pregnancy and delivery complications and presence of co-morbid health conditions in the child.

## 2.5 Statistical Analysis

We used quantile-based g-computation (QG-comp) (Keil et al., 2020) to estimate the overall effect of childhood hair metal mixture levels with neurodevelopmental and behavioral

outcomes. QG-comp estimates the change in outcome per quantile increase in all metals in the mixture and the relative contribution of each component to that direction (positive or negative). The estimated overall mixture effect ( $\psi$ ) can be interpreted as the change in outcome per quantile increase in all exposures together. We present results from deciles for ease of interpretation; specification of other quantiles produced very similar findings. The component weights are essentially the metal-specific beta divided by the total mixture effect across all metals acting in the same direction. Comparing the component weights for metals acting in the same direction can indicate which metals are driving the association between mixture and outcome. Additionally, we used Bayesian Kernel Machine Regression (BKMR) models (Bobb et al., 2018; Bobb et al., 2015) to assess potential non-linearity and interactions between metals as well as to corroborate the overall mixture results observed from QG-comp. For BKMR models, we scaled exposures and outcomes and specified Gaussian distributions and performed 50,000 iterations.

Although there was minimal missing data for any single variable (Supplemental Table 2), the cumulative missing-ness would have reduced our sample size and potentially introduced bias. To address this, we created 10 imputed datasets using the “mice” package in R (van Buuren and Groothuis-Oudshoorn, 2011) and pooled the QG-comp and linear regression estimates from each model using Rubin’s Rule (Rubin, 2004). To calculate the weights from QG-comp, we averaged across the 10 models from the imputed datasets. BKMR models were fitted using only complete case data. Missing data for teacher ratings were not imputed.

All multivariable models were adjusted for the same set of covariates based on *a priori* decisions or a covariate’s association with an exposure and outcome. PFOA did not meet confounding criteria (Rothman and Greenland, 1998) for this study of metal exposure (i.e., null associations between PFOA and metals (Stein et al., 2013, 2014)) so was not included as a covariate. Additionally, we had no hypothesis for why PFOA and these metals would have a synergistic impact on neurodevelopment. Analyses adjusted for child age at assessment (continuous); sex (female, male); secondhand smoke exposure from birth to 2 years of age (yes, no); cognitive and emotional HOME scores (continuous); maternal education (less than high school graduate, high school graduate, some college, college graduate); maternal IQ (continuous); and examiner (for WASI, WIAT, NEPSY, CPT outcomes only). Additionally, we ran QG-comp models stratified by sex to explore the possibility of sex-specific effects of metals on cognition and development. Lastly, to complement our mixture analysis and for comparison with previous publications, we also used linear regression models to estimate the relationships between each individual metal and subtest scores. In linear regression models, all metal biomarkers were log<sub>2</sub>-transformed and the resulting estimates are interpreted as the change in outcome per doubling of metal concentration.

### 3. RESULTS

Most of the 222 children included in the hair metal analyses were non-Hispanic white (95.9%) and female (76.1%), with an average age of 9.8 (SD 1.7) years (Table 1). Mothers reported that 9.5% of children had ever been diagnosed with ADHD. The plurality of mothers had some college. Mean measures of children’s cognitive skills and development,

attention/impulsivity, and behavior were all close to the standardized population means. Hair metal concentration varied by metal (Table 2); cadmium was measured at the lowest concentration (median 0.007, interquartile range (IQR) 0.013  $\mu\text{g/g}$ ) and lead at the highest (median 0.152, IQR 0.252  $\mu\text{g/g}$ ). The four metals were moderately correlated (Supplemental Figure 2).

### 3.1 Cognitive Skills and Development

Using QG-comp, a simultaneous increase in all four metal mixture components was associated with worse IQ scores on the WASI. Each decile increase in all metals was associated with a Full Scale IQ reduction of 1.01 points (95% confidence interval (CI)  $-1.88, -0.15$ ) and a Verbal IQ reduction of 1.11 points (95% CI  $-1.97, -0.25$ ), adjusted for child age, sex, secondhand smoke exposure, HOME score, maternal education, maternal IQ, and examiner (Figure 1, Supplemental Table 3). Performance IQ ( $-0.48, 95\% \text{ CI } -1.35, 0.39$ ) was not associated with the metals mixture. Based on mixture component weights, manganese and lead were driving the associations with IQ (Figure 2). In adjusted BKMR models, when all metals were at their respective 75<sup>th</sup> percentiles compared to their respective 25<sup>th</sup> percentiles, there was a reduction in Full Scale IQ of 0.15 SD, or 1.5 points (Supplemental Figure 3). There was no evidence of interactions among metals in relation to any scale on the WASI, although manganese exhibited non-monotonic and non-linear associations (data not shown), which reflects manganese's dual role as an essential element that is toxic at higher concentrations.

While there was a pattern of worse reading fluency and arithmetic skills on the WIAT in relation to a decile increase in all metals, the magnitude of the effect was small in relation to the SD (Figure 1, Supplemental Table 3). To the extent that there was an adverse association between metal mixture and academic skills manganese again appeared to be the key component (Figure 2). BKMR models exhibited similar patterns of small, negative, non-significant associations (Supplemental Figure 3), with no evidence of interactions or non-linearity among metals on the WIAT (data not shown).

Across QG-COMP and BKMR models there was no evidence of an association between the metal mixture and performance on the NEPSY (Figure 1, Supplemental Table 3, Supplemental Figure 3).

### 3.2 Attention/Impulsivity

In direct assessment of the children using Conners' Continuous Performance Test-II, there were no associations between the metal mixture and measures of attention or impulsivity across QG-comp and BKMR models (Figure 1, Supplemental Table 3, Supplemental Figure 3). Using the CADS, however, mother report on child's ADHD-like behavior showed worse scores on Inattentive (0.90, 95% CI 0.12, 1.68) and Combined (0.80, 95% CI 0.04, 1.57) scales per decile increase in all metals, adjusted for child age, sex, secondhand smoke exposure, HOME score, maternal education, and maternal IQ (Figure 1, Supplemental Table 3). These findings were primarily driven by arsenic and cadmium, with lead also a factor for the Inattentive scale (Figure 2). BKMR models exhibited comparable patterns (Supplemental Figure 3), with no indication of interactions or non-linearity (data not shown). Teacher



report on the CADS showed null associations between the metal mixture and child ADHD-like behaviors across QG-comp and BKMR models (Figure 1, Supplemental Table 3, Supplemental Figure 3).

### 3.3 Behavior

Using the BRIEF, metal mixture exposure was associated with less favorable child executive functioning by maternal, but not teacher report. A decile increase in all metals was associated with an increase in the Global Executive Composite of 0.78 (95% CI -0.02, 1.58) points and an increase in the Metacognition Index of 0.83 (95% CI 0.01, 1.65) points, adjusted for child age, sex, secondhand smoke exposure, HOME score, maternal education, and maternal IQ (Figure 1, Supplemental Table 3). Arsenic and cadmium were the key components of the change in Global Executive Composite; arsenic and manganese were the key components of the change in Metacognition Index (Figure 2). BKMR models exhibited comparable patterns (Supplemental Figure 3), with no indication of interactions although there was a non-linear, non-monotonic association between cadmium and the BRIEF scales (data not shown), which indicates lack of a dose-response relationship.

On the BASC-2, the metal mixture was in the direction of worse Adaptive Skills for on both mother and teacher reports and more Externalizing Problems based on mother report (Figure 1, Supplemental Table 3). In QG-comp models, the magnitude of the decrement in Adaptive Skills, while not statistically significant, was comparable across mother and teacher report. BKMR models exhibited similar patterns (Supplemental Figure 3), with no indication of interactions or non-linearity (data not shown).

In sex-stratified analyses (Supplemental Figure 4), the adverse associations between metal mixture and IQ were more pronounced among females compared to males and the associations with executive function were observed exclusively among males. An examination of results from adjusted linear regression models estimating associations between individual metals and neurodevelopmental and behavioral outcomes (Supplemental Figure 5) did not alter the interpretation of results from the mixture models.

## 4. DISCUSSION

In this cross-sectional study of 6–12 year old children, greater exposure to a mixture of four metals measured in hair was associated with a decrease in Full Scale and Verbal IQ, increase in maternal report of ADHD-like behaviors, increase in maternal report of executive dysfunction, and reduced adaptive skills based on both mother and teacher report. However, exposure was not associated to any notable degree with Performance IQ, atypical cognitive development, or direct assessment of attention and impulsivity. We did observe some sex-specific associations with both IQ and behavior, although only 24% of the sample was comprised of males. The magnitudes of these adverse associations between childhood hair metal mixture exposure and neurobehavioral development may be small for a decile increase in all metals, but could impart meaningful decrements across exposure ranges at the population level. Also, estimates from metal mixture and individual metal models were similar. These results indicate that school-aged children may be susceptible to the neurotoxic effects of recent joint exposure to multiple metals. This finding is important because

middle childhood is a period of rapid neuro-adaption when cognition, language, and social skills are consolidated and gains in cognitive control permit the effective processing and understanding of material. Disruptions to these processes may lead to long-term negative consequences in academic achievement and social-emotional development.

Despite numerous studies showing adverse associations between prenatal and/or early childhood exposure to select metals and neurobehavioral development, few studies provide a direct comparison when considering the specific metal mixture (arsenic, cadmium, manganese, lead) and age range (6–12 year olds) of this analysis. A 2013 systematic review and meta-analysis evaluated associations of pre or postnatal exposure to arsenic, cadmium and manganese with neurodevelopment and behavioral disorders in children through 16 years of age (Rodríguez-Barranco et al., 2013). In meta-analysis, a 50% increase in urinary arsenic concentration was associated with a  $-0.39$  point decrease (95% CI  $-0.84, 0.06$ ) in Full Scale IQ, but not behavior. There were too few studies to conduct meta-analyses for cadmium; separate study results were mixed with only some studies reporting associations between cadmium exposure and lower IQ or higher frequency of attention problems. Manganese, however, showed consistent negative associations with IQ. In meta-analysis, a 50% increase in hair manganese concentration was associated with a  $-0.70$  point decrease (95% CI  $-1.07, -0.34$ ) in Full Scale IQ,  $-1.26$  point decrease (95% CI  $-2.20, -0.33$ ) in Verbal IQ, and  $-0.42$  point decrease ( $-0.82, -0.02$ ) in Performance IQ. Manganese was also consistently associated with more behavioral problems. Our study showed IQ decrements of similar magnitude, but with a 10% increase in components in a mixture of four metals rather than a 50% increase in a single metal, underscoring the need to examine exposures in mixtures as they occur in real world situations.

A more recent review of the association between metals and neurodevelopment through a mean age of 8 years focused on susceptibility factors, including metal co-exposures (Bauer et al., 2020b). While there are mixed findings, adverse interactive associations with neurodevelopment were most frequently observed between lead and manganese (Al-Saleh et al., 2019; Frndak et al., 2019; Menezes-Filho et al., 2018), lead and arsenic (Freire et al., 2018), and lead and cadmium (Levin-Schwartz et al., 2019). Importantly, the review noted the limited availability of research on co-exposures, particularly research employing statistical mixture methods to investigate joint exposure, which is a strength of our study.

There are limited benchmark values for hair metal concentrations. ATSDR suggests arsenic concentrations in hair should not exceed  $1 \mu\text{g/g}$  ((ATSDR), 2007), which is higher than our 95<sup>th</sup> percentile, but lower than our maximum value. Studies among children in neighboring regions in Ohio more directly impacted by manganese pollution reported geometric mean (SD) hair metal concentrations of  $0.4165 \mu\text{g/g}$  (0.0024) (Haynes et al., 2015) and  $0.3602 \mu\text{g/g}$  (0.0022) (Haynes et al., 2018), compared to  $0.1194 \mu\text{g/g}$  (0.0089) in our population, although direct comparison of hair metal levels between studies is challenging due to differences in hair cleaning and analysis methods (Eastman et al., 2013; Jursa et al., 2018). In an Italian study of adolescents living in regions with current and historic ferroalloy activity, the median concentration of hair manganese was  $0.08 \mu\text{g/g}$  (interquartile range 0.10) (Bauer et al., 2020a), which is close to the median in our study (both studies used identical hair cleaning and analysis methods). Overall, hair metal levels in our study population

are generally lower than other studies in children, likely reflecting differences both in endogenous metal exposure and incorporation of metals from the circulation into hair as well as different hair cleaning methodologies (Jursa et al., 2018).

A key strength of this study is the inclusion of a direct assessment of the child as well as reports from mothers and teachers. We know, though, that different informants differentially report on child behavior, with parents typically reporting more problems than teachers (Briggs-Gowan, 1996; Touliatos, 1981). While parents observe their child's behavior across a range of situations and teachers primarily observe behavior only in the classroom, parent report of child behavior is colored by their emotional connection to the child as well as their own mental health (Briggs-Gowan, 1996; Phares, 1989). Teachers, however, are able to gauge a child's behavior in relation to the child's peers, and comment on difficulties related to academic or social success that parents may never see. Reports on boys tend to have more agreement across raters than do reports on girls (Briggs-Gowan, 1996; Touliatos, 1981). Consequently, the concordance between mother and teacher reports on the BASC-2 Adaptive Skills scale is notable.

Associations between environmental exposures and worse adaptive skills have been observed previously. The New Hampshire Birth Cohort Study reported an association between prenatal exposure to manganese in a mixture of six metals and worse Adaptive Skills scores based on maternal report of preschool-aged children (Doherty et al., 2020). The HOME study found evidence of associations between blood lead concentration and worse Adaptive Skills among females, but not males, at age 8 years (Sears et al., 2021). PROGRESS found that higher concentrations of airborne PM<sub>2.5</sub> during the first trimester of pregnancy were associated with worse Adaptive Skills by mother or caregiver report at ages 4–6 years (McGuinn et al., 2020). The New Bedford Cohort reported associations between increased concentrations of phthalates and worse teacher-reported Adaptive Skills among adolescents (Shoaff et al., 2019).

This study's greatest limitation is its cross-sectional design, reliant on measures of recent metals exposure in hair and concurrent associations with outcomes. Without prenatal and early childhood measures of exposure we are unable to discern whether the observed pattern of results reflects adverse impacts of early childhood exposure combined with a strong correlation between early and middle childhood exposure levels. Exposure pathways, however, are known to change as children age (United States. Environmental Protection Agency. Risk Assessment Forum., 2005). Previous examinations in this population showed that hair metal levels varied considerably between subjects and less within subjects when comparing across proximal, medial, and distal hair segments representing approximately a six-month exposure period (Jursa et al., 2018). Still, the relevance of this exposure period during this age range is uncertain. Additionally, this study population is overwhelming white and female, which restricts its generalizability. The predominantly white study sample does, however, reflect that of the original study as well as the original study's recruitment pool. The skewed sex distribution, and consequent differences between children included and excluded from the analyses, results from more males having insufficient hair for sampling and analysis. Also, hair lead as a biomarker is not widely used because of the possibility of exogenous contamination. The use of hair lead measures may have

reduced our ability to observe lead effects in this study. While we employed appropriate statistical methods to examine a mixture of four metals this still represents only a fraction of total metal exposures. Given the limited volume of hair we focused on the three metals (arsenic, cadmium manganese) that exceeded ATSDR and U.S. EPA health-based comparison values during their investigation, as well as lead, which we were able to quantify on the same panel. It is unclear how to compare the pattern of results we observed with the mixtures to what may occur for individual exposures. It is also possible the pattern of substantive associations arose because of the precision of measurement differs among neuropsychological instruments. Some of these associations were based on low concentrations of metals leading to imprecise measures and potentially unstable estimates.

#### 4.1 Conclusion

Despite the above methodological constraints we demonstrate that middle childhood is a developmental window of susceptibility to the adverse neurodevelopmental consequences of environmental toxicant exposure. No segment of the childhood age spectrum appears to be spared from the negative consequences of exposure to environmental neurotoxicants.

### Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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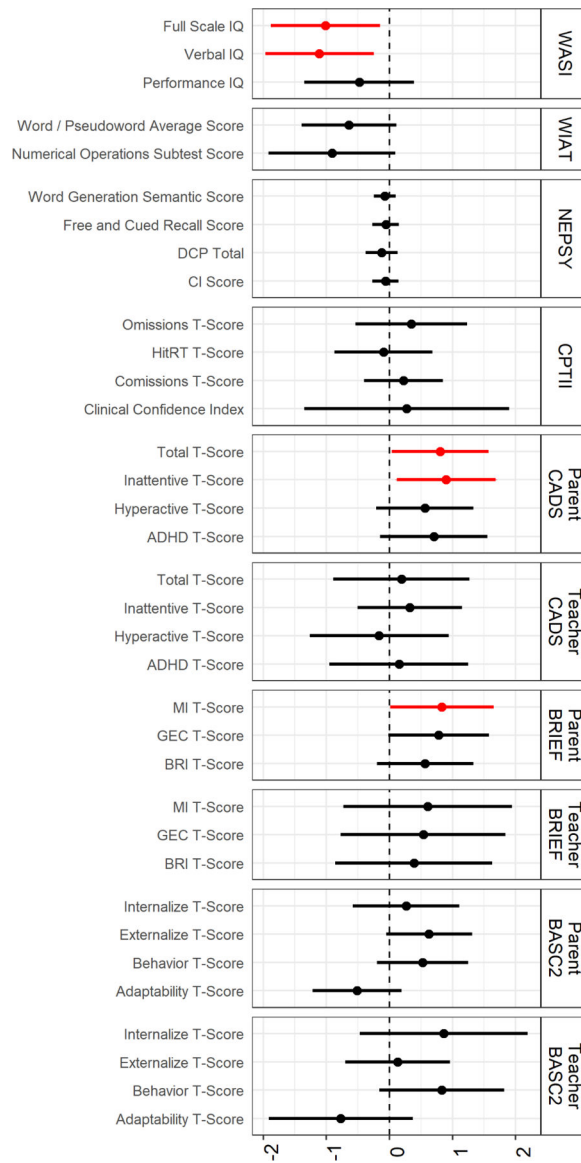
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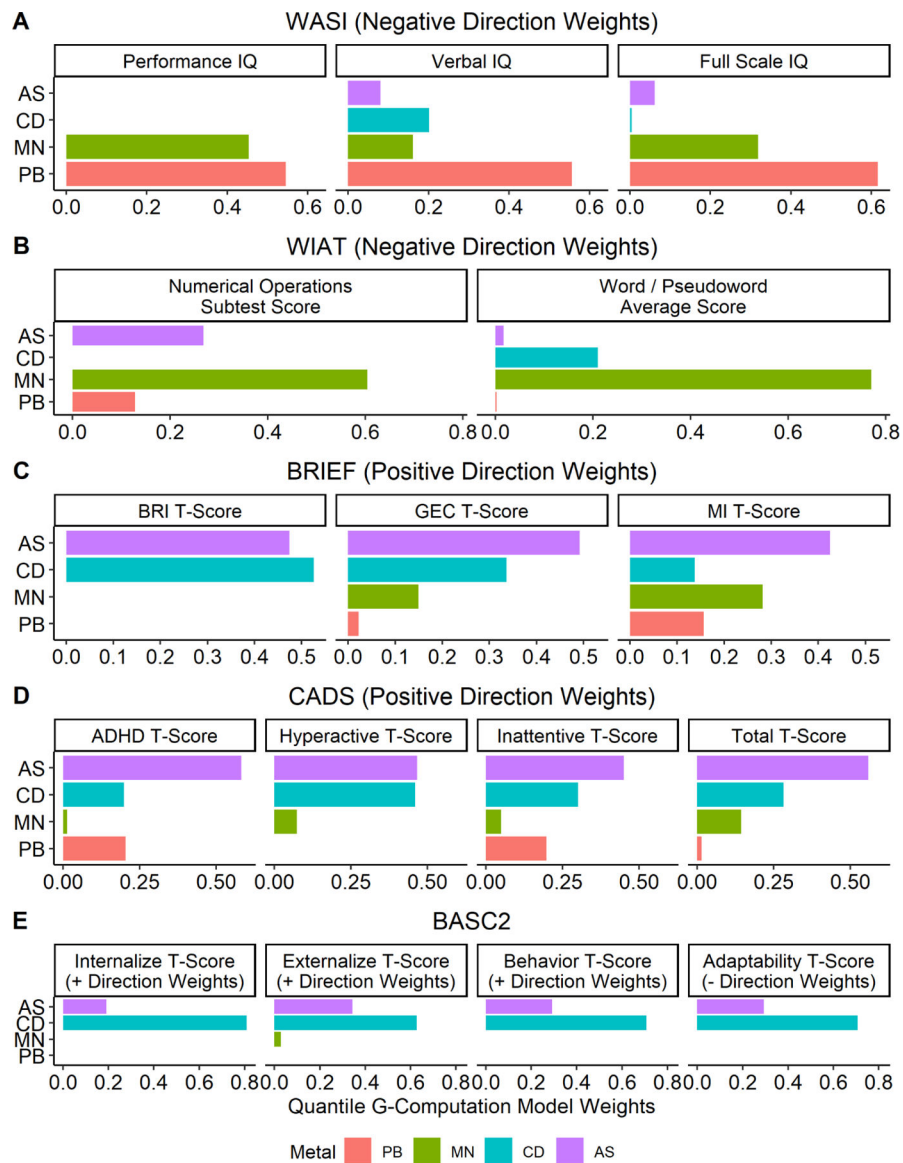


**HIGHLIGHTS**

- For some individual metals, even exposure at low concentrations may be neurotoxic
- Statistical analysis of metal mixtures is more representative of real world exposure
- Metal mixtures were associated with neuropsychological outcomes among children aged 6–12
- Higher metal mixture concentration was associated with lower IQ
- Higher metal mixture concentration was associated with worse maternal-reported behaviors



**Figure 1.** Estimated adjusted change ( $\psi$ ; 95% confidence interval) in neuropsychological measure per decile increase in all metals. Children aged 6–12 years, C8 Health Project Neurobehavioral Follow-up Study, Mid-Ohio Valley, 2009–2010 (n = 222). Effect estimates from Quantile G-Computation are interpreted as change in outcome per decile increase in all metals (arsenic, cadmium, manganese, lead). Adjusted for child age, sex, secondhand smoke exposure from birth to 2 years of age, HOME score (cognitive and emotional), maternal education, maternal IQ, and child examiner (WASI, WIAT, NEPSY, CPT).



**Figure 2.** Estimated weights from Quantile G-Computation indicating contribution of each metal to adjusted association between hair metal mixture and neuropsychological measure averaged across 10 imputed datasets. Children aged 6–12 years, C8 Health Project Neurobehavioral Follow-up Study, Mid-Ohio Valley, 2009–2010 ( $n = 222$ ). All models adjusted for child age, sex, secondhand smoke exposure from birth to 2 years of age, HOME score (cognitive and emotional), maternal education, maternal IQ, and child examiner (WASI, WIAT, NEPSY, CPT). Only weights for the direction of the mixture effect are shown. For example, the metal mixture is negatively associated with WASI scores so only weights from the negative direction are presented. Note: BASC-2 values are for parent report.

**Table 1.**

Characteristics of children aged 6–12 years with hair metal measurements, C8 Health Project Neurobehavioral Follow-up Study, Mid-Ohio Valley, 2009–2010 (n = 222)

Characteristic	N	Percent or Mean	Standard Deviation
Child age, years	222	9.8	1.7
Child sex			
Male	53	23.9	
Female	169	76.1	
Secondhand smoke for age 0–2 years			
Yes	41	18.0	
No	181	82.0	
HOME score, maternal section only			
Cognitive scale	222	6.9	1.7
Emotional scale	222	9.8	1.5
Maternal age, years	221	38.6	6.0
Maternal education			
Less than high school	10	4.5	
High school graduate	64	28.8	
Some college	87	39.2	
College graduate	61	27.5	
Maternal Full Scale IQ (M 100, SD 15) ▲	221	99.4	12.6
Child Neuropsychological Measures			
WASI (M 100, SD 15) ▲			
Full Scale IQ	220	101.9	12.9
Verbal IQ	221	103.2	13.1
Performance IQ	220	99.5	13.0
WIAT (M 100, SD 15) ▲			
Word Reading/Pseudoword Decoding standard score mean	212	102.4	11.1
Numerical Operations standard score	221	97.8	14.3
NEPSY (M 10, SD 3) ▲			
Comprehension of Instructions scaled score	221	10.5	2.9
Design Copying Total scaled score	209	8.6	3.4
Narrative Memory Free and Cued Recall scaled score	221	9.3	2.9
Semantic/Initial Letter scaled score mean	209	9.7	2.5
CPT (M 50, SD 10) ▼			
Clinical Confidence Index	215	47.8	23.5
Omissions T-score	215	53.6	12.3
Commissions T-score	215	56.3	8.2
Hit Reaction Time T-score	215	47.5	11.0

Characteristic	N	Percent or Mean	Standard Deviation
CADS, mother report (M 50, SD 10) ▼			
ADHD Index	221	54.1	11.6
Combined Type	221	53.9	10.6
Inattentive Type	221	52.3	10.7
Hyperactive-Impulsive Type	221	55.3	10.7
CADS, teacher report (M 50, SD 10) ▼			
ADHD Index	134	53.5	12.1
Combined Type	134	51.9	11.5
Inattentive Type	134	48.2	9.2
Hyperactive-Impulsive Type	134	51.7	11.5
BRIEF, mother report (M 50, SD 10) ▼			
Global Executive Composite	219	50.5	11.3
Behavioral Regulation Index	220	49.6	10.9
Metacognition Index	219	50.9	11.3
BRIEF, teacher report (M 50, SD 10) ▼			
Global Executive Composite	134	56.2	14.2
Behavioral Regulation Index	134	54.0	13.3
Metacognition Index	134	56.7	14.7
BASC-2, mother report (M 50, SD 10) ▼			
Behavioral Symptom Index	214	50.2	10.2
Adaptive Skills (▲)	214	50.5	10.0
Internalizing Problems	214	52.3	11.8
Externalizing Problems	214	50.3	9.4
BASC-2, teacher report (M 50, SD 10) ▼			
Behavioral Symptom Index	104	48.9	8.8
Adaptive Skills (▲)	104	51.9	9.9
Internalizing Problems	104	51.3	11.2
Externalizing Problems	104	47.8	7.2

M, mean; SD, standard deviation

▲ higher score reflects better/more favorable performance

▼ lower score reflects better/more favorable performance

**Table 2.**

Hair metal concentrations ( $\mu\text{g/g}$ ) among children ages 6–12 years, C8 Health Project Neurobehavioral Follow-up Study, Mid-Ohio Valley, 2009–2010 (n = 222)

	N	LOD	Minimum	Mean	SD	Median	Interquartile Range	95 <sup>th</sup> -tile	Maximum
Arsenic	215	0.0018	0.0041	0.045	0.206	0.018	0.020	0.098	2.964
Cadmium	221	0.0004	0.0003	0.026	0.084	0.007	0.013	0.113	1.040
Manganese	222	0.0038	0.0025	0.237	0.441	0.109	0.215	0.696	4.100
Lead	221	0.0077	0.0080	0.380	0.829	0.152	0.252	1.330	7.726

LOD, limit of detection

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