Published in final edited form as:
Sci Total Environ. 2024 July 20; 935: 173387. doi:10.1016/j.scitotenv.2024.173387.

Associations of a Toenail Metal Mixture with Attention and Memory in the Gulf Long-Term Follow-Up (GuLF) Study

Joyce J.Y. Lin[1], **Jordan R. Kuiper**[2], **Aisha S. Dickerson**[3], **Jessie P. Buckley**[4], **Heather E. Volk**[5], **Diane S. Rohlman**[6], **Kaitlyn G. Lawrence**[7], **W. Braxton Jackson II**[8], **Dale P. Sandler**[7], **Lawrence S. Engel**[4,7], **Ana M. Rule**[1]

[1]Department of Environmental Health and Engineering, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA.

[2]Department of Environmental and Occupational Health, The George Washington University Milken Institute School of Public Health, Washington, DC, USA.

[3]Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA.

[4]Department of Epidemiology, University of North Carolina Gillings School of Global Public Health, Chapel Hill, NC, USA.

[5]Department of Mental Health, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA.

[6]Department of Occupational and Environmental Health, University of Iowa College of Public Health, Iowa City, IA, USA.

[7]Epidemiology Branch, National Institute of Environmental Health Sciences, National Institutes of Health, Research Triangle Park, NC, USA.

[8]Social & Scientific Systems, Inc., Durham, NC, USA.

Abstract

Background: Research on metal-associated neurodegeneration has largely focused on single metals. Since metal exposures typically co-occur as combinations of both toxic and essential elements, a mixtures framework is important for identifying risk and protective factors. This study examined associations between toenail levels of an eight-metal mixture and attention and memory in men living in US Gulf states.

Corresponding author: Joyce J.Y. Lin, Johns Hopkins Bloomberg School of Public Health, Department of Environmental Health and Engineering, 615 N. Wolfe Street, Baltimore, MD 21205, E6628, Jlin103@jhu.edu.

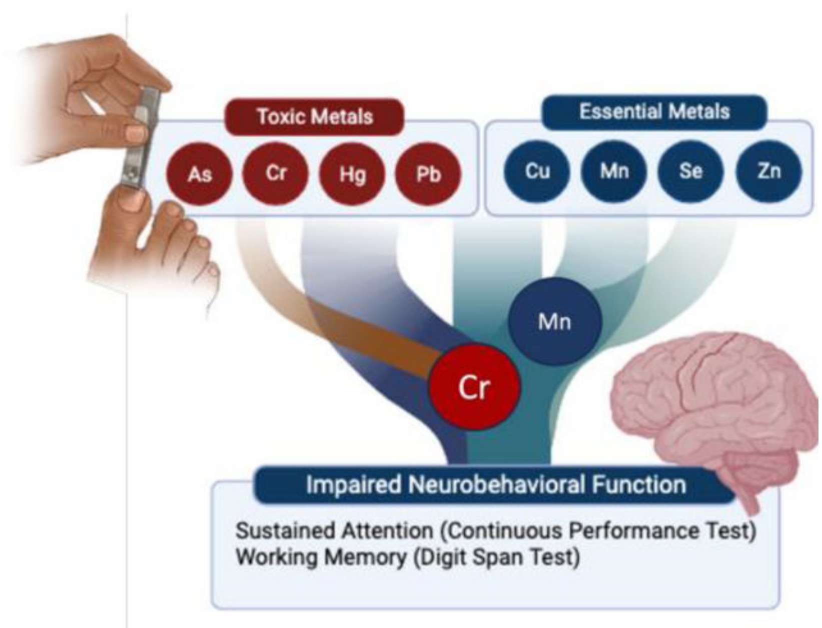
Declaration of interests
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Methods: We measured toenail concentrations of toxic (arsenic, chromium, lead, and mercury) and essential (copper, manganese, selenium, and zinc) metals in 413 non-smoking men (23 – 69 years, 46% Black) from the Gulf Long-Term Follow-Up (GuLF) Study. Sustained attention and working memory were assessed at the time of toenail sample collection using the continuous performance test (CPT) and digit span test (DST), respectively. Associations between toenail metal concentrations and performance on neurobehavioral tests were characterized using co-pollutant adjusted general linear models and Bayesian Kernel Machine Regression.

Results: Adjusting for other metals, one interquartile range (IQR) increase in toenail chromium was associated with a 0.19 (95% CI: −0.31, −0.07) point reduction in CPT D Prime score (poorer ability to discriminate test signals from noise). One IQR increase in toenail manganese was associated with a 0.20 (95% CI: −0.41, 0.01) point reduction on the DST Reverse Count (fewer numbers recalled). Attention deficits were greater among Black participants compared to White participants for the same increase in toenail chromium concentrations. No evidence of synergistic interaction between metals or adverse effect of the overall metal mixture was observed for either outcome.

Conclusions: Our findings support existing studies of manganese-related memory deficits but are some of the first to show chromium related attention deficits in adults. Longitudinal study of cognitive decline is needed to verify chromium findings. Research into social and chemical co-exposures is also needed to explain racial differences in metal-associated neurobehavioral deficits observed in this study.

Graphical Abstract



Keywords

Metals; Toenail Biomarker; Neurobehavioral Outcomes; Mixtures

Author Manuscript
Author Manuscript
Author Manuscript

1. Introduction

The rapid aging of the global population has led to an unprecedented burden of neurodegenerative disease and, consequently, strong interest in identifying potentially modifiable factors that can decelerate cognitive decline (1,2). While much of this work has been in older participants, there is growing attention on identifying risk factors and cognitive performance among younger populations before the onset of clinical disease (3–5). Metals and metalloids, hereafter referred to as metals, are ubiquitous in the environment and have been topics of extensive research, in part, for their wide-ranging effects on neurobehavioral health. Chronic exposures to metals like lead, mercury, and manganese have been shown to induce subtle but irreversible functional impairment in the adult brain (6,7) These deficits have been associated with heightened risks of physical disability, hospitalization, and dementia diagnoses in geriatric populations (8–12).

Despite the long history of research into the health effects of metals, the impact of chronic, low-level exposure to metals such as chromium, copper, and zinc remains understudied due to exposure window limitations in traditional biomonitoring methods (1,13). Even less is known about the combined effects of multiple metal exposures. Metals typically co-occur, as both toxic metals (i.e., arsenic, chromium, lead, and mercury) and essential metals (i.e., copper, manganese, selenium, and zinc), and often share exposure sources. While there is growing concern about the potential synergistic or compounding effects of simultaneous toxic exposures, there is also interest in identifying the potentially neuroprotective role of certain metals within metal mixtures (1). Multiple studies in children and adolescents have investigated multi-metal exposures on cognition and intelligence, finding evidence of adverse joint associations and interactive effects of a metal mixture (14–17). While fewer studies have examined neurotoxic and potentially neurodegenerative effects of combined metal exposures in adults, some have found promising protective effects of selenium and high antioxidant diet on the effect of toxic metals and cognition (18,19). However, most existing studies have relied on metal exposure assessment using blood or urine biomarkers, both of which have metabolic and exposure window limitations for multi-metal panels depending on the metals of interest (20). Toenail metal biomarkers can reflect metal exposures averaged up to 12 months (21) and since metals deposited in the toenail are metabolically inert, we can compare metal concentrations averaged across the same exposure window for all metals of interest.

Studying potentially neurotoxic metals in a mixtures context, as well as vulnerabilities across certain populations, may provide important clues for identifying risk factors and intervention points to delay or reduce the onset of neurodegenerative diseases. We hypothesize that co-exposure to multiple metals may intensify the negative effects of individual toxic metals on cognitive function and that adverse effects may be more pronounced among individuals from more marginalized communities in the US. In this study, we examined individual, joint, and interactive associations between toenail levels of a multi-metal mixture and attention and memory outcomes in a racially diverse cohort of non-smoking men in the US Gulf states.

2. Material and Methods

2.1 Study Population

This study was conducted in a sample of men from the Gulf Long-term Follow-up (GuLF) Study, a prospective cohort of 32,608 individuals (ages 21 years and older at enrollment) from 5 US states (Alabama, Florida, Louisiana, Mississippi, and Texas) formed after the 2010 Deepwater Horizon Disaster. GuLF Study participants either participated in oil spill response activities or received safety training but did not work on the cleanup. Between August 2014 and June 2016, 3,401 individuals who lived within 60 miles of study clinics in Mobile, Alabama, or New Orleans, Louisiana participated in a clinical exam in which trained examiners collected data on health, work history, and residential address, as well as toenail biospecimens, anthropometric measures and neurobehavioral test results. Further details of study events have been described previously (22).

Participants of the overall GuLF Study cohort were selected into the present study if they completed a full neurobehavioral assessment at the clinical exam (n = 2,652), if their biospecimens had been used in an existing GuLF Study biomarker sub-study of kidney function and injury (males only) to maximize biomarker overlap (n=667), and if they provided sufficient toenail specimens for metals analysis and self-reported as a non-smoker at the clinical exam/toenail sample collection (n = 413). Current smokers at the time of toenail sample collection were excluded due to funding limitations with ICP-MS analysis and to maximize the sensitivity of toenail samples to ambient, environmental exposures in a previous study (23). The final analytic sample consisted of 413 non-smoking men. Participants in this study had otherwise comparable characteristics to those in the larger cohort except for smoking status, which was purposefully selected for exclusion. A comparison of participant characteristics from this study to the larger cohort is provided in Supplemental Table 1.

2.2 Metal Measurement in Toenails

Toenail samples, collected at the time of neurobehavioral exam administration, were analyzed for 18 metals/metalloids (aluminum (Al), arsenic (As), cadmium (Cd), calcium (Ca), chromium (Cr), cobalt (Co), copper (Cu), iron (Fe), mercury (Hg), magnesium (Mg), manganese (Mn), molybdenum (Mo), nickel (Ni), lead (Pb), antimony (Sb), selenium (Se), vanadium (V), and zinc (Zn)) using inductively coupled plasma mass spectrometry (ICP-MS).

The present analysis focused on elements with relevance to neurobiology (identified from the literature) (13,24,25), those that have been validated with external exposure studies or are commonly reported in toenails (20,26–28), and those that were detected in >85% of our toenail samples and reliably measured in our previous toenail reliability study (26) (As, Cr, Cu, Hg, Mn, Pb, Se, and Zn). Details of the metals analysis protocol and reliability summaries by metal are provided in a previous study (26). In brief, toenail samples were cleaned using a multi-stage process involving acetone, detergent, and Milli-Q water and then digested in nitric acid (HNO_3, OptimaTM grade) and hydrogen peroxide (H_2O_2, Optima™, grade) with an open vessel microwave-assisted digestion method to 110°C, adapted from the Dartmouth Trace Element Analysis Core (29). Samples were analyzed by ICP-MS (8900 Triple Quad; Agilent technologies, Inc., Santa Clara, CA) in 7 batches. Data quality was monitored via multipoint calibration curves for each analyte at the beginning and end of each batch, analysis of laboratory and digestion blanks, duplicates, spikes, and comparison with two reference materials: human hair Japan NIES #13 (National Institute for Environmental Studies, Ibaraki, Japan) and caprine horn NYS RM 1801 (New York State Department of Health Wadsworth Center, Albany, NY) (30). The limit of detection (LOD) ranged from 0.0003 µg/g (As) to 0.007 µg/g (Zn) (Supplemental Table 2). Values below the LOD were assigned a value of the LOD divided by $\sqrt{2}$ (31).

2.3 Neurobehavioral Outcome Ascertainment

The early signs of neurotoxic dysfunction associated with low-level metal exposure are subtle and can go undetected without standardized testing (6). As a result, the quantification of sub-clinical cognitive impairment often relies on neurobehavioral test batteries that have undergone testing for sensitivity to neurotoxins with test-retest reliability (1,32,33). The GuLF study administered a battery of neurobehavioral tests spanning a wide range of functions using the Behavioral Assessment and Research System (BARS) test to capture as many potential manifestations of neurotoxicity as possible (34). BARS is designed to measure neurobehavioral function in people with limited formal education, literacy, or computer skills and consists of a series of computerized tests with simple verbal instructions and graphics which uses a response keyboard with nine large buttons for ease of use (34). BARS tests have been used to identify neurobehavioral deficits in early-stage Parkinson's patients, symptomatic Persian Gulf War veterans, pesticide-exposed agricultural workers, and workers with oil spill related exposures in the GuLF study (35–38). Here we focused on tests of attention and memory, which have been identified as predictors of future neurodegeneration.

The A-X version of the Continuous Performance Test (CPT) was used to measure selective and sustained attention and the Digit Span Test (DST) was used to measure attention, working memory and memory span. Both tests were administered at the in-person clinical exam in a computerized format that required limited education or literacy to complete (34). Details about the neurobehavioral test battery and setup are provided elsewhere (39,40). Briefly, the CPT presents various stimuli on a screen every 50 milliseconds and participants are required to press a button only when target stimuli are presented. Performance on the CPT is typically reported using multiple outcome measures (omission errors, commission errors, and response latency). In this manuscript, we report the raw (unstandardized) CPT D prime score as the primary outcome as it represents a composite score of the rate of correct hits minus the rate of incorrect hits, which reflects the ability of the participant to discriminate between signal and noise. Secondary outcome measures such as the hit fraction (number of correctly identified targets over total number of targets, "omission error"), and the false alarm fraction (number of false alarms over total responses, "commission error") provide additional details on specific attention related functions, results from which are reported in the supplemental materials. The DST requires participants to type, from memory, a series of 9 digits that were presented visually in both forward and reverse order. We only report results from the raw reverse DST as this measure is more sensitive to working memory deficits than results from the forward DST (41).

2.4. Covariate Selection

We used a conceptual framework informed by existing literature to identify covariates associated with toenail metal concentrations and attention and memory (Supplemental Figure 1). Covariates included age at toenail collection/cognitive assessment (years), body mass index (BMI) calculated using measured height and weight at study visit (continuous), self-reported smoking history (former, never), educational attainment (years), and marital status (single/never married, married/living with partner). Self-reported caffeine use within 2 hrs of the neurobehavioral exam (yes/no; n = 148), frequent alcohol consumption (>14 drinks/week; n = 52), and history of head injury with loss of consciousness (yes/no; n = 36), and oil spill cleanup involvement, measured using estimated cumulative average total hydrocarbon (THC) exposure, did not change effect estimates by more than 10% and thus were not included as covariates or considered as exclusion factors.

2.5. Statistical Analysis

Toenail metal concentrations were log10-transformed to reduce the influence of outliers and to improve model fit (42). Bivariate correlations between metal concentrations were examined using Spearman's rank correlation coefficients. We modeled CPT D Prime and DST Reverse Count outcomes as continuous outcome variables, and secondary CPT outcomes (Correct Response Fraction and Hit Fraction) as dichotomous variables split at above or below 80% based on the distribution of participant scores (Supplemental Figure 2). We standardized all neurobehavioral scores for direction so that a negative regression coefficient indicated worse performance with increasing toenail metal levels.

To investigate confounding by co-exposure while adjusting for covariates, we used co-pollutant adjusted general linear models (GLM) to estimate differences and 95% confidence intervals (CIs) in attention and memory test scores per interquartile range increase of log10-transformed toenail metal concentrations adjusted for the covariates described above. Given differences in participant characteristics by race, as well as disproportionate burdens of cognitive impairment and dementia in Black populations in the US (43), we also assessed effect modification by self-identified race with an interaction term and using stratified analyses, which, due to sample size limitations was categorized into White and Black subpopulations. With only 32 participants comprising the "other" (Asian, American Indian/Alaskan Native, Mixed Race, or other) category, race stratified analyses focused on differences between White and Black participants in this study. As only 11 of the 413 participants in this study identified as Hispanic, we did not account for Hispanic ethnicity. To investigate whether potential effect measure modification by race was driven by differences in education level we additionally stratified our analyses by educational attainment. We examined all models for normality, influence of outliers, multi-collinearity, and heteroscedasticity using added variable plots, distribution of residuals, and variance inflation factors.

To allow for potential non-additive associations and higher-order interactions between metals, we additionally conducted Bayesian Kernel Machine Regression (BKMR) to model flexible dose-response associations with the attention and memory outcomes (44). This method allowed us to examine potential metal interactions as well as the effects of an overall metal mixture on attention and memory outcomes while accounting for correlated mixture components and adjusting for confounders. Component wise variable selection also controls for multiple testing and allowed us to assess the relative importance of individual metals of the mixture.

We standardized log10-transformed metal concentrations and outcomes before BKMR analysis. We fit separate BKMR models for CPT D Prime and DST Reverse Count; both were modeled as continuous outcomes using a Gaussian kernel and adjusted for the covariates identified above. We estimated parameters using five parallel chains, each with 40,000 iterations and default priors using the *bkmrhat* package in R version 4.2.0 (The R Foundation for Statistical Computing Platform). We reported four primary outputs from these models: a) the exposure response relationship for each metal on CPT D Prime and DST Reverse Count when all other metals are fixed at their median; b) the pairwise dose–response relationship for each metal on attention/memory at varying levels (25^{th}, 50^{th}, and 75^{th} percentiles) of a second metal, with remaining metals fixed at their medians; c) the association of an interquartile range (IQR; i.e., 25th to 75th percentile) increase in a particular metal on each outcome, at varying levels of the mixture (i.e., at the 25th, 50th, and 75th); and d) the joint association of incremental percentile increases in the overall 8-metal mixture on attention and memory outcomes, compared with when all metals are at their medians. BKMR results are reported as the difference estimate and 95% credible intervals (CrI).

2.6. Sensitivity Analyses

Two sensitivity analyses were conducted. First, we ran the above-mentioned analyses after removing the 99^{th} percentile of metal concentrations to account for potential outlier observations in metal exposure. Second, we ran the above mentioned GLM models additionally adjusting for each participant’s state of residence.

3. Results

3.1 Participant Characteristics

Study participants comprise 413 adults ranging 21 — 69 (median = 49) years of age. The sample was racially diverse with 46% of participants (n = 191) self-identifying as Black, 46% of participants self-identifying as White (n = 190), and the remaining 8% (n = 32) identifying as Asian, American Indian/Alaskan Native, Mixed Race, or other. Compared to White participants, Black participants were younger, had lower educational attainment, were less often former smokers, and less often never married. Black participants also had lower median toenail concentrations of all metals measured as well as poorer recorded performance on both tests of attention and memory (Table 1).

The detection frequency was above 90% for all metals of interest except As, which was detected in 87% of the toenail samples. Limits of detection and summary information for each metal are provided in Supplemental Table 2. Toenail metal concentrations were low to moderately correlated (Spearman's rho ranging from 0.03 — 0.62), with the highest correlations observed between Mn and Pb (0.62), Mn and As (0.60), and As and Pb (0.55) (Supplemental Figure 3). Adjusted GAMs did not suggest any significant non-linear relationships between metals or continuous covariates with the outcomes (not shown). Thus, metals and continuous covariates were modeled as linear variables. We did not find statistically significant evidence of effect measure modification using interaction analyses between metals and race or education but present suggestive evidence of effect measure modification by self-identified race in race stratified analyses.

3.2 CPT D Prime (selective and sustained attention)

Figure 1 and Supplemental Table 3 summarize the difference in attention associated with an interquartile range (IQR) increase in each of the log10-transformed metal concentrations. In co-pollutant adjusted GLM models, we observed inverse associations between toenail Cr concentration and CPT D Prime score. One IQR increase in toenail Cr concentration was associated with a 0.19 (95% CI: −0.31, −0.07) point reduction in CPT D Prime score, which ranges from 0 – 5.3 among participants in this study. In race-stratified analysis, Black participants exhibited 0.22 (95% CI: −0.42, −0.04) point reduction in CPT D Prime score per IQR increase in toenail Cr. Yet, White participants exhibited only a 0.09 (95% CI: −0.24, 0.05) reduction in CPT D Prime score for the same change in toenail Cr (Figure 1). One IQR increase in toenail Cu appeared to improve CPT D Prime scores by 0.16 (95% CI: 0.03, 0.28) points among White participants but not Black participants who did not exhibit significant differences in CPT D Prime score ($\beta = -0.05$, 95% CI: −0.20, 0.11). We also observed a race-specific improvement in CPT D Prime score for an IQR increase in toenail Zn, but only among Black participants ($\beta = 0.17$, 95% CI: 0.04, 0.30). This same Zn increase did not appear to have any effect on CPT D Prime performance among White participants ($\beta = 0.01$, 95% CI: −0.14, 0.17). Results for the secondary CPT outcome measures (CPT hit fraction, and CPT correct response fraction) followed similar trends to those described above, with inverse associations driven by toenail Cr. The remaining metals (As, Hg, Pb, and Se) did not appear to be significantly associated with performance on any of the CPT tests of sustained attention. Results were robust to sensitivity analyses removing outlier Cr concentrations and, separately, additionally adjusting for state of residence (Supplemental Table 4).

3.3 DST Reverse Count (working memory and memory span)

The difference in working memory test scores associated with an IQR increase in each of the log10-transformed metal concentrations is also presented in Figure 1. We observed inverse associations between toenail Mn concentration and DST Reverse Count score. Specifically, an IQR increase in toenail Mn was associated with 0.19 (95% CI: −0.41, 0.01) point lower performance on the DST Reverse Count (scale from 1 – 9). While the magnitude of the associations were comparable between White ($\beta = -0.30$, 95% CI: −0.69, 0.08) and Black participants ($\beta = -0.32$, 95% CI: −0.61, −0.04), there was greater uncertainty in the estimate for White participants. We also observed positive associations between toenail

Cu and DST Reverse Count scores, with an IQR increase in toenail Cu concentration associated with 0.20 (95% CI: 0.03, 0.36) point better DST Reverse Count performance. However, this association was driven primarily by White participants (β = 0.29, 95% CI: 0.02, 0.56) and was negligible among Black participants (β = 0.08, 95% CI: −0.14, 0.31). We did not observe significant associations with As, Hg, Pb, or Se on performance on the DST Reverse Count of working memory. Sensitivity analyses revealed stronger inverse associations between toenail Mn and DST Reverse Count among Black participants when removing the 99^{th} percentile of Mn concentrations (β = −0.44, 95% CI: −0.84, −0.05) and when, separately, additionally adjusting for state of participation (β = −0.42, 95% CI: −0.82, −0.02). Results of sensitivity analyses are summarized in Supplemental Table 4.

3.4 Stratification by education status

Fifty-six percent of White participants reported having at least some college education or more compared to 41% of Black participants. To evaluate whether race specific associations observed were driven by differences in educational attainment between groups, we additionally stratified our analysis by education using two groups defined as “high school or less” and “some college or more”. Plots comparing the magnitude of associations across stratified categories of race and education are shown in Supplemental Figure 4.

Inverse relationships between toenail Cr with CPT D Prime score observed in race stratified analyses persisted across stratified levels of educational attainment. One IQR increase in toenail Cr was associated with a 0.16 (95% CI: −0.32, 0.01) point decrease in CPT D Prime score among participants with some college education or more and a 0.22 (95% CI: −0.40, −0.03) decrease in CPT D Prime score among participants with high school or less education. On the other hand, an IQR increase in toenail Mn concentration was associated with a 0.32 (95% CI: −0.64, −0.01) decrease in DST Reverse Count score among participants with some college education or more but was more weakly related to DST Reverse Count score among participants with high school or less education (β = −0.11, 95% CI: −0.41, 0.19). Positive associations between toenail Cu and Zn with neurobehavioral test performance that were observed in race-stratified analyses were attenuated in education stratified models.

3.5 Bayesian Kernel Machine Regression (BKMR)

Univariate BKMR analyses evaluating associations of each of the metals in the eight-metal mixture with sustained attention and working memory did not indicate any non-linear relationships or interactions between exposures (Figure 2). Consistent with the findings from general linear regression, when all other metals were held at their median concentrations, an increase in toenail Cr concentration from the 25^{th} to 75^{th} percentile was associated with 0.18 (95% CrI: −0.33, −0.03) lower CPT D Prime score (Figure 3). Inverse trends were also observed for Mn and DST Reverse Count score, but the estimates were imprecise (β = −0.09, 95% CrI: −0.21, 0.03). A positive association between toenail Cu was observed for both outcomes with stronger effects observed for DST Reverse Count (β = 0.09, 95% CrI: −0.001, 0.19) and results crossing the null for CTP D Prime score (β = 0.09, 95% CrI: −0.03, 0.20) (Figure 3).

The posterior inclusion probabilities (PIPs) for each metal in the model also supported the GLM findings with Cr (PIP = 0.63) being the most important metal to the CPT D Prime outcome of sustained attention and Mn (PIP = 0.13) being the most important metal (with a noticeable inverse trend) to the DST Reverse Count outcome of working memory. PIPs for Cu were high compared to other metals in both CPT D Prime (PIP = 0.49) and DST Reverse Count (PIP = 0.16) models, suggesting the relative importance of Cu to both outcomes.

Examination of pairwise metal exposure-response functions for CPT D Prime was suggestive of potential interactions between Cr and Cu, Cr and Hg, and Cr and Mn, but the divergence in effect for Cu, Hg, and Mn was minimal across various quantiles (0.25, 0.50, 0.75) of Cr (Supplemental Figure 5a & 5b). We observed no evidence of interactions for the DST Reverse outcome (Supplemental Figure 5c). When we evaluated the individual associations of an IQR increase in each metal, holding all other metals in the mixture at the 25^{th}, 50^{th}, and 75^{th} percentiles, we observed that Cu was particularly influential for both outcomes in the positive direction (Figure 3). Similarly, we observed that Cr and Mn were separately influential for CPT D Prime and DST Reverse Count, respectively (Figure 3). There was no evidence of an overall joint mixture effect for either sustained attention or working memory outcomes (Figure 4A1, Figure 4B1).

3.5.1 BKMR by race categories—Univariate BKMR results by race categories were consistent with race-stratified results from co-pollutant adjusted multiple linear regression suggesting greater influence of Cr and Mn among Black participants compared to their White counterparts after adjusting for education level. Race specific plots of the univariate relationship between toenail metals and CPT D Prime are provided in Supplemental Figure 6a and for DST reverse count in Supplemental Figure 6b.

An inverse trend for Cr and CPT D Prime was observed for both racial groups, but the relative importance of Cr to the CPT D Prime outcome was stronger among Black participants (PIP = 0.77) compared to White participants (PIP = 0.22) (Supplemental Figure 6a). The same was observed for Mn and DST Reverse count with the PIPs for Mn among Black participants (PIP = 0.55) more than twice that of the Mn PIP among White participants (PIP = 0.20) (Supplemental Figure 6b). Similar to findings from GLM, the positive effects of Cu on CPT D Prime and DST Reverse Count scores were exclusive to White participants. Cu was minimally influential among Black participants with no visible positive trend and low PIPs for both CPT D Prime (Cu PIP = 0.29) and DST Reverse Count (PIP = 0.11) (Supplemental Figure 6a, 6b).

No evidence of synergistic metal interactions was observed in different race categories for BKMR models (results not shown). However, there was an inverse joint mixture effect of the eight-metal mixture and CPT D Prime score among Black participants (Figure 4A1). The overall effect was predominantly driven by the inverse association between toenail Cr and CPT D Prime score (PIP = 0.77) but supported by inverse associations for As (PIP = 0.34), Hg (PIP = 0.27), Mn (PIP = 0.35), and Se (PIP = 0.42). A negative trend was similarly observed for the joint effect of the eight-metal mixture and DST Reverse Count among Black participants, but the estimates were imprecise and crossed the null (Figure 4B1). No evidence of a joint mixture association with CPT D Prime score was observed among White participants, but there appeared to be slight a positive effect of the joint overall mixture with DST Reverse Count score among White participants above the 75^{th} quantile of the joint exposure compared to the median (Figure 4B2).

3.6 Differences in toenail metal concentration by race

Concentrations of all toenail metals were 23% (Se) to 71% (As) lower among Black participants compared to White participants after adjusting for personal and environmental factors such as age, BMI, smoking history, passive smoke exposure, and state of residence, although the difference was not statistically significant for Pb (Supplemental Figure 7). Compared to White participants, Black participants had greater variability in toenail metal concentrations, though the overall distribution of observed concentrations largely overlapped between both groups (Supplemental Figure 8).

4. Discussion

Metals play important roles in health and disease, and the mechanism by which metals induce neurodegeneration is dependent on various factors, such as the metal and its valence state, the dose, and the route of exposure (13). While the mechanisms of neurotoxicity are well-established for certain toxic metals like Pb, the neurotoxic mechanisms for other, lesser studied metals like Cr remain unclear, though oxidative stress and inflammatory processes are suspected to be unifying pathways (24,45–47). In this study, we examined the association of toenail concentrations of an eight-metal mixture on sustained attention and working memory outcomes in a racially diverse sample of non-smoking men in the US Gulf states. We considered the independent (co-pollutant adjusted) associations of each metal, interactions between metals in the mixture, and associations of the joint eight-metal mixture. Results from BKMR were consistent with those for the GLM and showed that deficits in sustained attention were driven by toenail Cr concentrations, while deficits related to working memory were driven by toenail Mn concentrations. In race-stratified analyses, we found stronger inverse associations of Cr and Mn with sustained attention and working memory performance among Black participants and positive associations between Cu and both outcomes only among White participants. There was limited evidence of synergistic interactions between metals within the mixture and either outcome. The joint effects of the overall eight-metal mixture and performance on both sustained attention and working memory tests were null for the overall sample, but an inverse trend was observed for CPT D Prime among Black participants in race specific analyses. We did not observe adverse sustained attention and working memory effects from established neurotoxic metals such Pb using measurements taken from the toenail.

4.1 Essential metals

Toenail Mn was inversely associated with performance on the Reverse DST of working memory using both GLM and BKMR. Manganese is a well-studied metal that is required for normal physiological functioning but is toxic outside of a “normal” physiological range (i.e., low or high/excess levels). Yet, the ideal physiologic range is not well established as it varies by individual, genetic, and environmental factors, and has not been quantified for toenail concentrations. The underlying mechanisms of Mn neurotoxicity include oxidative stress, mitochondrial dysfunction, autophagy dysregulation, accumulation of intracellular toxic metabolites, and apoptosis (48). Occupational studies have consistently shown the adverse effects of Mn exposure on attention and memory at high concentrations (49–52). Several studies have also found working memory deficits related to chronic Mn exposure at ambient environmental concentrations in children and non-human primates (17,53,54). Our findings are consistent with several existing studies of the adverse effects of Mn exposure on working memory, but it is one of few studies to highlight this association at ambient Mn concentrations in adults.

Increasing toenail Cu was associated with improvements in performance on both tests of sustained attention and working memory, driven by stronger association among White participants. However, a positive relationship was observed between toenail Zn and CPT D Prime score only among Black participants. Both Cu and Zn are essential elements that are known to have both neurotoxic and neuroprotective effects at varying concentrations (1). Both elements also serve as cofactors of major antioxidant enzymes that play important roles in reducing oxidative stress (55), which may explain their positive associations with performance on sustained attention and working memory tests. At higher exposures, Cu and Zn have been shown to induce oxidative stress and disrupt homeostatic mechanisms (1).

The neurotoxicity of acute Se exposure has been shown in laboratory and animal studies but the effect of chronic Se exposure in human epidemiological studies is less well-characterized (56). Our null findings for toenail Se concentration and sustained attention and working memory outcomes in this study suggest that Se may require longer or more frequent, cumulative exposures to elicit a neurotoxic response in humans, but more research is needed to determine this.

4.2 Toxic metals

We observed poorer performance on all three outcome measures of the CPT of sustained attention with increasing toenail Cr concentrations. Hexavalent Cr [Cr(VI)] is a known human carcinogen that is increasingly being recognized as a potential neurotoxicant due to its rapid reduction to Cr(III) in the body, a process that produces reactive oxygen species that contribute to the oxidative deterioration of biological macromolecules (57). Animal studies have also found elevated proinflammatory cytokines in rats following Cr(VI) exposure, highlighting potential neuroinflammatory pathways for Cr(VI) neurotoxicity (58,59). While human studies remain limited, studies have identified inverse relationships between Cr measured in hair and urine and neurobehavioral tests of attention in children (60) and topological brain metrics in adolescents (61). Our results are consistent with the few published studies on Cr neurotoxicity and represent one of the first reports of ambient Cr-related sustained attention impairment in adults.

Despite the well-documented neurotoxic effects of Hg, As, and Pb, none of these metals were associated with sustained attention or working memory deficits in this sample. Mercury in the toenail is predominantly comprised of methylmercury, a common indicator of seafood intake (62,63). As a result, the neurotoxic effects of Hg may be offset, to some degree, by the positive effects of fish consumption on cognitive performance in this sample (64). Arsenic is a neurotoxicant that is known to induce neurological damage through a variety of cellular pathways (46); yet, epidemiologic studies frequently report inconsistent associations with neurocognitive performance depending on the functional tests used to measure deficits (66,67). Thus, it is possible that our selected neurobehavioral tests (CPT, DST) were not sensitive to the neurotoxic effects of As concentrations measured in the toenail. Lead is an extensively studied neurotoxic metal that has shown consistent associations with memory deficits when measured in blood and bone (68–70). Yet, in this study, we found no significant associations between Pb and either sustained attention or working memory, which may be attributed to the timing of exposures relevant for toenail measures.

4.3 Toenail metal biomarkers

Toenail samples can reflect stable metal concentrations averaged over 4 – 6 months from as much as 6 – 12 months prior to clipping (71,72), which reduces many of the metabolic and exposure window limitations that complicate the interpretation of multi-metal panels in blood or urine. The toenail biomarker is still a growing area of research and has proven to be an appropriate biomarker of external exposure to As, Hg, Cu, Mn, Se, and Zn, but the validity of this matrix for the measurement of Cr and Pb is less clear despite frequent reporting of both elements in toenail metal studies (20,28,73). However, it is important to note that the utility of a biomarker relies on a combination of measurement precision and accuracy as well as the ability of the biomarker to predict health outcomes of interest (74). Since measurement reliability in the toenail matrix has already been shown (26), understanding the utility of the toenail biomarker for Cr and Pb exposure assessment now relies on the inclusion of these metals in epidemiologic research. This study adds to a growing literature highlighting the potential utility of toenail as a source of Cr and Mn exposure assessment for studies investigating longer exposure windows and neurodegenerative risk. On the other hand, while toenail Pb may be useful for population level surveillance or biomonitoring (23), its utility in health studies remains unclear. A 2020 study investigating Pb exposure and cognition in children using both blood and toenail measures of Pb found that while concentrations in both matrices were correlated, blood Pb concentration was predictive of cognition at relative levels where toenail Pb was not (75). Our null Pb findings, when combined with previous reports of null toenail Pb findings, raise further questions about their usefulness as exposure measures in studies of cognitive function.

Racial differences in metal exposures were corroborated by blood metal measurements of Hg, Mn, Pb, and Se collected in 723 participants of the same age and gender in another GuLF sub-study (23). Blood concentrations of Pb were not significantly different by race but concentrations of Hg, Mn, and Se were significantly lower among Black participants than White participants after adjusting for age, BMI, smoking history, passive smoke exposure, and employment status. Blood concentrations of As, Cu, Cr, and Zn were not measured.

4.4 Heterogeneity of effect across racial groups

Our ability to detect statistically significant effect measure modification may be limited by our sample size. However, since few studies have had sufficient diversity to address questions related to heightened susceptibility due to race/social factors, we stratified the analyses by race to investigate potential susceptibilities to metal neurotoxicity to the extent that race serves as a proxy of unmeasured social factors or other health-related conditions in the historically segregated US Gulf region. In race-stratified analysis, we found suggestive, though not statistically significant, evidence of effect modification by race. While confidence intervals overlapped, we observed greater Cr and Mn related neurobehavioral deficits among Black participants despite their having lower median toenail concentrations of both metals compared to White participants. The positive effect of toenail Zn on attention was similarly observed only among Black participants. While greater exposure variability among Black participants could, in part, explain this heterogeneity of effect by race for Cr, Mn, and Zn, it does not explain the positive Cu associations with both neurobehavioral outcomes that were observed among White participants.

Another consideration is that metal concentrations observed in this study are unlikely to cover the full range of exposures along each metal's dose response curve. This is particularly relevant when examining metals that have non-linear or U-shaped dose response curves where the point along the continuum of exposure and response can change both the direction and magnitude of the reported association. Considering that toenail Cu and Zn concentrations among Black participants were lower compared to White participants, it is possible that race-specific associations reflect different directions of association at varying concentrations of the metal.

Education-stratified results mirrored race-stratified results for Cr and CPT D Prime performance suggesting that racial differences in the magnitude of this association may be influenced by differences in educational attainment between racial groups in the Gulf region. However, the interpretations of education-stratified results for Mn, Cu, and Zn are less clear, as they did not follow race-stratified trends. It is possible that other unmeasured factors such as diet or occupation, which may differ systematically between racial groups in the Gulf region, could contribute to the race specific protective or adverse effects. Previous work in this cohort showed that Black participants tended to reside closer to industrial facilities and in census tracts with lower median household income (23), potentially disproportionately adding harmful exposures or influencing the way they interact with their environments in ways that could negatively impact cognition (e.g., *less walkable neighborhoods leading to less physical activity)*.

Racial disparities on standard neuropsychological tests have also been observed previously, with older Black individuals performing worse on a range of tests than older White individuals, even when matched for level of education (76,77). While differences in education quality may be a factor in these disparities, social stressors such as segregation and discrimination may also play a role (76). In the US, for a given level of education, Black individuals often earn less than their White counterparts (78) leading to potential differences in diet and healthcare access which may be tangible downstream manifestations of social phenomena like segregation and racial discrimination. Furthermore, social stressors have been shown to have similar biological mechanisms as chemical (and other environmental) exposures, thereby introducing potential synergistic effects of chemical and social co-exposures (43,79). Co-occurring psychosocial stressors and metal exposures throughout life can increase allostatic load and hypothalamic-pituitary-adrenal (HPA) axis dysregulation, which have been indicated in the pathophysiology of neurodegenerative and behavioral disorders (80–83). Therefore, the same magnitude of chemical exposures may have greater adverse impacts on socially disadvantaged populations who experience greater cumulative burden of chronic stress and life events such as racism and discrimination (79). It may also be difficult to detect associations between metal exposures and cognitive impairment among individuals with several other primary risk factors for adverse neurobehavioral outcomes. Future studies might examine potential interactions between Cr and allostatic load as a potential explanation for heterogeneity of effect by race.

4.5 Limitations

There are several limitations of this study that should be considered. First, we cannot infer causality in this study design without longitudinal follow-up for cognitive decline. Since performance on neurobehavioral tests can be influenced by a variety of non-exposure related factors, follow-up for decline over time would provide a clearer picture of the extent of impairment and clarify the temporal relationship. Second, the study has a relatively small sample size, and we could not undertake metal speciation reporting only total metal concentrations in the toenail. While speciation is less of a concern for metals like Hg, which we know is comprised primarily of methylmercury in the toenail, it is important when considering metals like chromium for which Cr(VI) is far more toxic than Cr(III). Third, we recognize the tradeoffs in using metal biomonitoring over external exposure assessment and acknowledge that the route of exposure (not captured using biomonitoring methods) may be critical to the severity of any resulting neurobehavioral impairment. Last, although we controlled for several factors that might affect toenail metal concentrations and cognitive performance, potential sources of bias may remain. Notably, there may be unmeasured confounding by other neuroactive exposures (including metals not measured in this sample) or dietary factors. Our use of self-reported smoking history could have led to misclassification or residual confounding related to issues of social desirability. Moreover, while our exclusion of self-reported current smokers resulted in participants with otherwise similar characteristics to those in the overall cohort, there may be factors related to non-smoking status that limit the generalizability of our findings to broader populations.

Despite these limitations, we provide novel contributions to metal neurotoxicity research on two fronts. First, our results raise unique questions about lesser-studied metals and their effects on sustained attention in adults. The application of BKMR allowed us to reduce the dimension of correlated metals and identify toenail Cr and Mn as major drivers of sustained attention and working memory deficits in this population. Our results for Mn are consistent with the existing literature showing the adverse effects of Mn exposure on working memory and demonstrate the utility of Mn biomonitoring in the toenail matrix. Our results for Cr, when combined with the growing Cr neurobehavioral literature, highlight the importance of expanding research into the mechanisms of Cr neurotoxicity and validation studies for toenail Cr biomonitoring. In particular, longitudinal research involving Cr biomonitoring is needed to understand whether Cr-related neurobehavioral deficits observed cross-sectionally also translate to neurobehavioral deficits over time. Second, neurobehavioral studies are rarely racially and ethnically diverse, and Black participants are frequently under-represented in neurotoxicity studies (43). Leveraging the racial diversity in the GuLF Study, we found significant differences in the magnitude of both positive and negative associations between metals and neurobehavioral test performance by self-reported race. Our findings suggest that Black participants in this study may experience greater attention impairments at lower concentrations of Cr than their White counterparts, supporting the need for future mixtures research to incorporate interactions between social and chemical stressors.

5. Conclusion

In this sample of non-smoking adult male Gulf state residents, we identified inverse associations between toenail Cr concentration with selective and sustained attention and toenail Mn concentrations with working memory and memory span, drawing greater attention to the neurotoxic potential of these metals in adults. Heterogeneity of metal-associated neurobehavioral performance by race identified potential vulnerabilities among Black participants and highlights the need to address potential interactions between social and chemical stressors in neuro-epidemiology research.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

We thank all GuLF study participants, the field visit examiners who assisted with data collection, and Kate Christenbury for data management on this project.

Funding

This research was supported in part by a grant from the US Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health to the Johns Hopkins Education and Research Center for Occupational Safety and Health (award number T42 OH0008428), by the Office of The Director of the National Institutes of Health under Award Number S10OD030355, the NIH Common Fund, the Intramural Research Program of the NIH, National Institute of Environmental Health Sciences (ZO1 ES 102945), and the 21st Century Cities Initiative at Johns Hopkins University. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Data Availability

The data and code can be requested by email to the corresponding author.

References

1. Bain L, Norris SP, Stroud C, editors. Environmental Neuroscience: Advancing the Understanding of How Chemical Exposures Impact Brain Health and Disease: Proceedings of a Workshop [Internet]. Washington, D.C.: National Academies Press; 2020 [cited 2023 Jul 23]. Available from: https://www.nap.edu/catalog/25937
2. Feigin VL, Vos T, Nichols E, Owolabi MO, Carroll WM, Dichgans M, et al. The global burden of neurological disorders: translating evidence into policy. The Lancet Neurology. 2020 Mar 1;19(3):255–65. [PubMed: 31813850]
3. Schoenmaker N, Gool WAV. The age gap between patients in clinical studies and in the general population: a pitfall for dementia research. The Lancet Neurology. 2004 Oct 1;3(10):627–30. [PubMed: 15380160]

4. Röhr S, Pabst A, Baber R, Engel C, Glaesmer H, Hinz A, et al. Social determinants and lifestyle factors for brain health: implications for risk reduction of cognitive decline and dementia. Sci Rep. 2022 Jul 28;12(1):12965. [PubMed: 35902604]
5. Hendriks S, Ranson JM, Peetoom K, Lourida I, Tai XY, de Vugt M, et al. Risk Factors for Young-Onset Dementia in the UK Biobank. JAMA Neurology. 2024 Feb 1;81(2):134–42. [PubMed: 38147328]
6. White RF, Feldman RG, Travers PH. Neurobehavioral Effects of Toxicity Due to Metals, Solvents, and Insecticides. Clinical Neuropharmacology. 1990 Oct;13(5):392. [PubMed: 2272020]
7. Andrade VM, Aschner M, Marreilha Dos Santos AP. Neurotoxicity of Metal Mixtures. Adv Neurobiol. 2017;18:227–65. [PubMed: 28889271]
8. Gooch CL, Pracht E, Borenstein AR. The burden of neurological disease in the United States: A summary report and call to action. Annals of Neurology. 2017;81(4):479–84. [PubMed: 28198092]
9. Blacker D, Lee H, Muzikansky A, Martin EC, Tanzi R, McArdle JJ, et al. Neuropsychological Measures in Normal Individuals That Predict Subsequent Cognitive Decline. Archives of Neurology. 2007 Jun 1;64(6):862–71. [PubMed: 17562935]
10. Morris JC, Storandt M, Miller JP, McKeel DW, Price JL, Rubin EH, et al. Mild Cognitive Impairment Represents Early-Stage Alzheimer Disease. Archives of Neurology. 2001 Mar 1;58(3):397–405. [PubMed: 11255443]
11. Kawas CH, Corrada MM, Brookmeyer R, Morrison A, Resnick SM, Zonderman AB, et al. Visual memory predicts Alzheimer's disease more than a decade before diagnosis. Neurology. 2003 Apr 8;60(7):1089–93. [PubMed: 12682311]
12. Lopez OL, Jagust WJ, Dulberg C, Becker JT, DeKosky ST, Fitzpatrick A, et al. Risk Factors for Mild Cognitive Impairment in the Cardiovascular Health Study Cognition Study: Part 2. Archives of Neurology. 2003 Oct 1;60(10):1394–9. [PubMed: 14568809]
13. Wright RO, Baccarelli A. Metals and neurotoxicology. J Nutr. 2007 Dec;137(12):2809–13. [PubMed: 18029504]
14. Bauer JA, Devick Katrina L, Bobb Jennifer F, Coull Brent A, Bellinger David, Benedetti Chiara, et al. Associations of a Metal Mixture Measured in Multiple Biomarkers with IQ: Evidence from Italian Adolescents Living near Ferroalloy Industry. Environmental Health Perspectives. 2020;128(9):097002. [PubMed: 32897104]
15. Muñoz MP, Rubilar P, Valdés M, Muñoz-Quezada MT, Gómez A, Saavedra M, et al. Attention deficit hyperactivity disorder and its association with heavy metals in children from northern Chile. Int J Hyg Environ Health. 2020 May;226:113483. [PubMed: 32106053]
16. Skogheim TS, Weyde KVF, Engel SM, Aase H, Surén P, Øie MG, et al. Metal and essential element concentrations during pregnancy and associations with autism spectrum disorder and attention-deficit/hyperactivity disorder in children. Environment International. 2021 Jul 1;152:106468. [PubMed: 33765546]
17. Oppenheimer AV, Bellinger DC, Coull BA, Weisskopf MG, Korrick SA. Prenatal exposure to chemical mixtures and working memory among adolescents. Environmental Research. 2022 Apr 1;205:112436. [PubMed: 34843721]
18. Laouali N, Benmarhnia T, Lanphear BP, Weuve J, Mascari M, Boutron-Ruault MC, et al. Association between blood metals mixtures concentrations and cognitive performance, and effect modification by diet in older US adults. Environ Epidemiol. 2022 Jan 25;6(1):e192. [PubMed: 35169670]
19. Sasaki N, Carpenter DO. Associations between Metal Exposures and Cognitive Function in American Older Adults. International Journal of Environmental Research and Public Health. 2022 Jan;19(4):2327. [PubMed: 35206515]
20. Gutiérrez-González E, García-Esquinas E, de Larrea-Baz NF, Salcedo-Bellido I, Navas-Acien A, Lope V, et al. Toenails as biomarker of exposure to essential trace metals: A review. Environmental Research. 2019 Dec 1;179:108787. [PubMed: 31610392]
21. Longnecker MP, Stampfer MJ, Morris JS, Spate V, Baskett C, Mason M, et al. A 1-y trial of the effect of high-selenium bread on selenium concentrations in blood and toenails. The American Journal of Clinical Nutrition. 1993 Mar 1;57(3):408–13. [PubMed: 8438776]

22. Kwok RK, Engel LS, Miller AK, Blair A, Curry MD, Jackson WB, et al. The GuLF STUDY: A Prospective Study of Persons Involved in the Deepwater Horizon Oil Spill Response and Clean-Up. Environ Health Perspect. 2017;125(4):570–8. [PubMed: 28362265]
23. Lin JJY, Werder EJ, Lawrence KG, Braxton Jackson W, Sandler DP, Dickerson AS, et al. Residential Proximity to Metal-Emitting Industries and Toenail Metal Concentration in the US Gulf States. Expo Health [Internet]. 2024 Jan 2 [cited 2024 Jan 7]; Available from: 10.1007/s12403-023-00618-0
24. Aschner M, Costa LG, editors. Neurotoxicity of Metals [Internet]. Cham: Springer International Publishing; 2017 [cited 2022 Nov 9]. (Advances in Neurobiology; vol. 18). Available from: http://link.springer.com/10.1007/978-3-319-60189-2
25. Cicero CE, Mostile G, Vasta R, Rapisarda V, Signorelli SS, Ferrante M, et al. Metals and neurodegenerative diseases. A systematic review. Environmental Research. 2017 Nov 1;159:82–94. [PubMed: 28777965]
26. Lin JJY, Koffman LJ, Tehrani MW, Chen R, Han SG, Sandler DP, et al. Reliability of low mass toenail samples as biomarkers of chronic metal exposure. J Expo Sci Environ Epidemiol. 2023 Jun 9;1–9.
27. Garland M, Morris JS, Rosner BA, Stampfer MJ, Spate VL, Baskett CJ, et al. Toenail trace element levels as biomarkers: reproducibility over a 6-year period. Cancer Epidemiol Biomarkers Prev. 1993 Oct;2(5):493–7. [PubMed: 8220096]
28. Martinez-Morata I, Sobel M, Tellez-Plaza M, Navas-Acien A, Howe CG, Sanchez TR. A State-of-the-Science Review on Metal Biomarkers. Curr Envir Health Rpt [Internet]. 2023 Jun 20 [cited 2023 Jun 30]; Available from: 10.1007/s40572-023-00402-x
29. Andrew AS, O’Brien KM, Jackson BP, Sandler DP, Kaye WE, Wagner L, et al. Keratinous biomarker of mercury exposure associated with amyotrophic lateral sclerosis risk in a nationwide U.S. study. Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration. 2020 Jul 2;21(5–6):420–7. [PubMed: 32329357]
30. Tehrani MW, Yang KX, Parsons PJ. Development and characterization of reference materials for trace element analysis of keratinized matrices. Anal Bioanal Chem. 2020 Mar;412(8):1847–61. [PubMed: 32020317]
31. Helsel DR. Nondetects and data analysis: Statistics for censored environmental data. 2005 [cited 2022 Oct 4]; Available from: https://pubs.er.usgs.gov/publication/70180734
32. Rohlman DS, Bailey SR, Brown M, Blanock M, Anger WK, McCauley L. Establishing stable test performance in tests from the Behavioral Assessment and Research System (BARS). Neurotoxicology. 2000 Oct;21(5):715–23. [PubMed: 11130275]
33. Palta P, Snitz B, Carlson MC. Chapter 7 - Neuropsychologic assessment. In: Aminoff MJ, Boller F, Swaab DF, editors. Handbook of Clinical Neurology [Internet]. Elsevier; 2016 [cited 2022 Dec 6]. p. 107–19. (Neuroepidemiology; vol. 138). Available from: https://www.sciencedirect.com/science/article/pii/B9780128029732000070 [PubMed: 27637955]
34. Rohlman DS, Gimenes LS, Eckerman DA, Kang SK, Farahat FM, Kent Anger W. Development of the Behavioral Assessment and Research System (BARS) to Detect and Characterize Neurotoxicity in Humans. NeuroToxicology. 2003 Aug 1;24(4):523–31. [PubMed: 12900065]
35. Camicioli R, Grossmann SJ, Spencer PS, Hudnell K, Anger WK. Discriminating mild parkinsonism: Methods for epidemiological research. Movement Disorders. 2001;16(1):33–40. [PubMed: 11215590]
36. Storzbach D, Rohlman DS, Anger WK, Binder LM, Campbell KA. Neurobehavioral deficits in Persian Gulf veterans: additional evidence from a population-based study. Environ Res. 2001 Jan;85(1):1–13. [PubMed: 11161646]
37. Rohlman DS, Lasarev M, Anger WK, Scherer J, Stupfel J, McCauley L. Neurobehavioral Performance of Adult and Adolescent Agricultural Workers. NeuroToxicology. 2007 Mar 1;28(2):374–80. [PubMed: 17141876]
38. Quist AJL, Rohlman DS, Kwok RK, Stewart PA, Stenzel MR, Blair A, et al. Deepwater Horizon oil spill exposures and neurobehavioral function in GuLF study participants. Environmental Research. 2019 Dec 1;179:108834. [PubMed: 31703974]

39. Rohlman DS, Gimenes LS, Eckerman DA, Kang SK, Farahat FM, Kent Anger W. Development of the Behavioral Assessment and Research System (BARS) to Detect and Characterize Neurotoxicity in Humans. NeuroToxicology. 2003 Aug 1;24(4):523–31. [PubMed: 12900065]
40. Rohlman DS, Lucchini R, Anger WK, Bellinger DC, van Thriel C. Neurobehavioral testing in human risk assessment. NeuroToxicology. 2008 May;29(3):556–67. [PubMed: 18539229]
41. Banken JA. Clinical utility of considering Digits Forward and Digits Backward as separate components of the wechsler adult intelligence Scale-Revised. Journal of Clinical Psychology. 1985;41(5):686–91.
42. Choi G, Buckley JP, Kuiper JR, Keil AP. Log-transformation of Independent Variables: Must We? Epidemiology. 2022 Nov 1;33(6):843–53. [PubMed: 36220581]
43. Barnes LL. Alzheimer disease in African American individuals: increased incidence or not enough data? Nat Rev Neurol. 2022 Jan;18(1):56–62. [PubMed: 34873310]
44. Bobb JF, Valeri L, Claus Henn B, Christiani DC, Wright RO, Mazumdar M, et al. Bayesian kernel machine regression for estimating the health effects of multi-pollutant mixtures. Biostatistics. 2015 Jul;16(3):493–508. [PubMed: 25532525]
45. Farina M, Avila DS, da Rocha JBT, Aschner M. Metals, oxidative stress and neurodegeneration: A focus on iron, manganese and mercury. Neurochemistry International. 2013 Apr 1;62(5):575–94. [PubMed: 23266600]
46. Huat TJ, Camats-Perna J, Newcombe EA, Valmas N, Kitazawa M, Medeiros R. Metal Toxicity Links to Alzheimer's Disease and Neuroinflammation. Journal of Molecular Biology. 2019 Apr 19;431(9):1843–68. [PubMed: 30664867]
47. Chen P, Miah MR, Aschner M. Metals and Neurodegeneration. F1000Res [Internet]. 2016 Mar 17 [cited 2020 Oct 19];5. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4798150/
48. Bakulski KM, Seo YA, Hickman RC, Brandt D, Vadari HS, Hu H, et al. Heavy Metals Exposure and Alzheimer's Disease and Related Dementias. J Alzheimers Dis. 2020;76(4):1215–42. [PubMed: 32651318]
49. Bowler RM, Roels HA, Nakagawa S, Drezgic M, Diamond E, Park R, et al. Dose-effect relationships between manganese exposure and neurological, neuropsychological and pulmonary function in confined space bridge welders. Occup Environ Med. 2007 Mar;64(3):167–77. [PubMed: 17018581]
50. Hobson A, Seixas N, Sterling D, Racette BA. Estimation of Particulate Mass and Manganese Exposure Levels among Welders. The Annals of Occupational Hygiene. 2011 Jan 1;55(1):113–25. [PubMed: 20870928]
51. Rodier J Manganese Poisoning in Moroccan Miners. Br J Ind Med. 1955 Jan;12(1):21–35. [PubMed: 14351643]
52. Chang Y, Lee JJ, Seo JH, Song HJ, Kim JH, Bae SJ, et al. Altered working memory process in the manganese-exposed brain. NeuroImage. 2010 Dec 1;53(4):1279–85. [PubMed: 20620213]
53. Lucchini RG, Guazzetti S, Renzetti S, Conversano M, Cagna G, Fedrighi C, et al. Neurocognitive impact of metal exposure and social stressors among schoolchildren in Taranto, Italy. Environmental Health. 2019 Jul 19;18(1):67. [PubMed: 31324194]
54. Schneider JS, Decamp E, Clark K, Bouquio C, Syversen T, Guilarte TR. Effects of Chronic Manganese Exposure on Working Memory in Non-Human Primates. Brain Res. 2009 Mar 3;1258:86–95. [PubMed: 19133246]
55. Collins JF. Chapter 24 - Copper. In: Marriott BP, Birt DF, Stallings VA, Yates AA, editors. Present Knowledge in Nutrition (Eleventh Edition) [Internet]. Academic Press; 2020 [cited 2023 Jul 25]. p. 409–27. Available from: https://www.sciencedirect.com/science/article/pii/B978032366162100024X
56. Vinceti M, Mandrioli J, Borella P, Michalke B, Tsatsakis A, Finkelstein Y. Selenium neurotoxicity in humans: bridging laboratory and epidemiologic studies. Toxicol Lett. 2014 Oct 15;230(2):295–303. [PubMed: 24269718]
57. Stohs SJ, Bagchi D, Hassoun E, Bagchi M. Oxidative Mechanisms in the Toxicity of Chromium and Cadmium Ions. JEP(T) [Internet]. 2001 [cited 2023 Jul 24];20(2). Available from: https://www.dl.begellhouse.com/journals/0ff459a57a4c08d0,467349702fb3f660,4ccf7c2c372fd17f.html

58. Hegazy R, Mansour D, Salama A, Hassan A, Saleh D. Exposure to intranasal chromium triggers dose and time-dependent behavioral and neurotoxicological defects in rats. Ecotoxicology and Environmental Safety. 2021 Jun 15;216:112220. [PubMed: 33845363]
59. Salama A, Hegazy R, Hassan A. Intranasal Chromium Induces Acute Brain and Lung Injuries in Rats: Assessment of Different Potential Hazardous Effects of Environmental and Occupational Exposure to Chromium and Introduction of a Novel Pharmacological and Toxicological Animal Model. PLOS ONE. 2016 Dec 20;11(12):e0168688. [PubMed: 27997619]
60. Caparros-Gonzalez RA, Giménez-Asensio MJ, González-Alzaga B, Aguilar-Garduño C, Lorca-Marín JA, Alguacil J, et al. Childhood chromium exposure and neuropsychological development in children living in two polluted areas in southern Spain. Environ Pollut. 2019 Sep;252(Pt B):1550–60. [PubMed: 31277024]
61. Invernizzi A, Rechtman E, Oluyemi K, Renzetti S, Curtin P, Colicino E, et al. Topological network properties of resting-state functional connectivity patterns are associated with metal mixture exposure in adolescents. Front Neurosci. 2023 Feb 6;17:1098441. [PubMed: 36814793]
62. Castro-González MI, Méndez-Armenta M. Heavy metals: Implications associated to fish consumption. Environmental Toxicology and Pharmacology. 2008 Nov 1;26(3):263–71. [PubMed: 21791373]
63. Alfthan GV. Toenail mercury concentration as a biomarker of methylmercury exposure. Biomarkers. 1997;2(4):233–8. [PubMed: 23899215]
64. Butler LJ, Janulewicz PA, Carwile JL, White RF, Winter MR, Aschengrau A. Childhood and Adolescent Fish Consumption and Adult Neuropsychological Performance: An Analysis from the Cape Cod Health Study. Neurotoxicol Teratol. 2017 May;61:47–57. [PubMed: 28263856]
65. Mochizuki H Arsenic Neurotoxicity in Humans. Int J Mol Sci. 2019 Jul 11;20(14):3418. [PubMed: 31336801]
66. Rodríguez-Barranco M, Gil F, Hernández AF, Alguacil J, Lorca A, Mendoza R, et al. Postnatal arsenic exposure and attention impairment in school children. Cortex. 2016 Jan;74:370–82. [PubMed: 25682472]
67. Wasserman GA, Liu X, Parvez F, Ahsan H, Factor-Litvak P, Kline J, et al. Water Arsenic Exposure and Intellectual Function in 6-Year-Old Children in Araihazar, Bangladesh. Environ Health Perspect. 2007 Feb;115(2):285–9. [PubMed: 17384779]
68. Weisskopf MG, Proctor SP, Wright RO, Schwartz J, Spiro AI, Sparrow D, et al. Cumulative Lead Exposure and Cognitive Performance Among Elderly Men. Epidemiology. 2007 Jan;18(1):59–66. [PubMed: 17130688]
69. Shih RA, Hu H, Weisskopf MG, Schwartz BS. Cumulative Lead Dose and Cognitive Function in Adults: A Review of Studies That Measured Both Blood Lead and Bone Lead. Environ Health Perspect. 2007 Mar;115(3):483–92. [PubMed: 17431502]
70. Bandeen-Roche K, Glass TA, Bolla KI, Todd AC, Schwartz BS. Cumulative Lead Dose and Cognitive Function in Older Adults. Epidemiology. 2009;20(6):831–9. [PubMed: 19752734]
71. Hopps HC. The biologic bases for using hair and nail for analyses of trace elements. Science of The Total Environment. 1977 Jan 1;7(1):71–89. [PubMed: 319530]
72. Yaemsiri S, Hou N, Slining MM, He K. Growth rate of human fingernails and toenails in healthy American young adults. J Eur Acad Dermatol Venereol. 2010 Apr;24(4):420–3. [PubMed: 19744178]
73. Mordukhovich I, Wright RO, Hu H, Amarasiriwardena C, Andrea Baccarelli, Litonjua A, et al. Associations of Toenail Arsenic, Cadmium, Mercury, Manganese, and Lead with Blood Pressure in the Normative Aging Study. Environ Health Perspect. 2012 Jan;120(1):98–104. [PubMed: 21878420]
74. Hu H, Shih R, Rothenberg S, Schwartz BS. The Epidemiology of Lead Toxicity in Adults: Measuring Dose and Consideration of Other Methodologic Issues. Environ Health Perspect. 2007 Mar;115(3):455–62. [PubMed: 17431499]
75. Dantzer J, Ryan P, Yolton K, Parsons PJ, Palmer CD, Cecil K, et al. A comparison of blood and toenails as biomarkers of children’s exposure to lead and their correlation with cognitive function. Sci Total Environ. 2020 Jan 15;700:134519. [PubMed: 31698272]

76. Chin AL, Negash S, Xie S, Arnold SE, Hamilton R. Quality, and not just quantity, of education accounts for differences in psychometric performance between African Americans and White Non-Hispanics with Alzheimer's disease. J Int Neuropsychol Soc. 2012 Mar;18(2):277–85. [PubMed: 22300593]
77. Zahodne LB, Manly JJ, Azar M, Brickman AM, Glymour MM. Racial Disparities in Cognitive Performance in Mid- and Late Adulthood: Analyses of Two Cohort Studies. J Am Geriatr Soc. 2016 May;64(5):959–64. [PubMed: 27225354]
78. Huffman ML, Cohen PN. Racial Wage Inequality: Job Segregation and Devaluation Across U.S. Labor Markets. American Journal of Sociology. 2004;109(4):902–36.
79. Guidi J, Lucente M, Sonino N, Fava GA. Allostatic Load and Its Impact on Health: A Systematic Review. Psychother Psychosom. 2021;90(1):11–27. [PubMed: 32799204]
80. Thomson EM. Air Pollution, Stress, and Allostatic Load: Linking Systemic and Central Nervous System Impacts. J Alzheimers Dis. 69(3):597–614. [PubMed: 31127781]
81. deFur PL, Evans GW, Hubal EAC, Kyle AD, Morello-Frosch RA, Williams DR. Vulnerability as a Function of Individual and Group Resources in Cumulative Risk Assessment. Environ Health Perspect. 2007 May;115(5):817–24. [PubMed: 17520073]
82. Geronimus AT, Hicken M, Keene D, Bound J. "Weathering" and age patterns of allostatic load scores among blacks and whites in the United States. Am J Public Health. 2006 May;96(5):826–33. [PubMed: 16380565]
83. Lupien SJ, Juster RP, Raymond C, Marin MF. The effects of chronic stress on the human brain: From neurotoxicity, to vulnerability, to opportunity. Front Neuroendocrinol. 2018 Apr;49:91–105. [PubMed: 29421159]

Highlights

- Toenail chromium inversely associated with sustained attention
- Toenail manganese inversely associated with working memory
- Greater attention deficits at lower chromium levels for Black vs White participants
- Future mixtures research should investigate social and chemical co-exposures

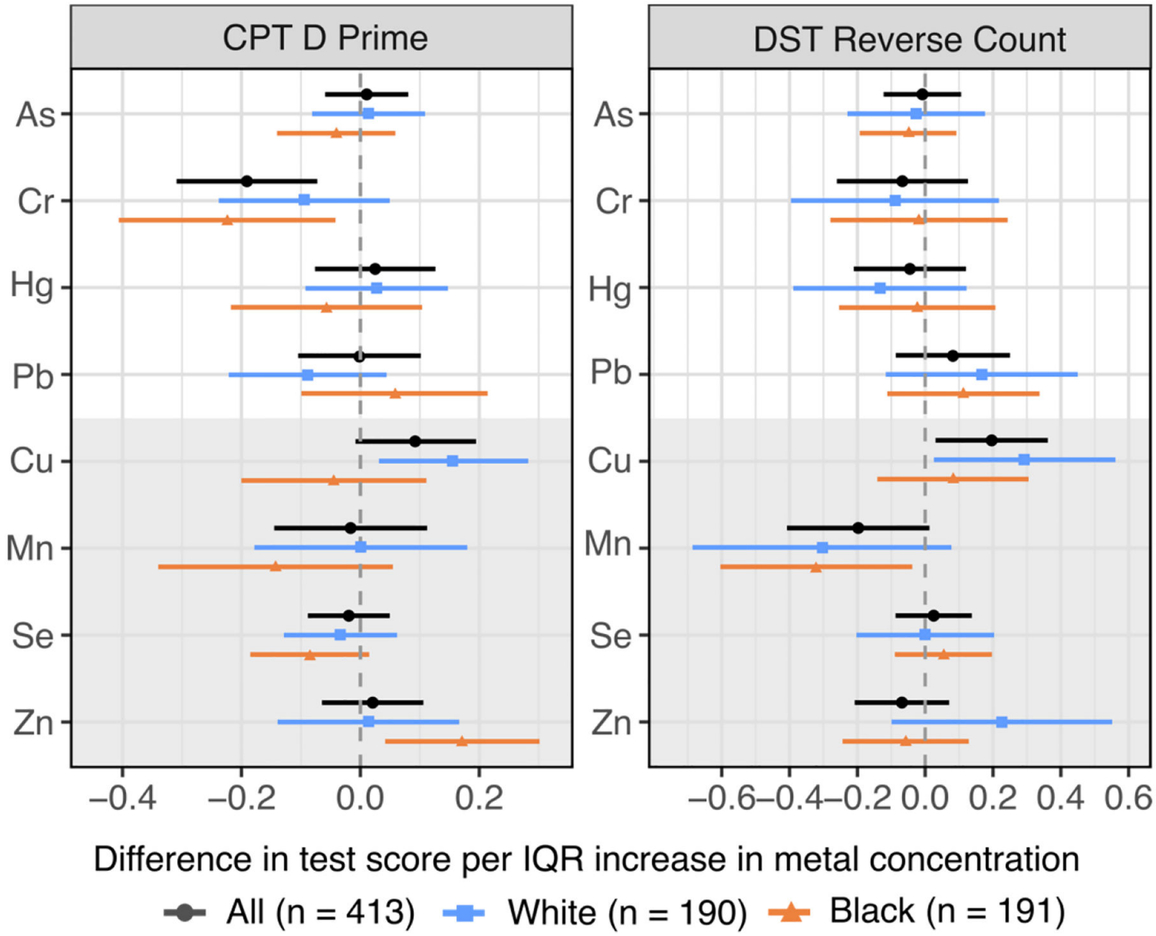


Figure 1.

Difference in sustained attention (CPT D Prime) and working memory (DST Reverse Count) performance associated with an IQR change in log10-transformed concentration of each individual metal, adjusted for covariates. Lower scores indicate poorer performance. Estimates for non-essential metals with no known benefits to human health (white background), and essential metals with potential neurotoxic effects at deficient or excess levels (grey shading). GLM estimates were calculated by scaling the regression coefficients by the interquartile range.

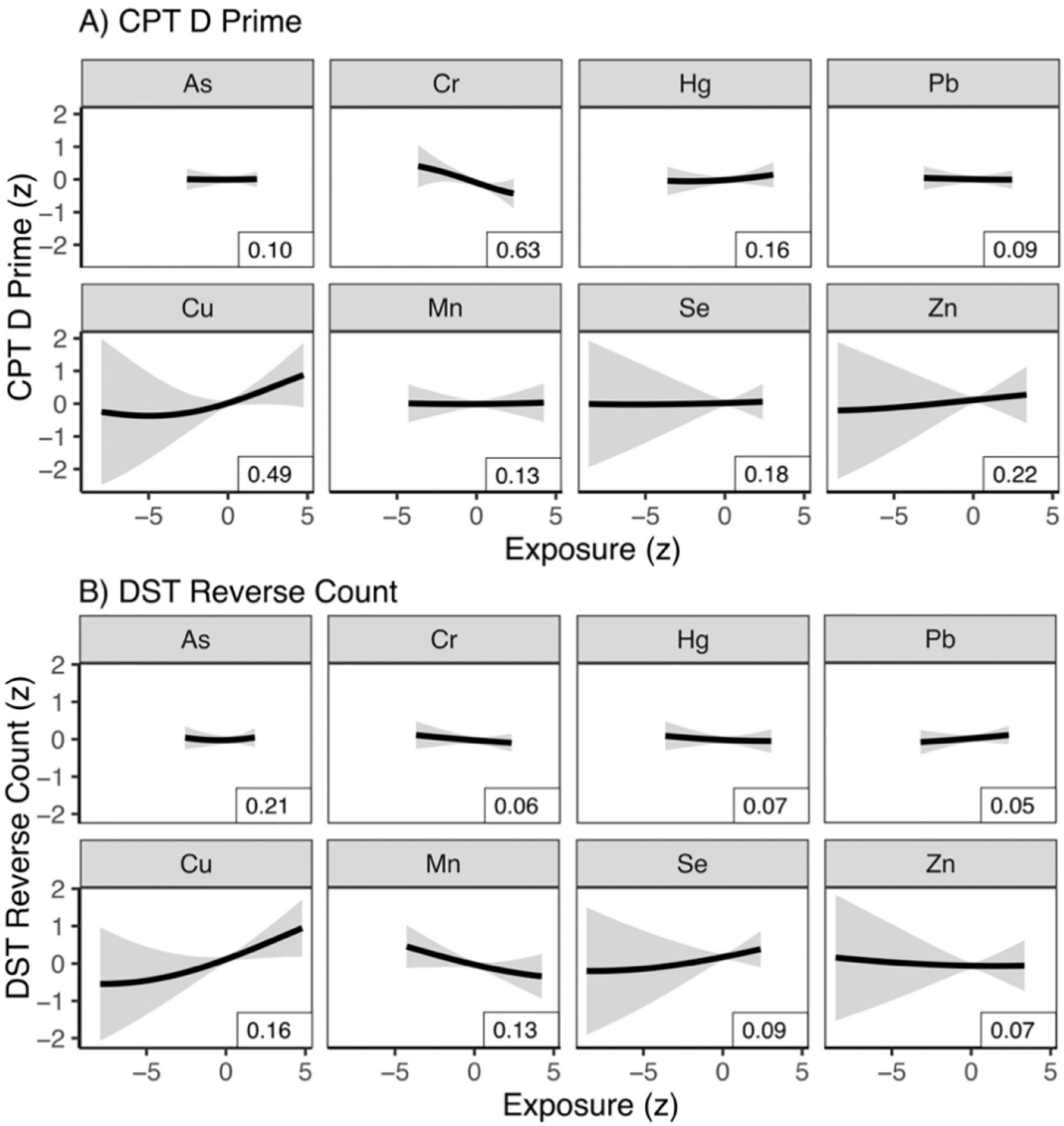


Figure 2.

Univariate relationship between toenail metals and A) CPT D Prime and B) DST Reverse Count in the study population (n = 413), modeled with Bayesian kernel machine regression (five parallel chains, each with 40,000 iterations and default priors). Outcomes have been standardized. Exposures have been log10-transformed and standardized. Models included all 8 metals while adjusting for age, BMI, education level, smoking history, and marital status. Posterior inclusion probabilities (PIPs) are denoted in boxes on the bottom right corner of each plot.

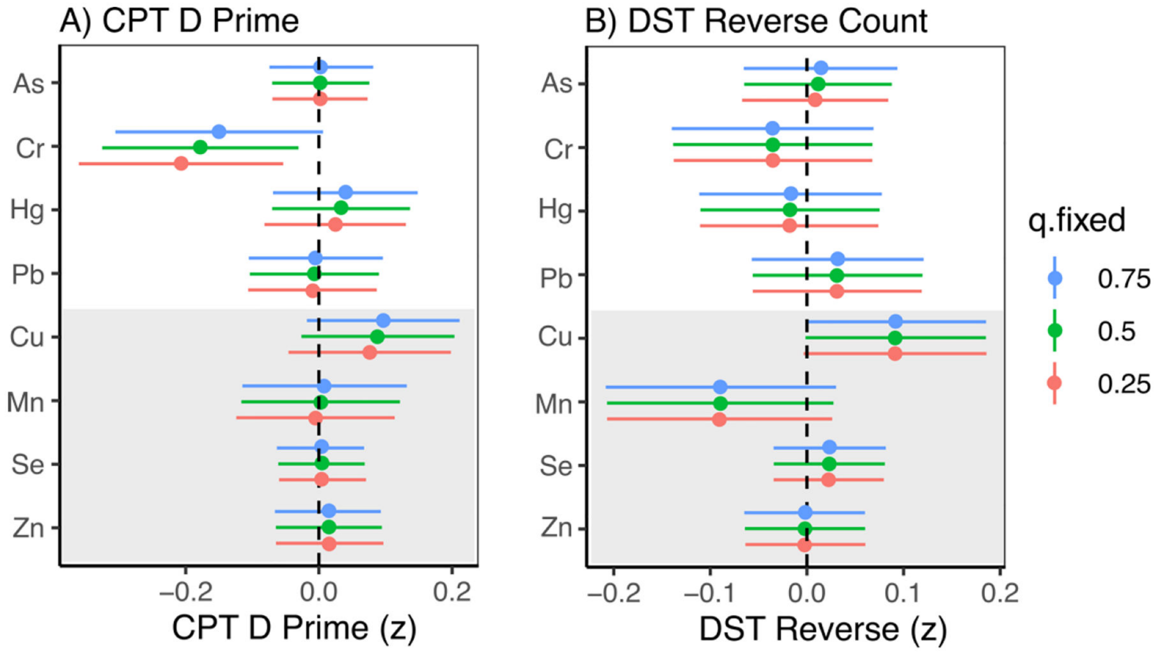


Figure 3.

Associations (estimates and 95% credible intervals) for an IQR increase in each metal with A) CPT D Prime and B) DST Reverse Count, when other metals are fixed at either the 25th, 50th, or 75th percentile.

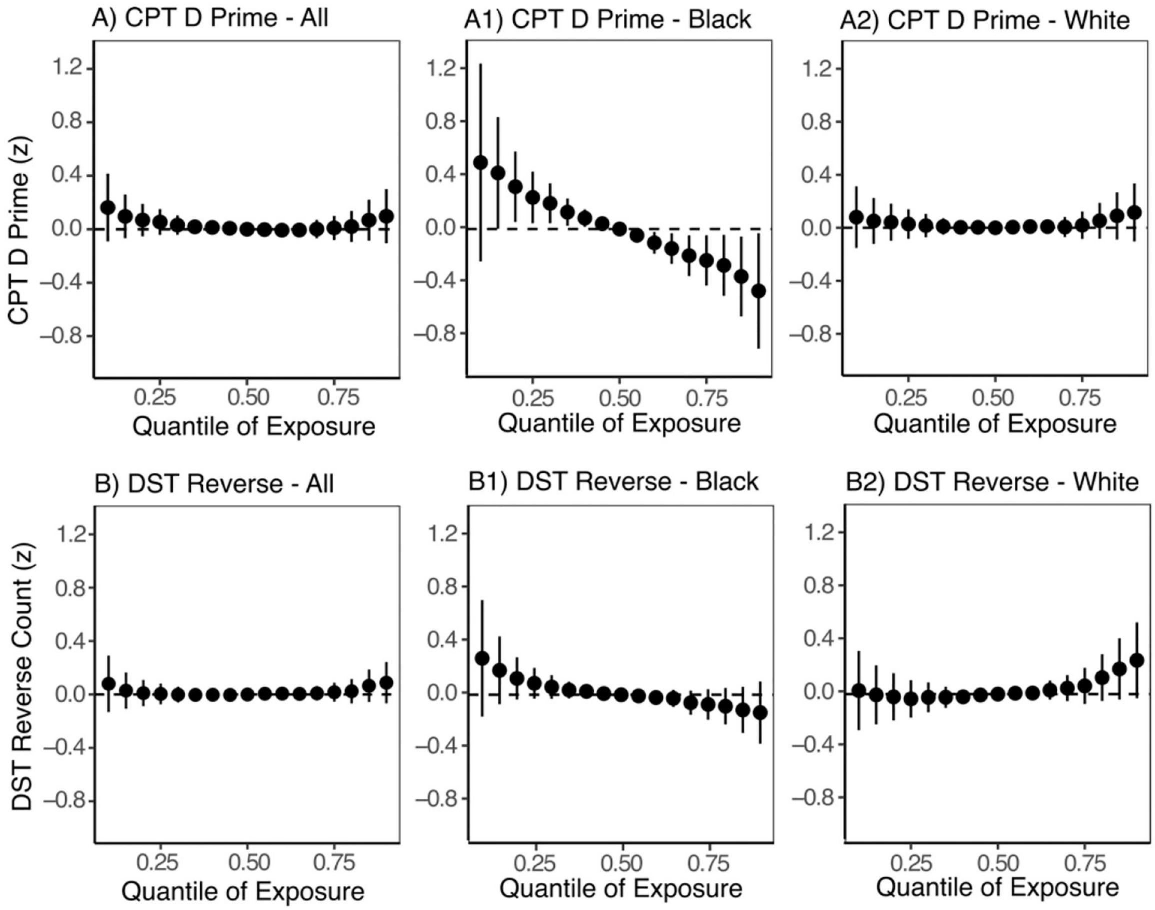


Figure 4.

Joint association (estimates and 95% credible intervals) of the 8-metal mixture with the A) CPT D Prime and B) DST Reverse Count scores among all men in the analysis group (N = 413). A1 (Black) and A2 (White) reflect race specific results for CPT D Prime. B1 (Black) and B2 (White) reflect race specific results for DST reverse Count. Metal mixture levels at each percentile are compared to its median level. Exposures have been log10-transformed and standardized. Models have been adjusted for age, BMI, education level, smoking history, and marital status.

Table 1.

Descriptive characteristics of the study population by race (N = 413).

	Total n = 413	White n = 190	Black n = 191	Other n = 32
Age (years), n (%)				
20 – 39	120 (29.1)	42 (22.1)	71 (37.2)	7 (21.9)
40 – 59	222 (53.8)	100 (52.6)	104 (54.5)	18 (56.3)
60 – 69	71 (17.2)	48 (25.3)	16 (8.4)	7 (21.9)
Educational Attainment, n (%)				
< High school	72 (17.4)	30 (15.8)	30 (15.7)	12 (37.5)
High school	145 (35.1)	54 (28.4)	83 (43.5)	8 (25.0)
Some college	132 (32.0)	60 (31.6)	64 (33.5)	8 (25.0)
College or more	64 (15.5)	46 (24.2)	14 (7.3)	4 (12.5)
Marital Status, n (%)				
Married/living together	228 (55.2)	134 (70.5)	74 (38.7)	20 (62.5)
Widowed/separated	78 (18.9)	34 (17.9)	37 (19.4)	7 (21.9)
Never married	106 (25.7)	22 (11.6)	80 (41.9)	4 (12.5)
Missing	1 (0.2)	0 (0)	0 (0)	1 (3.1)
Smoking History, n (%)				
Former	139 (33.7)	88 (46.3)	42 (22.0)	9 (28.1)
Never	274 (66.3)	102 (53.7)	149 (78.0)	23 (71.9)
BMI, n (%)				
Underweight	3 (0.7)	0 (0)	3 (1.7)	0 (0)
Healthy	74 (17.9)	26 (13.7)	42 (21.9)	6 (18.8)
Overweight	137 (33.2)	68 (35.8)	56 (29.3)	13 (40.6)
Obese	199 (48.2)	96 (50.5)	90 (47.1)	13 (40.6)
Toenail metal concentration μg/g [geometric mean (GSD)]				
Arsenic	0.019 (13.7)	0.032 (10.2)	0.013 (14.8)	0.011 (26.9)
Chromium	0.11 (7.5)	0.15 (6.9)	0.077 (8.3)	0.12 (5.2)
Copper	3.2 (2.4)	3.8 (1.7)	2.6 (2.9)	3.9 (1.9)
Lead	0.091 (9.3)	0.11 (8.6)	0.082 (9.3)	0.062 (13.3)
Manganese	0.30 (6.1)	0.38 (4.3)	0.23 (7.1)	0.26 (12.6)
Mercury	0.13 (6.3)	0.21 (5.3)	0.082 (6.7)	0.18 (5.4)
Selenium	0.68 (2.4)	0.77 (1.4)	0.58 (3.5)	0.76 (1.4)
Zinc	88 (3.1)	104 (1.4)	74 (5.1)	100 (1.5)
Neurobehavioral Test Scores [(Mean (SD)]				
CPT D Prime	3.9 (0.98)	4.2 (0.8)	3.6 (1.0)	3.4 (1.3)
DST Reverse Count	4.3 (1.7)	4.5 (1.8)	4.1 (1.5)	4.4 (1.5)

"Other" consists of participants identifying as Asian, American Indian/Alaskan Native, Mixed Race, or Other. BMI calculated using height and weight measurements taken during the clinical exam.

Underweight = BMI< 18.5; Healthy = BMI 18.5—24.9, Overweight = BMI 25.0—29.9; Obese = BMI > 30. Lower neurobehavioral test scores = worse performance.