



Associations of a toenail metal mixture with attention and memory in the Gulf long-term follow-up (GuLF) study

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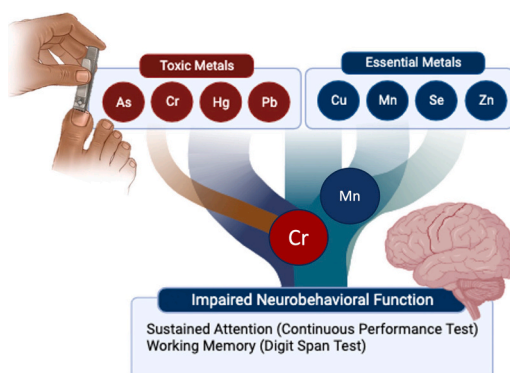
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HIGHLIGHTS

- 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.

GRAPHICAL ABSTRACT



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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.

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

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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 and 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.

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 (Bain et al., 2020; Feigin et al., 2020). 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 (Schoenmaker and Gool, 2004; Röhr et al., 2022; Hendriks et al., 2024). 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 (White et al., 1990; Andrade and Aschner, 2017). These deficits have been associated with heightened risks of physical disability, hospitalization, and dementia diagnoses in geriatric populations (Gooch et al., 2017; Blacker et al., 2007; Morris et al., 2001; Kawas et al., 2003; Lopez et al., 2003).

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 (Bain et al., 2020; Wright and Baccarelli, 2007). 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 (Bain et al., 2020). 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 (Bauer et al., 2020; Muñoz et al., 2020; Skogheim et al., 2021; Oppenheimer et al., 2022). 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 (Laouali et al., 2022; Sasaki and Carpenter, 2022). 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 (Gutiérrez-González et al., 2019). Toenail metal biomarkers can reflect metal exposures averaged up to 12 months (Longnecker et al., 1993) 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, 3401 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 (Kwok et al., 2017).

Participants of the overall GuLF Study cohort were selected into the present study if they completed a full neurobehavioral assessment at the clinical exam ($n = 2652$), 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 (Lin et al., 2024). 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) (Wright and Baccarelli, 2007; Aschner and Costa, 2017; Cicero et al., 2017), those that have been validated with external exposure studies or are commonly reported in toenails (Gutiérrez-González et al., 2019; Lin et al., 2023; Garland et al., 1993; Martínez-Morata et al., 2023), and those that were detected in >85 % of our toenail samples and reliably measured in our previous toenail reliability study (Lin et al., 2023) (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 (Lin et al., 2023). In brief, toenail samples were cleaned using a multi-stage process involving acetone, detergent, and Milli-Q water and then digested in nitric acid (HNO₃, Optima™ grade) and hydrogen peroxide (H₂O₂, Optima™, grade) with an open vessel microwave-assisted digestion method to 110 °C, adapted from the Dartmouth Trace Element Analysis Core (Andrew et al., 2020). 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) (Tehrani et al., 2020). 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 $\sqrt{2}$ (Helsel, 2005).

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 (White et al., 1990). 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 (Bain et al., 2020; Rohlman et al., 2000; Palta et al., 2016). 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 (Rohlman et al., 2003a). 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 (Rohlman et al., 2003a). 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 (Camicoli et al., 2001; Storzbach et al., 2001; Rohlman et al., 2007; Quist et al., 2019). 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 (Rohlman et al., 2003a). Details about the neurobehavioral test battery and setup are provided elsewhere (Rohlman et al., 2003b; Rohlman et al., 2008). 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 (Banken, 1985).

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 Fig. 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 h 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 >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 (Choi et al., 2022). 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 Fig. 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 (Barnes, 2022), 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, multicollinearity, 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 (Bobb et al., 2015). 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 (25th, 50th, and 75th 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 99th 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 to 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 Fig. 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.

Table 1
Descriptive characteristics of the study population by race (*N* = 413).

	Total <i>n</i> = 413	White <i>n</i> = 190	Black <i>n</i> = 191	Other <i>n</i> = 32
Age (years), <i>n</i> (%)				
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, <i>n</i> (%)				
< 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, <i>n</i> (%)				
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, <i>n</i> (%)				
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, <i>n</i> (%)				
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.

3.2. CPT D Prime (selective and sustained attention)

Fig. 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 to 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 (Fig. 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 (β = −0.05, 95 % CI:

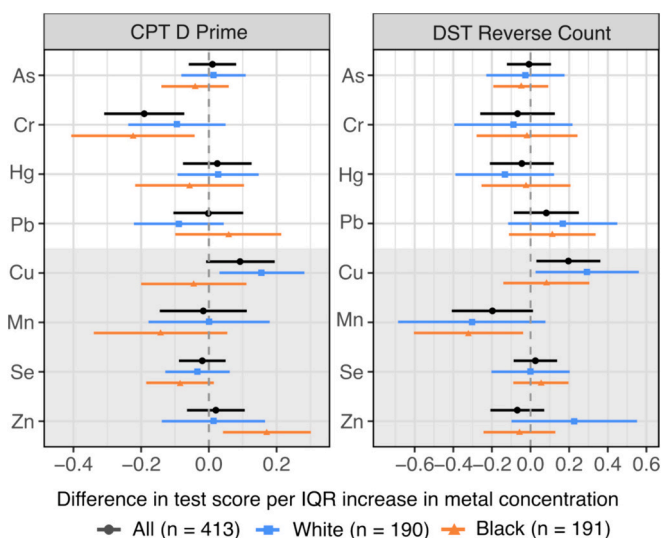


Fig. 1. Difference in sustained attention (CPT D Prime) and working memory (DST Reverse Count) performance associated with an IQR change in log10-transformed concentration of each individual metal, adjusted for covariates. Lower scores indicate poorer performance. Estimates for non-essential metals with 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.

−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 Fig. 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 to 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 ($\beta = 0.29$, 95 % CI: 0.02, 0.56) and was negligible among Black participants ($\beta = 0.08$, 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 99th percentile of Mn concentrations ($\beta = -0.44$, 95 % CI: −0.84, −0.05) and when, separately, additionally adjusting for state of participation ($\beta = -0.42$, 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 Fig. 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 ($\beta = -0.11$, 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 (Fig. 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 25th to 75th percentile was associated with 0.18 (95 % CrI: −0.33, −0.03) lower CPT D Prime score (Fig. 3). Inverse trends were also observed for Mn and DST Reverse Count score, but the estimates were imprecise ($\beta = -0.09$, 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 ($\beta = 0.09$, 95 % CrI: −0.001, 0.19) and results crossing the null for CPT D Prime score ($\beta = 0.09$, 95 % CrI: −0.03, 0.20) (Fig. 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 Fig. 5a & 5b). We observed no evidence of interactions for the DST Reverse outcome (Supplemental Fig. 5c). When we evaluated the individual associations of an IQR increase in each metal, holding all other metals in the mixture at the 25th, 50th, and 75th percentiles, we observed that Cu was particularly influential for both outcomes in the positive direction (Fig. 3). Similarly, we observed that Cr and Mn were separately influential for CPT D Prime and DST Reverse Count, respectively (Fig. 3). There was no evidence of an overall joint mixture effect for either sustained attention or working memory outcomes (Fig. 4A1, B1).

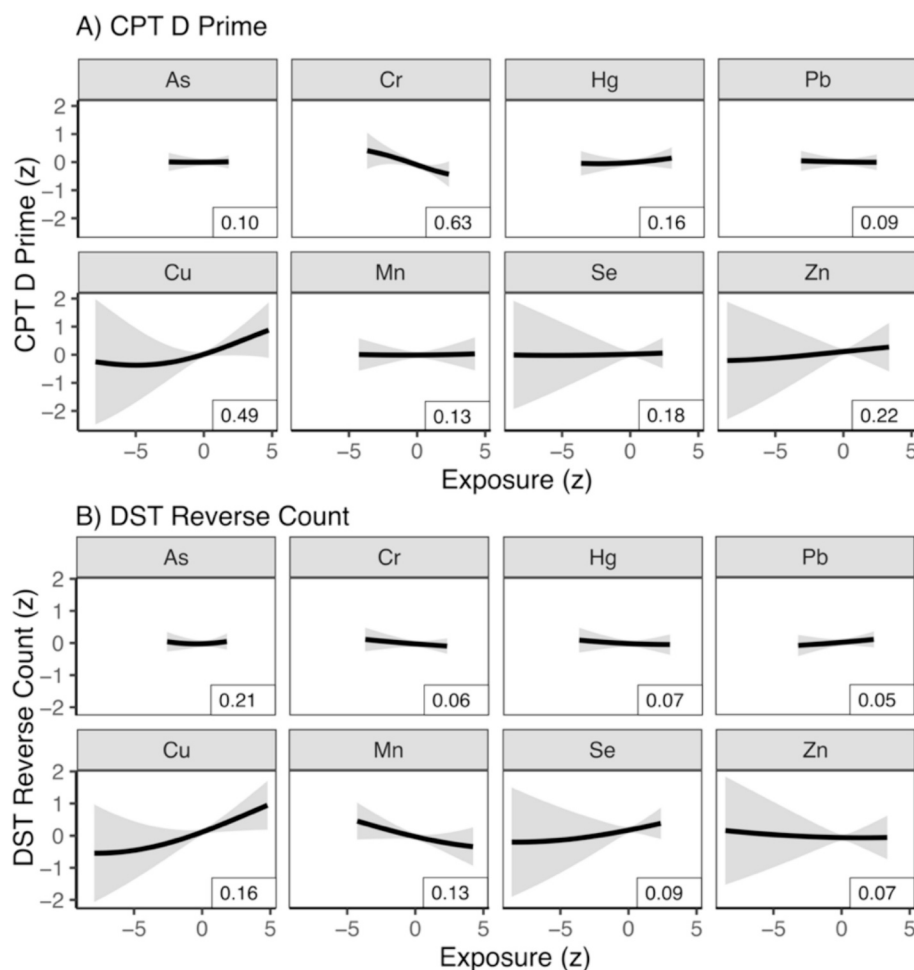


Fig. 2. Univariate relationship between toenail metals and A) CPT D Prime and B) DST Reverse Count in the study population ($n = 413$), modeled with Bayesian kernel machine regression (five parallel chains, each with 40,000 iterations and default priors). Outcomes have been standardized. Exposures have 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.

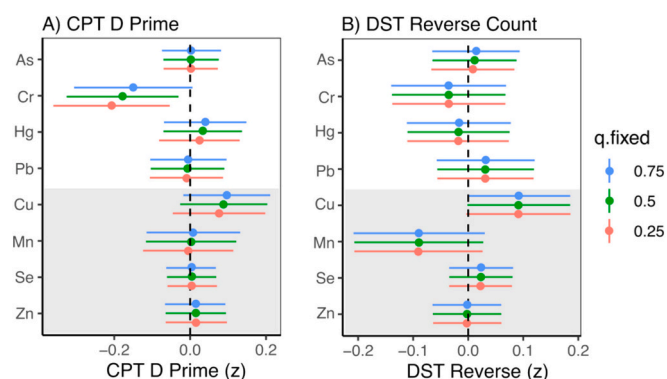


Fig. 3. Associations (estimates and 95 % credible intervals) for an IQR increase in each metal with A) CPT D Prime and B) DST Reverse Count, when other metals are fixed at either the 25th, 50th, or 75th percentile.

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

Fig. 6a and for DST reverse count in Supplemental Fig. 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 Fig. 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 Fig. 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 Fig. 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 (Fig. 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 (Fig. 4B1). No evidence of a joint mixture association with CPT D Prime score was observed among White participants, but there appeared to be

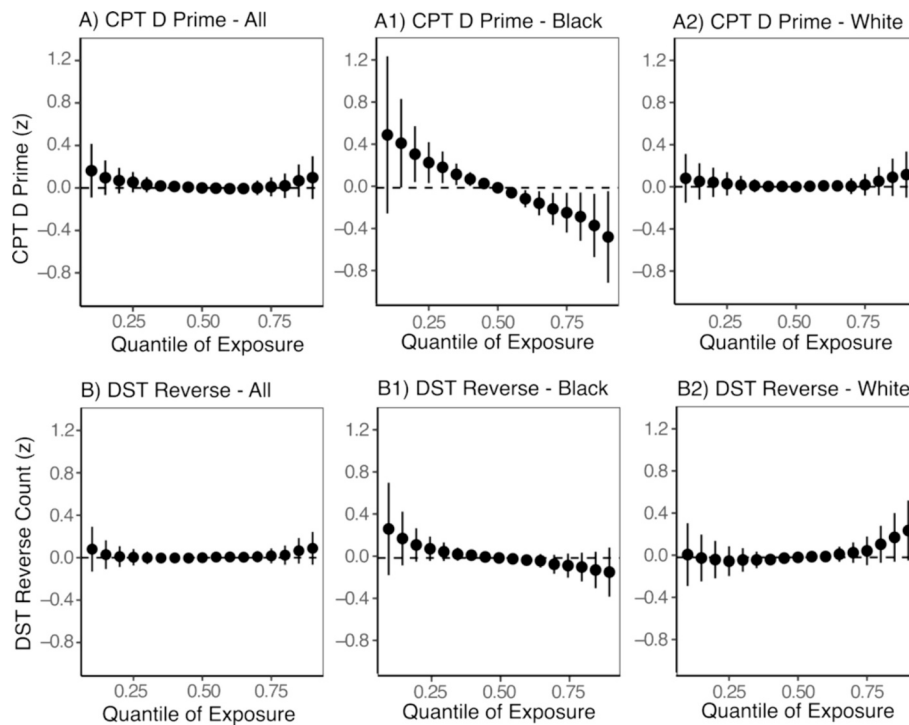


Fig. 4. Joint association (estimates and 95 % credible intervals) of the 8-metal mixture with the A) CPT D Prime and B) DST Reverse Count scores among all men in the analysis group ($N = 413$). A1 (Black) and A2 (White) reflect race specific results for CPT D Prime. B1 (Black) and B2 (White) reflect 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.

slight a positive effect of the joint overall mixture with DST Reverse Count score among White participants above the 75th quantile of the joint exposure compared to the median (Fig. 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 Fig. 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 Fig. 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 (Wright and Baccarelli, 2007). 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 (Aschner and Costa, 2017; Farina et al., 2013; Huat et al., 2019; Chen et al., 2016). 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 (Bakulski et al., 2020). Occupational studies have consistently shown the adverse effects of Mn exposure on attention and memory at high concentrations (Bowler et al., 2007; Hobson et al., 2011; Rodier, 1955; Chang et al., 2010). Several studies have also found working memory deficits related to chronic Mn exposure at ambient environmental concentrations in children and non-human primates (Oppenheimer et al., 2022; Lucchini et al., 2019; Schneider et al., 2009). 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 (Bain et al., 2020). Both elements also serve as cofactors of major antioxidant enzymes that play important roles in reducing oxidative stress (Collins, 2020), 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 (Bain et al., 2020).

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 (Vinceti et al., 2014). 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 (Stohs et al., 2001). Animal studies have also found elevated proinflammatory cytokines in rats following Cr(VI) exposure, highlighting potential neuroinflammatory pathways for Cr(VI) neurotoxicity (Hegazy et al., 2021; Salama et al., 2016). While human studies remain limited, studies have identified inverse relationships between Cr measured in hair and urine and neurobehavioral tests of attention in children (Caparros-Gonzalez et al., 2019) and topological brain metrics in adolescents (Invernizzi et al., 2023). 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 (Castro-González and Méndez-Armenta, 2008; Alfthan, 1997). 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 (Butler et al., 2017). Arsenic is a neurotoxicant that is known to induce neurological damage through a variety of cellular pathways (Huat et al., 2019); yet, epidemiologic studies frequently report inconsistent associations with neurocognitive performance depending on the functional tests used to measure deficits (Rodríguez-Barranco et al., 2016; Wasserman et al., 2007). 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 (Weisskopf et al., 2007; Shih et al., 2007; Bandeen-Roche et al., 2009). 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 (Hopps, 1977; Yaemsiri et al., 2010), 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 (Gutiérrez-González et al., 2019; Martínez-Morata et al., 2023; Mordukhovich et al., 2012). 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 (Hu et al., 2007). Since measurement reliability in the toenail matrix for metals in this study has already been shown (Lin et al., 2023), 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 (Lin et al., 2024), 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 (Dantzer et al., 2020). 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 (Lin et al., 2024). 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 may not 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 (Lin et al., 2024), 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 (Chin et al., 2012; Zahodne et al., 2016). While differences in education quality may be a factor in these disparities, social stressors such as segregation and discrimination may also play a role (Chin et al., 2012). In the US, for a given level of education, Black individuals often earn less than their White counterparts (Huffman and Cohen, 2004) 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 (Barnes, 2022; Guidi et al., 2021). 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 (Thomson, n.d.; deFur et al., 2007; Geronimus et al., 2006; Lupien et al., 2018). 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 (Guidi et al., 2021). 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 additional 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 (Barnes, 2022). Leveraging the racial diversity in the GuLF Study, we found 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.

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CRedit authorship contribution statement

Joyce J.Y. Lin: Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Jordan R. Kuiper:** Writing – review

& editing, Methodology. **Aisha S. Dickerson:** Writing – review & editing. **Jessie P. Buckley:** Writing – review & editing, Methodology. **Heather E. Volk:** Writing – review & editing. **Diane S. Rohlman:** Writing – review & editing, Data curation. **Kaitlyn G. Lawrence:** Writing – review & editing. **W. Braxton Jackson:** Data curation. **Dale P. Sandler:** Supervision. **Lawrence S. Engel:** Writing – review & editing, Supervision, Methodology, Investigation, Conceptualization. **Ana M. Rule:** Writing – review & editing, Supervision, Resources, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

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.

Data availability

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2024.173387>.

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