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Integrated measures of lead and manganese exposure improve estimation of their joint effects on cognition in Italian school-age children

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ABSTRACT

Every day humans are exposed to mixtures of chemicals, such as lead (Pb) and manganese (Mn). An underappreciated aspect of studying the health effects of mixtures is the role that the exposure biomarker media (blood, hair, etc.) may play in estimating the effects of the mixture. Different biomarker media represent different aspects of each chemical's toxicokinetics, thus no single medium can fully capture the toxicokinetic profile for all the chemicals in a mixture. A potential solution to this problem is to combine exposure data across different media to derive integrated estimates of each chemical's internal concentration. This concept, formalized as a multi-media biomarker (MMB) has proven effective for estimating the health impacts of Pb exposure, but may also be useful to estimate mixture effects, such as the joint effects of metals like Pb and Mn, while factoring in how the association changes based upon the biomarker media. Levels of Pb and Mn were quantified in five media: blood, hair, nails, urine, and saliva in the Public Health Impact of Metals Exposure (PHIME) project, a study of Italian adolescents aged 10–14 years. MMBs were derived for both metals using weighted quantile sum (WQS) regression across the five media. Age-adjusted Wechsler Intelligence Scale for Children (WISC) IQ scores, measured at the same time as the exposure measures, were the primary outcome and models were adjusted for sex and socioeconomic status. The levels Pb and Mn were relatively low, with median blood Pb of 1.27 (IQR: 0.84) μg/dL and median blood Mn of 1.09 (IQR: 0.45) μg/dL. Quartile increases in a Pb-Mn combination predicted decreased Full Scale IQ of 1.9 points (95% CI: 0.3, 3.5) when Pb and Mn exposure levels were estimated using MMBs, while individual regressions for each metal were not associated with Full Scale IQ. Additionally, a quartile increase in the WQS index of Pb and Mn, measured using MMBs, were associated with reductions in Verbal IQ by 2.8 points (1.0, 4.5). Weights that determine the contributions of the metals to the joint effect highlighted that the contribution of the Pb-Mn was 72–28% for Full Scale IQ and 42–58% for Verbal IQ. We found that the joint effects of Pb and Mn are strongly affected by the medium used to measure exposure and that the joint effects of the Pb and Mn MMBs on cognition were the stronger than any individual biomarker. Thus, increase power and accuracy for measuring mixture effects compared to individual biomarkers. As the number of chemicals in mixtures increases, appropriate biomarker selection will become increasingly important and MMBs are a natural way to reduce bias in such analyses.

1. Introduction

Understanding the impact of environmental exposures on children's health is critical when designing interventions to prevent detrimental health effects and requires embracing the complexity of exposure assessment. Traditionally, investigations into environmental exposures

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that impact cognitive development have focused on single chemical exposures measured in a single biological matrix (Pohl et al., 1997; Stafoggia et al., 2017). However, this modeling strategy ignores the fact that mixtures of exposures are the default exposure scenario in daily life. Furthermore, individual exposure biomarkers provide incomplete data on exposure and methods to integrate information across different biomarkers can lead to better estimates of the health impact of chemicals (Levin-Schwartz, 2020). Therefore, we need to develop the methods that can maximize our understanding of both mixtures and exposure biomarkers if we are to understand how environment impacts health.

Metals are a ubiquitous class of chemicals which commonly co-occur (Fairbrother et al., 2007, 2007; Sexton, 2006), leading to interactive effects on health outcomes (Valeri, 2017; Pan, 2018; Kupsco, 2019). Manganese (Mn) and lead (Pb) act non-additively to disrupt neurodevelopment in both animal models (Shukla and Chandra, 1987; Chandra et al., 1981) and observational studies in children (Kim, 2009; Lin, 2013; Claus Henn, 2012). Accurately characterizing the health risk of metal mixtures requires accurate estimates of exposure for each metal. When assessing the effects of metal exposure, the level of exposure is estimated through the use of exposure biomarkers–the measurement of a compound or its metabolite in a specific medium (e.g., blood, urine, etc.) to approximate the internal concentration (Phoon, 1988). However, different metals distribute differently across the body due to their unique toxicokinetic properties, i.e., the process by which chemicals enter the body and are metabolized and excreted. Additionally, exposure may occur from multiple sources that can alter the kinetics and dynamics, particularly when first pass metabolism does not occur. Manganese, for example, more readily enters the circulation from ambient exposure (via inhalation) than from oral ingestion for this reason (Leggett, 2011). Thus, a single exposure biomarker may not fully characterize the internal levels of a metal, let alone multiple chemicals simultaneously (Grandjean and Budtz-Jorgensen, 2010; Grandjean and Budtz-Jorgensen, 2007). This can result in inaccurate or even biased estimates of exposure for different metals, leading to exposure misclassification when they are combined in a mixture analysis that uses only a single biomarker medium. Such misclassified exposure estimates hinder our ability to understand the neurocognitive effects of metals both individually and as part of a mixture (Weisskopf et al., 2018; Grandjean and Herz, 2011; Zeger, 2000; Grandjean et al., 2003).

Multiple media, including blood, hair, urine, nails, and even teeth, have been used to measure exposure to Pb and Mn individually (Abadin, 2007, 2012). For Pb, blood is considered to be the most reliable biomarker for research, clinical use, and public health surveillance (Abadin, 2007; Barbosa et al., 2005). However, there is a lack of consensus regarding an ideal biomarker for Mn (Williams, 2012; Jursa et al., 2018; Viana, 2014; Hassani et al., 2016). Researchers assessing the relationship between Mn levels, measured using different biomarkers, and cognition have found associations using Mn levels measured using certain biomarkers, but not others (Menezes-Filho et al., 2011; Riojas-Rodriguez, 2010). Determining an ideal biomarker for Mn is complicated by the role of Mn as a nutrient (Aschner and Aschner, 2005), making it biologically different than Pb, which has no known natural biological role. Nutrient metals are transported in blood to tissues to serve biological functions and therefore levels in fluids and tissues reflect more than exposure and biotransformation. They reflect physiologic levels in the context of nutritional status for that nutrient and their nutritional need typically varies over the lifespan. Thus, levels of Mn are tightly regulated in blood with excess Mn in the blood being rapidly removed by the liver and excreted in the bile and urine (Klaassen, 1974; Malecki et al., 1996). This may limit the utility of blood as a biomarker of Mn exposure, as blood Mn may not represent excess exposure until levels are very high. Measurements of Mn using hair as the biomarker have been most consistently associated with neurodevelopmental deficits (Coetzee, 2016); however, it may not be ideal as a measurement of occupational exposure due to its contamination from Mn in dust (Bader et al., 1999). Saliva is a more recent and promising biomarker of Mn exposure, however relatively little is known about its utility to reflect exposure levels or associated health risks (Butler, 2018).

Though blood is not an ideal biomarker of Mn exposure, most research into the joint effects of Pb and Mn exposure on cognition have nonetheless used blood Mn (Valeri, 2017; Liu, 2018; Kim, 2009; Lin, 2013; Claus Henn, 2012). Use of blood as an exposure biomarker may lead to an underestimation of the risks posed by excess Mn as well as the joint effects of Pb and Mn. No single biomarker media may be ideal to estimate both Pb and Mn exposure. However, all biomarker media provide some information about the level of internal concentration across individuals and this information is likely to be complementary as they represent different kinetic properties (excretion, secretion, plasma compartmentalization, etc.). A potential solution to estimating the joint impact of Pb-Mn co-exposure is to quantify exposure using multi-media biomarkers (MMBs) (Levin-Schwartz, 2020). MMBs are constructs derived using statistical methods for environmental mixtures across exposure biomarkers measured in different biological media. Since they integrate exposure information across biomarkers, MMBs can be better suited to estimate the effects of metals on cognition than selecting a single biomarker as the exposure metric, which discards exposure information from the other biomarkers (Levin-Schwartz, 2020; Menezes-Filho, 2018).

In this study, we test two hypotheses. The first is that the choice of biomarker medium used to measure exposure affects the strength of the joint effects as well as the contribution of each metal to the joint effect. The second hypothesis is that integrating information across biomarker media, through the use of MMBs, can improve the estimation of the joint impact of environmental exposures on health outcomes compared to traditional single biomarker media approaches. We derive MMBs using weighted quantile sum regression (WQS), a mixtures analysis technique. In order to test these hypotheses, we study a relatively straightforward combination of two metals, Pb and Mn. We estimate the combined effects of Pb and Mn on cognition using MMBs and the Third Edition of the Wechsler Intelligence Scale for Children (WISC-III) in participants in the Public Health Impact of Manganese Exposure (PHIME) cohort in Italy. We estimate the combined effect using WQS and measure Pb and Mn in blood, hair, nails, urine, and saliva. We compare the combined effects, estimated using each medium individually, to combined effects estimated when the level of exposure to Pb and Mn is estimated using MMBs.

2. Materials and methods

2.1. Description of the study population

The subjects in this study were drawn from the PHIME cohort based in the northern Italian province of Brescia, Lombardy. Between 2010 and 2013, a total of 720 participants were recruited through the local public schools. Eligibility criteria for PHIME included: being both born and raised within the study area, being between 10 and 14 years of age, and being from a family that had lived in the study area for at least two generations. The exclusion criteria included: having a neurodevelopmental disease or a family history of neurodegenerative disease, using medications with known neuro-psychological side effects, and having known visual or motor deficits. A fuller description of both the recruitment process and study design has been previously published (Lucchini, 2012). Eligible adolescents as well as their parents received detailed explanation of the study procedures prior to providing written informed consent to participate. The study protocols were approved by the following Institutional Review Boards (IRBs): the University of Brescia, the University of California, Santa Cruz, and the Icahn School of Medicine at Mount Sinai. Complete data for all biomarkers, outcomes, and covariates of interest were available from 259 adolescents and these comprise the final sample used in this analysis.

2.2. Pb and Mn biomarker measurements

The collection of biological samples (blood, urine, hair, nails, and saliva) from the PHIME participants, which occurred concurrently with the neurological testing, has been described previously (Lucas, 2015; Lucchini, 2012). Whole blood samples were collected using butterfly catheters into trace metal-free vacutainers. Spot urine samples were collected into sterile polyethylene containers. Hair samples were collected using stainless steel scissors and stored in paper envelopes. Fingernail samples were collected with stainless steel nail clippers and stored in paper envelopes. Saliva samples were drawn through plastic straws into trace metal free microfuge tubes. The concentrations of Mn and Pb in all media were measured using magnetic sector inductively coupled plasma mass spectrometry (Thermo Element XR ICP–MS), described in detail elsewhere (Smith, 2007; Eastman et al., 2013).

2.3. Covariate data

Trained study staff collected sociodemographic information through standardized questionnaires either at in-person visits or over the phone. Socioeconomic status (SES, categorized as low, medium, and high) was determined using an Italy-specific methodology that accounts for parental occupation and education (Cesana et al., 1995).

2.4. Wechsler Intelligence Scale for children (WISC-III)

The measure of cognition used in this study was the WISC-III, Italian language version, normed for children aged 6–16 years (Orsini and Picone, 2006). The WISC-III is composed of 10 subtests, grouped into two domains. These domains are verbal (measuring language-based skills) and performance (measuring non-verbal, perceptual organization). Verbal IQ and Performance IQ composite scores were created by combining the five subtests in each domain. The subtests comprising the Verbal IQ score are Information, Similarities, Arithmetic, Vocabulary and Comprehension. The subtests comprising the Performance IQ score are Picture Completion, Coding, Picture Arrangement, Block Design and Object Assembly. The Verbal and Performance composite scores are combined to derive a measure of overall general intelligence, Full Scale IQ. The WISC-III was administered by two trained neuro-psychologists who were blind to the participants' level of metal exposure. Age-adjusted standard scores from the Italian normative WISC-III population were used for all analyses. The three composite scores, Verbal IQ, Performance IQ, and Full Scale IQ, were the outcomes in the analyses.

2.5. Statistical analyses

2.5.1. Weighted quantile sum regression (WQS)

A fuller explanation of WQS has been presented previously (Carrico et al., 2014). Briefly, WQS is a technique that estimates a set of weights, $\boldsymbol{w}$, that maximize the likelihood of the following regression equation

$$E[y] = \beta_0 + \beta_1 \left(\sum_{b=1}^{B} w_b q_b \right) + z^{\mathrm{T}} \boldsymbol{\phi}, \quad (1)$$

where y is the outcome; β_0 is the intercept; β_1 is the regression coefficient for the weighted sum of the quantiled exposures, q_b; B is the number of exposures; $z = [z_1, \cdots, z_C]$ is the set of covariates; and $\boldsymbol{\phi}$ is the set of regression coefficients corresponding to z. The weights are constrained such that $0 \leq w_b \leq 1$ and $\sum_{b=1}^{B} w_b = 1$ (Carrico et al., 2014). For these analyses, the exposure data were grouped into quartiles. Additionally, 1000 bootstrap datasets were used and $w_b$was estimated as the average across the bootstrap estimates. The directionality of the association of the WQS index was constrained to be non-positive. The WQS assumptions of linearity and directional homogeneity were validated through visual inspection of residuals and comparing the fit of the linear models to nonlinear (quadratic) models. Due to our limited sample size, the data were not separated into training and testing datasets, thus the derived WQS indices may suffer from overfitting and, therefore, may not generalize to data from other populations. As with all observational studies, additional validation of these result in other populations is required. Since $0 \leq w_b \leq 1$ and $\sum_{b=1}^{B} w_b = 1$, the weights can be used to determine the relative contribution of each exposure, in this case Pb and Mn, to the combined or joint effect.

2.5.2. Multi-media biomarkers (MMBs)

To create our MMBs, we integrated metal levels from blood, urine, hair, nails, and saliva using WQS into separate indexes representing Pb exposure and Mn exposure. MMBs integrate exposure information across multiple biomarkers and derive exposure estimates that we propose better represent the overall body burden (Levin-Schwartz, 2020). The derivation of an MMB enables the estimation of weights that highlight the contributions of each biomarker medium to the MMB. When using MMBs to compute the joint effect of Pb and Mn on IQ, three WQS models were estimated per outcome (Verbal IQ, Performance IQ, and Full Scale IQ) (Fig. 1). The first WQS was performed with all Pb exposure biomarkers to estimate the Pb MMB. The second WQS was performed with all Mn exposure biomarkers to estimate the Mn MMB. The final WQS model estimated the joint effect of the Pb MMB, estimated in first WQS model, and the Mn MMB, estimated in the second WQS model. Thus, these analyses can be seen as hierarchical WQS analyses, where the first level is across media for a single metal and the second level is across metals (Levin-Schwartz, 2019).

2.5.3. Data screening and analysis

First, the Spearman correlation between the biomarkers for each metal were estimated. The three main types of analyses are depicted in Fig. 1. Since each biomarker is on a different scale, all exposure levels were first converted into quartiles. The first set of analyses, shown in Fig. 1**a**, were linear regressions, where the outcome was the neurodevelopmental outcome and the exposure was the metal exposure level measured in each medium. These analyses were repeated for each combination of metal and biomarker medium, resulting in 10 regression models. In the second set of analyses, shown in Fig. 1**b**, WQS was used to estimate the combined effect of Pb and Mn on neurodevelopment, where the levels of Pb and Mn were quantified using each medium separately. Thus, a separate WQS index was estimated for each medium (i.e., a WQS index for blood Pb-blood Mn, a WQS index for urine Pb-urine Mn, etc.). Then, the associations between the derived WQS indices and neurodevelopment were assessed using linear regressions. In the final set of analyses, shown in Fig. 1**c**, MMBs for Pb and Mn were estimated by applying WQS across the exposure estimates for each metal separately, as discussed in the previous section. Once MMBs were estimated for Pb and Mn, they were categorized into quartiles and a second WQS was performed to estimate the joint effect of Pb and Mn on neurodevelopment. The association between the derived Pb-Mn index and neurodevelopment was determined using linear regressions. All linear regressions were adjusted for sex and SES (WISC IQ scores were adjusted for age). The selection of these covariates was based upon prior research in the same cohort (Butler, 2018; Lucchini, 2012; Levin-Schwartz, 2019; Bauer, 2017). All analyses were performed using SAS 9.4. Note that, generally in WQS, weights of components are not interpreted if the joint effect is not significant. However, for completeness, we also show the weights for non-significant models in the results.

3. Results

3.1. Descriptive statistics

The demographic information for the 259 subjects in this study are shown in Table 1. Slightly more than half of the subjects (51.0%) were male and the average age was 12.2 years. Over half of the subjects were

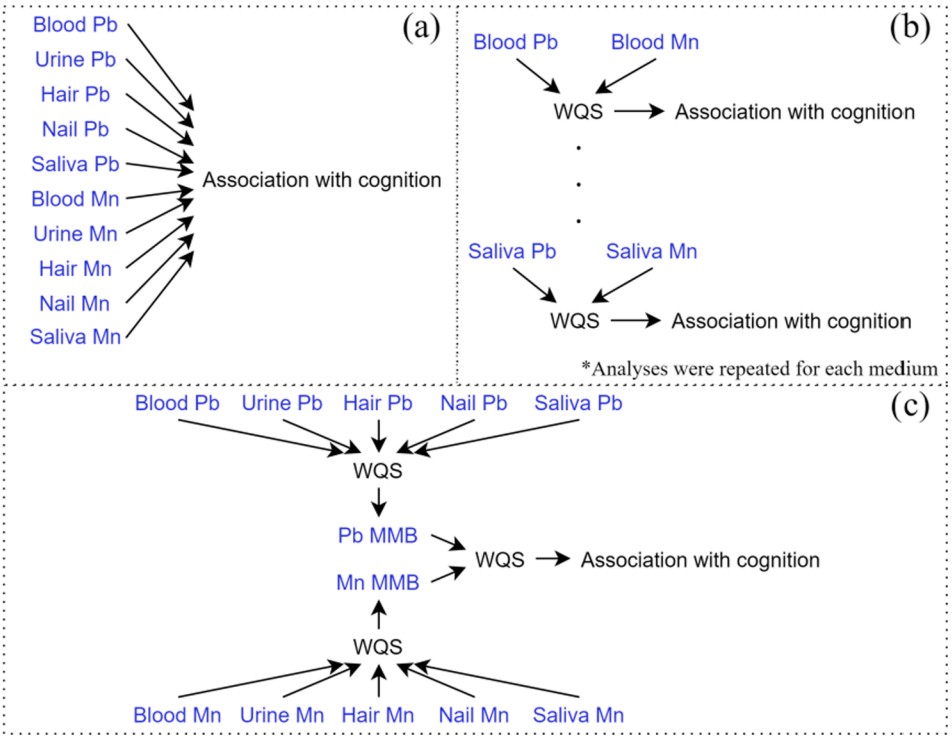

Fig. 1. Diagram of main analyses conducted to measure the effects of Pb and Mn on cognition. (a) The association between cognition and each biomarker for both Pb and Mn was assessed using traditional linear regression. (b) WQS was performed to measure the joint effect of Pb and Mn for each biomarker individually. (c) MMBs for Pb and Mn were derived by applying WQS to each biomarker for each metal. Then, the joint effect of Pb and Mn on cognition was assessed by applying WQS to the Pb and Mn MMBs. All models were adjusted for age, sex, and SES.

Table 1
Demographic information and descriptive statistics.

Demographics	Category	N (%)
Total		259 (100%)
Sex	Male	132 (51.0%)
	Female	127 (49.0%)
Socioeconomic Status	Low	57 (22.0%)
	Medium	138 (53.3%)
	High	64 (24.7%)
		Average ± SD (range)
Age (years)		12.2 ± 1.0 (10–14)

SD: Standard deviation.

from medium SES (53.3%), while 24.7% of participants were from high SES and 22.0% were from low SES. The concentrations of Pb and Mn in the different biological media are shown in Table 2. We show the Spearman correlation between each of the Pb biomarkers in Supplemental Table 1 and each of the Mn biomarkers in Supplemental Table 2. In general, the Pb biomarkers had stronger correlations amongst each other than the Mn biomarkers did. Blood Pb was correlated with urine Pb (r_s: 0.41, $p < 0.01$), hair Pb (r_s: 0.24, $p < 0.01$), and nail Pb (r_s: 0.26, $p < 0.01$). Urine Pb was correlated with nail Pb (r_s: 0.15, $p < 0.05$) and hair Pb was correlated with both hair Pb (r_s: 0.22, $p < 0.01$) as well as saliva Pb (r_s: 0.18, $p < 0.01$). Hair Mn was correlated with urine Mn (r_s: 0.14, $p < 0.05$), nail Mn (r_s: 0.17, $p < 0.01$), and saliva Mn (r_s: 0.22, $p < 0.01$). Nail Mn was also correlated with blood Mn (r_s: −0.12, $p < 0.05$) and urine Mn (r_s: 0.14, $p < 0.05$).

Table 2
Metal concentrations in exposure biomarkers (n = 259).

Metal	Biomarker Medium	Median	Interquartile Range
Lead	Blood (μg/dL)	1.27	0.84
	Urine (μg/L)	0.64	0.48
	Hair (μg/g)	0.09	0.19
	Nails (μg/g)	0.12	0.38
	Saliva (μg/L)	0.56	1.54
Manganese	Blood (μg/dL)	1.09	0.45
	Urine (μg/L)	0.22	0.30
	Hair (μg/g)	0.07	0.07
	Nails (μg/g)	0.16	0.25
	Saliva (μg/L)	3.98	9.29

3.2. Associations between combined Pb and Mn on cognition

In this section, we present the traditional linear regression analyses, followed by the single matrix WQS analyses, and finally the results of the MMBs analysis for each WISC-III outcome, Full Scale IQ, Verbal IQ, and Performance IQ.

3.2.1. Associations with Full Scale IQ

In covariate-adjusted models, though we found no association between metal levels in each medium individually and Full Scale IQ (Supplemental Figs. 1 and 2), saliva Mn was found to be marginally associated with Full Scale IQ (-1.2 [-2.5, 0.2], 0.09). For the media-specific mixture effects, when Pb and Mn levels were estimated using saliva, there was a marginal association between the Pb-Mn WQS index and Full Scale IQ (β [95% CI], p-value: −1.3 [−2.7, 0.2], 0.09).

In covariate adjusted models (Fig. 2), only the joint effect of Pb and Mn estimated using MMBs was associated with Full Scale IQ scores at a $p < 0.05$ threshold for significance. A quartile increase in this Pb-Mn WQS index was associated with a 1.9 point ([95% CI], p-value: [0.3, 3.5], 0.02) decrease in Full Scale IQ. Weights highlighting the contribution of each metal to the WQS index (Fig. 3) indicate that Pb contributed the majority of the weight (72%), with Mn contributing less (28%). Weights highlighting the contributions of each medium to the MMBs (Tables 3 and 4) indicate that top contributors to the Pb MMB were urine (26%), hair (23%), blood (22%), and saliva (20%). Saliva (49%) and hair (23%) were the top contributors to the Mn MMB.

3.2.2. Associations with Verbal IQ

When the association for each metal-biomarker combination with Verbal IQ were assessed individually (Supplemental Figs. 3 and 4), blood Pb and saliva Mn were found to be associated with Verbal IQ.

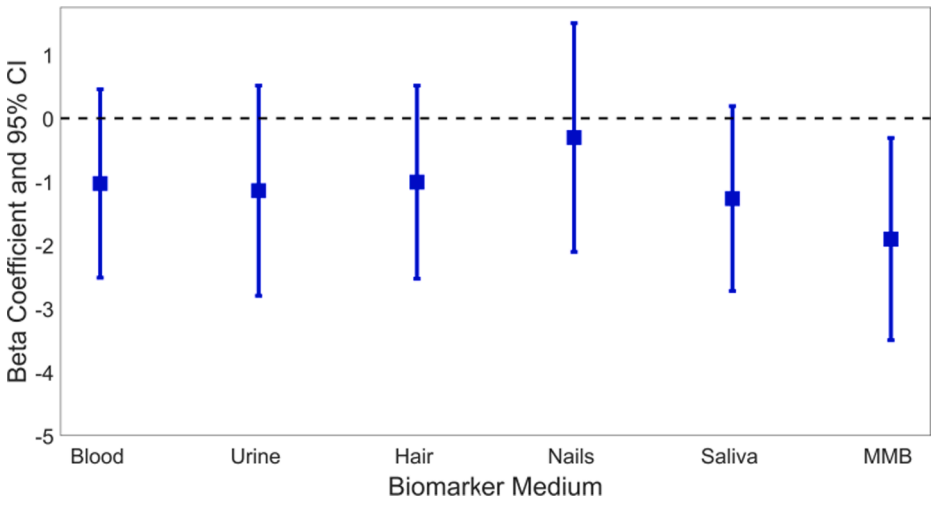

Fig. 2. Beta coefficients and 95% confidence intervals representing the association between the combination of Pb and Mn and age-adjusted Full Scale IQ. Beta coefficients reflect quartile changes in the combination. The joint effect is estimated using WQS. All models were adjusted for sex and SES.

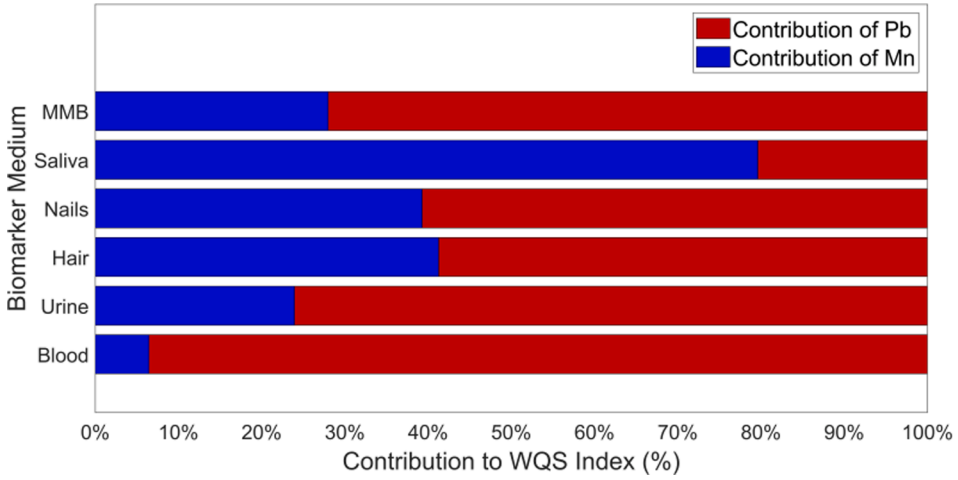

Fig. 3. Weights highlighting the contribution of Pb and Mn to the joint effect for Full Scale IQ. Higher weights indicate a greater contribution.

Table 3
Media contributions to the Pb MMBs.

Biomarker Medium	Verbal IQ	Performance IQ	Full Scale IQ
Blood	34%	10%	22%
Urine	17%	29%	26%
Hair	28%	21%	23%
Nails	8%	23%	9%
Saliva	13%	17%	20%

Table 4
Media contributions to the Mn MMBs.

Biomarker Medium	Verbal IQ	Performance IQ	Full Scale IQ
Blood	5%	9%	6%
Urine	14%	16%	12%
Hair	20%	28%	23%
Nails	18%	3%	10%
Saliva	44%	44%	49%

Quartile increases in blood Pb and saliva Mn were associated with 1.5 point (β [95% CI], p-value: [0.0, 2.9], 0.04) and 1.9 point ([0.6, 3.3], 0.01) decreases in Verbal IQ, respectively. Marginally significant associations were observed between Verbal IQ and hair Pb (-1.2 [-2.6, 0.2], 0.09) as well as hair Mn (-1.3 [-2.7, 0.1], 0.08).

The joint effects of Pb and Mn estimated using metal concentrations from blood, hair, and saliva were all associated with Verbal IQ scores (Fig. 4). Quartile increases in the Pb-Mn WQS indices were associated with 1.5 point ([95% CI], p-value: [0.0, 3.0], 0.05), 1.6 point ([0.1, 3.2], 0.04), and 2.0 point ([0.6, 3.4], $p < 0.001$) decreases in Verbal IQ for blood, hair, and saliva, respectively. The contributions of Pb and Mn to the WQS indices differed depending on the medium used to estimate exposure (Fig. 5). For blood, the contributions to the WQS index were 95% Pb and 5% Mn. For hair, the contributions were 43% Pb and 57% Mn. For saliva, the contributions were 3% Pb and 97% Mn.

The joint effect of Pb and Mn estimated using MMBs was Verbal IQ scores (Fig. 4). Quartile increases in the Pb-Mn WQS index was associated with 2.8 point (β [95% CI], p-value: [1.0, 4.5], $p < 0.001$) decreases in Verbal IQ. The contributions for Pb and Mn to this index were 42% and 58%, respectively (Fig. 5). Weights highlighting the contributions of each medium to the MMBs (Tables 3 and 4) indicate that top contributors to the Pb MMB were blood (34%), hair (28%), and urine (17%). Saliva (44%), hair (20%), and nails (18%) were the top contributors to the Mn MMB.

3.2.3. Associations with Performance IQ

Neither Pb nor Mn levels in any individual biomarker was found to

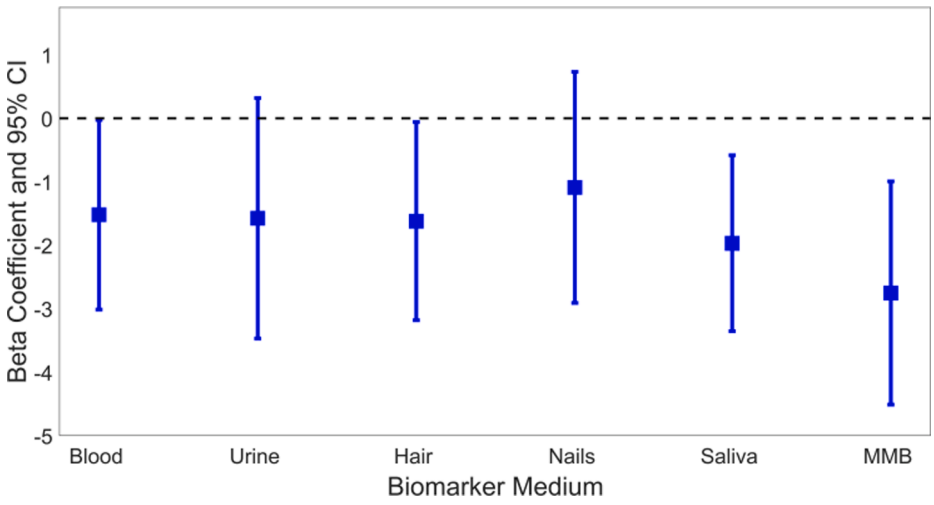

Fig. 4. Beta coefficients and 95% confidence intervals representing the association between the combination of Pb and Mn and age-adjusted Verbal IQ. Beta coefficients reflect quartile changes in the combination. The joint effect is estimated using WQS. All models were adjusted for sex and SES.

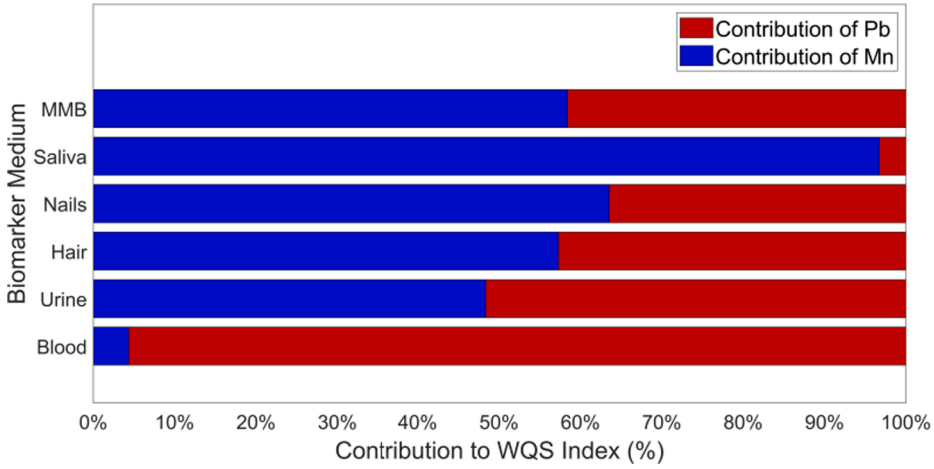

Fig. 5. Weights highlighting the contribution of Pb and Mn to the joint effect for Verbal IQ. Higher weights indicate a greater contribution.

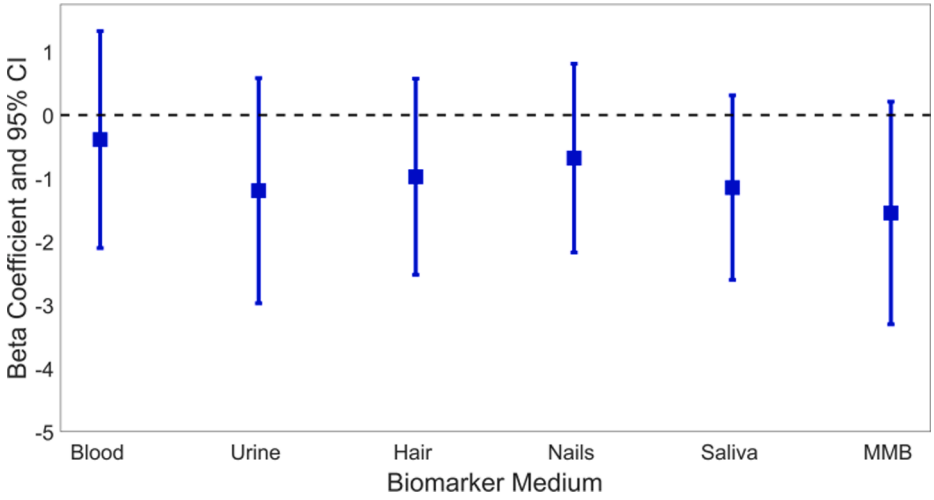

Fig. 6. Beta coefficients and 95% confidence intervals representing the association between the combination of Pb and Mn and age-adjusted Performance IQ. Beta coefficients reflect quartile changes in the combination. The joint effect is estimated using WQS. All models were adjusted for sex and SES.

be associated with Performance IQ (Supplemental Figs. 5 and 6). Additionally, none of the joint effects of Pb and Mn were associated with Performance IQ scores (Fig. 6). However, when Pb and Mn levels were estimated using MMBs, there was a marginal association between the Pb-Mn WQS index and Performance IQ (β [95% CI], p-value: −1.5 [−3.3, 0.2], 0.08). Weights highlighting the contribution of each metal to the WQS index (Fig. 7) indicate that Pb contributed 55% of the weight to the index and Mn contributed 45%.

4. Discussion

In this study, we examined the joint association between two metals, Pb and Mn, on childhood IQ, using novel methods–WQS and MMBs. Overall, we found that mixture results differed depending on the medium used to measure exposure and that the MMB approach had the most power to find an effect. We also found that MMBs uncovered consistent associations between the combination of Pb and Mn and neurodevelopment that no single biomarker media could replicate. We propose that this is because MMBs combine information across multiple exposure biomarkers and create a more comprehensive estimate of exposure than any individual biomarker. Our MMB approach also allows us to estimate the relative contributions of both the metals and the biomarker media to the mixture effect. The top contributors to the MMBs were blood for Pb and saliva for Mn, which also showed the strongest individual associations with cognition for Pb and Mn, respectively. Since the weights estimate the relative contribution of each biomarker media to the MMB, they can be used to inform future research on the biospecimens that are most critical to collect in order to explore the effects of metal mixtures on cognition. It is important to emphasize that the issues of bias in exposure estimation that we discuss become more acute as the mixture becomes larger, since the likelihood that a single biomarker is an accurate measure of exposure for every element of the mixture becomes increasingly less likely.

Biomarkers of exposure are influenced by toxicokinetic properties, i.e., the rate and manner in which the chemical is absorbed, distributed, metabolized, and excreted from the body, which may not uniformly correlate with toxicodynamics, i.e., the interaction with and alterations to biological processes that cause a toxic effect. The relationship between the two differs for each chemical and may not be fully captured by using a single biomarker medium, especially when each medium represents different aspects of kinetics (compartmentalization, secretion, excretion, etc.). By integrating exposure information across media, we can better capture the internal concentration. In this study, we observed strong evidence for the combined effect of Pb and Mn on Verbal IQ across multiple media. However, the strength of this association and the contribution of each metal to the joint effect (the WQS index) differed depending on the biomarker chosen to estimate exposure. For example, when comparing two significant WQS indices, one where exposure was measured using blood Pb/Mn and the other using saliva Pb/Mn, the contribution of Pb was 95% when blood was used for both exposures. However, when saliva was used, the relationship was very different as only 3% of the weight was attributed to saliva Pb. This range of values is likely due to toxicokinetic differences between these media as biomarkers for Pb and Mn. In other words, blood Pb is considered to be the gold standard biomarker of Pb exposure for research (Abadin, 2007; Barbosa et al., 2005) and blood Pb was significantly associated with Verbal IQ. In contrast, saliva may be a better biomarker of Mn exposure, as blood Mn is under physiologic control due to the role of Mn as a nutrient. In this study, blood Mn was not significantly associated with Verbal IQ; however, saliva Mn was significantly associated with Verbal IQ. We hypothesize that this may be because levels of blood Mn are more strictly regulated than saliva Mn, making saliva a better measure of excess Mn exposure than blood. It is important to note that the exact cause and dynamics of Mn secretion in saliva is not well understood, though there is some evidence that Mn is actively secreted and does not passively diffuse into saliva. Further research is needed to replicate and expand upon the current findings as well as further clarify the dynamics of Mn.

The health impacts of chemical exposures reflect the individual toxicodynamics of each chemical. MMBs address the potential misalignment between the toxicokinetics and toxicodynamics by integrating information across different media that reflect different aspects of toxicokinetics, such as excretion (urine), secretion (hair, nails, and saliva), and compartmentalization of dose (blood). By doing so, MMBs can better reflect the overall body burden of exposure and should better correlate with toxicodynamics. Our results are consistent with this hypothesis. We observed significant associations between the WQS indices derived when Pb and Mn exposure were estimated using MMBs and both Verbal IQ as well as Full Scale IQ. Traditional mixtures approaches would apply WQS to each medium individually (e.g., blood) rather than using a MMB. When we applied such traditional approaches, the associations found were uniformly weaker than when applying WQS to the MMBs. This is likely because we are integrating exposure information across media when using MMBs, therefore we do not introduce any bias through the selection of a single biomarker over another. Additionally, the weights estimated for the MMB enable the determination of the contributions of each medium and each metal in each medium to the MMB. Investigating these weights reveals that blood and then hair were the media that contributed most to the Pb MMBs across the different outcomes, while saliva followed by hair were the media that contributed most to the Mn MMBs. Note that we reached a similar conclusion about the media that best capture the internal concentration of Pb and Mn

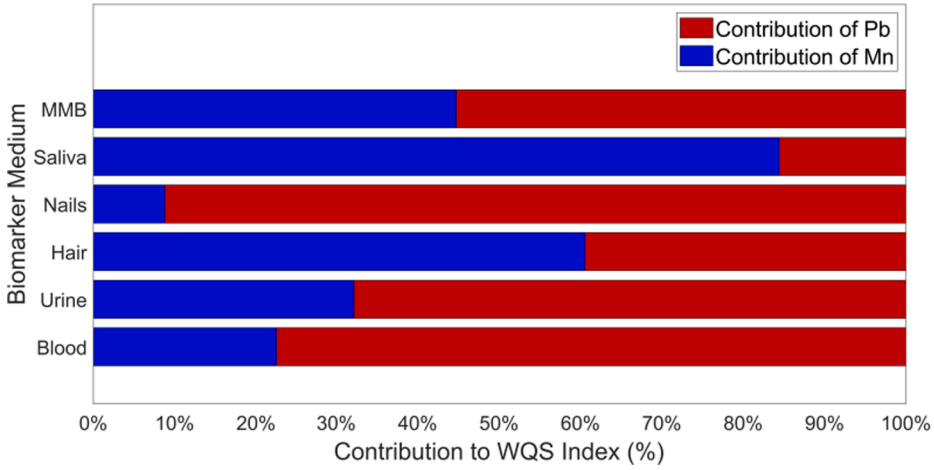

Fig. 7. Weights highlighting the contribution of Pb and Mn to the joint effect for Performance IQ. Higher weights indicate a greater contribution.

when comparing the WQS indices across media. However, by using MMBs we are able to leverage each biomarker's strengths as a surrogate measure of the internal level instead of ignoring this information by only selecting individual biomarkers. Additional work is needed to replicate and expand upon these findings.

Significant joint effects imply that there are underlying biological connections between Mn and Pb. There are multiple mechanisms by which Pb and Mn can jointly act to impact brain function and neurodevelopment (Neal and Guilarte, 2013; Stackelberg et al., 2015). First, co-exposure to Pb and Mn has been shown to increase uptake and levels of Pb in the brain (Malhotra et al., 1984; Chandra et al., 1984; Carfagna et al., 1996). Additionally, both Pb and Mn have been shown to interfere with the transmission of neurotransmitters, including dopamine, glutamate, and gamma aminobutyric acid (Chandra et al., 1981; Lasley and Gilbert, 1996; Lasley and Gilbert, 2002; Neal et al., 2010; Guilarte, 2006; Guilarte, 2008). These neurotransmitters are key to the proper function and development of the brain (Eshel et al., 2013), thus disruption in their levels impacts learning and development. Another mechanism by which Pb and Mn both affect the brain is disruption of the calcium (Ca) signaling. Both metals have been shown to increase the levels of cellular Ca^{2+} in the brain, hindering communication between neurons (Filipov and Dodd, 2012; Lidsky and Schneider, 2003; Neal and Guilarte, 2010). It is possible that some or all of these mechanisms may be contained in the same pathway and that these effects happen in sequence rather than simultaneously (Stackelberg et al., 2015). In all the above cases, joint exposure to higher doses would lead to greater toxicity.

There are multiple strengths to our study. We measured Pb and Mn levels in multiple media from the same people at the same time, which enables us to directly compare the joint effects of Pb and Mn across media. Such an approach is not common in epidemiological studies, but is important since doing so enables evaluation of the extent to which the joint effect is impacted by the selection of the exposure biomarker. The use of MMBs allows us to avoid the bias that is introduced through the selection of a single biomarker to measure the joint effects of Pb-Mn. This source of bias should not be underestimated in mixtures research as we found in our results that no single medium is an ideal biomarker for both Pb and Mn. Our use of a relatively straightforward two metal combination enables us to clearly highlight the problems of biomarker selection in mixtures studies. Highlighting these issues would be less clear with a more complex mixture effect. We used the same powerful mixture method, WQS, for all analyses facilitating comparisons of the joint effects of Pb and Mn and contributions of metals across the different media.

Our study also has some limitations. We had a relatively small sample size, though we were still able to detect robust associations even with this modest sample size. This may reflect added power from using MMBs. We did not split our data into training and testing datasets, thus we may have some issues with overfitting. The overall level of exposure for this population is relatively low compared to other populations, though these levels are likely similar to most adolescents in other developed countries (Pelc et al., 2016; da Rocha Silva, 2018). Since the distribution of Pb and Mn in the body can differ depending on the level of exposure, our conclusions about the biomarkers to measure Pb-Mn effects may not be fully generalizable to populations with higher levels of exposure. Our methods only considered additive effects, future work will explore the estimation of MMBs using methods that can account for multiplicative or other non-linear effects. Additionally, the performance of different biomarkers may also be dependent on the target organ under study, thus the results may not fully generalize to renal or cardiac endpoints.

5. Conclusion

In this work, we have examined the role that biomarker selection plays when estimating the joint effects of Pb and Mn on cognition. We have found that, though blood and saliva seem to be the best biomarkers to measure Pb and Mn exposure individually, hair was the best biomarker for the measurement of the joint effects of Pb and Mn. We also found that estimates of the combined effect of Pb and Mn using MMBs were stronger and more robust than when the same effects were estimated using the biomarkers individually. As the epidemiologic exploration of the impacts of chemical mixture progresses and mixtures become more complicated, appropriate biomarker selection will become even more important in order to estimate risk and MMBs are an important way to avoid bias in such estimation.

CRediT authorship contribution statement

Yuri Levin-Schwartz: Conceptualization, Formal analysis, Investigation, Methodology, Writing - original draft. **Birgit Claus Henn:** Writing - review & editing. **Chris Gennings:** Writing - review & editing. **Brent A. Coull:** Writing - review & editing. **Donatella Placidi:** Data curation, Writing - review & editing. **Megan K. Horton:** Funding acquisition, Writing - original draft. **Donald R. Smith:** Data curation, Funding acquisition. **Roberto G. Lucchini:** Funding acquisition. **Robert O. Wright:** Funding acquisition, Conceptualization, Supervision, Writing - review & editing.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2020.106312.

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