Full Length Article

Latent subgroups of cognitive performance in lead- and manganese-exposed Uruguayan children: Examining behavioral signatures

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ABSTRACT

Objectives: Lead (Pb) and manganese (Mn) are confirmed neurotoxins but it is unclear to what extent low-level exposure produces a unique behavioral signature. The objective of this study was to investigate latent cognitive profiles among children (6–8 years) from Montevideo, Uruguay co-exposed to these metals.

Method: Among 345 children, blood Pb and hair Mn were measured using atomic absorption spectroscopy and ICP-MS, respectively. Sixteen measures, reflecting multiple domains of cognitive functioning were gathered: (1) three tests from Cambridge Neuropsychological Test Automated Battery (CANTAB): Intra-Extra Dimensional Shift (IED), Spatial Span (SSP) and Stockings of Cambridge (SOC), (2) ten tasks from Woodcock-Muñoz Achievement Battery, Revised (W−M): Visual-Motor Integration, Verbal Comprehension (Vocabulary, Synonyms, Antonyms, Analogies), Visual-Auditory Comprehension, Concept Formation, Visual Spatial Thinking, Number Inversion and Spatial Relations, (3) Bender Gestalt task, and (4) Weschler block design task. Scores were modeled using latent profile analysis (LPA). Association between blood Pb and hair Mn on performance profiles was assessed using ordinal regression, controlling for confounders. An interaction between Pb and Mn was tested.

Results: Mean ± SD of blood Pb was 4.1 ± 2.1 μg/dL and 35% of children had blood Pb ≥ 5 μg/dL. Median [5%, 95%] hair Mn level was 0.8 [0.3, 4.1] ppb. Three latent cognitive performance profiles were identified: high (n = 46, 13%), average (n = 209, 61%) and low (n = 90, 26%). Each one-unit increase in blood Pb was associated with a 28% greater likelihood of belonging to a poorer-performing profile. The association was non-linear, with the effect of Pb on profile membership strongest at lower levels of exposure. There was no meaningful interaction between Pb and Mn.

Conclusions: A behavioral signature for low-level Pb & Mn exposure was not identified, but the likelihood of membership in low-performing profile was higher at lowest levels of blood Pb. There was no effect measure modification between Pb and Mn. Future research should address how complex environments created by chemical exposures and the social context relate to cognitive performance in young children.

1. Introduction

Lead (Pb) exposure negatively impacts child cognitive functioning, including IQ score attainment and performance on more specific neuropsychological tasks (Mason et al., 2014; Vrijheid et al., 2016), without a lower boundary for the detection of harm (Bellinger, 2008b; Canfield et al., 2003). In early studies of blood Pb and child cognition, Stiles and Bellinger (1993) noted that the neuropsychological impairments responsible for low IQ scores are not well understood. IQ scores were designed to assess global cognitive function rather than specific neuropsychological mechanisms. Therefore, neuropsychological tests may elucidate if Pb exposure results in a 'behavioral signature' defined by impairments in specific domains of cognitive functioning. Ultimately, Stiles and Bellinger found minimal effects on specific cognitive functions, but suggested that Pb exposure had greater detrimental effects on executive functioning and attention relative to

other domains (Stiles and Bellinger, 1993). Subsequent work among Pb-exposed children has continued to identify impairment across multiple domains, including attention, executive functioning and non-verbal reasoning (Canfield et al., 2004; Chiodo et al., 2004; Lanphear et al., 2000).

In recent years, children's blood Pb levels have generally been declining. In China, the geometric mean blood Pb in children (age 0–18) has decreased from ˜12 μg/dL in 2000 to ˜4 μg/dL in 2015 (Han et al., 2018). In the United States the geometric mean blood Pb among 1 to 5 year-olds has declined from 15 μg/dL in the late 1970s to 0.76 μg/dL by most recent estimates (Caldwell et al., 2017; CDC, 2013). Similar declines have been noted in Sweden, France, Belgium, Czech Republic and other European countries (Bierkens et al., 2011; Etchevers et al., 2014; Strömberg et al., 2008). Because of these trends, it is important to re-examine the potential existence of a specific cognitive profile or behavioral signature at low levels of Pb exposure to appropriately target interventions to affected children. Neuropsychological tests may become increasingly important for detecting cognitive impairment at these low levels of exposure.

At the same time, children's exposures to other metals, such as manganese (Mn), have been recognized as health threats, as have interactions among metals or effects of metal mixtures. For example, both cross sectional (Bouchard et al., 2018; Carvalho et al., 2018; Haynes et al., 2018, 2015) and prospective (Dion et al., 2018) studies have demonstrated an association between Mn exposure and children's IQ scores. Several studies have found evidence for an interaction between Mn and Pb exposure on cognitive performance among infants (Claus Henn et al., 2011; Lin et al., 2013), and among older children (Kim et al., 2009). Conversely, no effect modification between Mn and Pb exposure on infant development has also been reported (Rodrigues et al., 2016).

Using a sample of 6–8 year-old children exposed to low levels of Pb (70% of sample < 5 μg/dL) and low levels of Mn (50% of sample < 0.82 ppb), we had three objectives: (1) to derive cognitive performance profiles, (2) to investigate the association between blood Pb, hair Mn, and cognitive profile membership with the aim of identifying a specific behavioral signature of low-level metal exposure, and (3) to investigate a potential interaction between blood Pb and hair Mn on profile membership.

2. Materials and methods

2.1. Setting and recruitment

A total of 357 of 673 eligible first-grade children from Montevideo, Uruguay were enrolled in this cross-sectional study. Children were sampled from 11 private elementary schools located in areas of Montevideo with demonstrated or suspected exposure to metal contaminants. Children and parents who were enrolled in the study completed a series of questionnaires, neuropsychological tests and biological sampling consisting of a blood draw and hair sampling. Study protocols were approved by research ethics boards at the Pennsylvania State University, the Catholic University of Uruguay, the Faculty of Chemistry at the University of the Republic of Uruguay, and the University at Buffalo. Recruitment methods are fully described elsewhere (Roy et al., 2015). Of the 357 children enrolled in the study, 12 did not have a neuropsychological assessment, resulting in a final sample of 345.

2.2. Sociodemographic measures

Caregivers (typically mothers) reported on demographic characteristics with respect to themselves, the child, and the household environment. Descriptive variables collected on the child included: sex, height for age z-score and age. Caregivers completed an extensive questionnaire regarding parental education, age, smoking status, and

other sociodemographic information. Household crowding was measured as the ratio of the number of individuals living in the house to the number of rooms. To create a measure of wealth, principal components analysis (PCA) was applied to twelve household items (i.e. television, automobile, cellular phone, and dishwasher). A single component was identified consisting of five items, as described previously (Kordas et al., 2018a). A sum of these five items comprises the possessions of wealth variable (range 0–5). Household environment was measured during a home visit conducted by a social worker, using the Home Observation for Measurement of the Environment Inventory (HOME score) (Bradley, Caldwell, & Corwyn, 2003). Overall, the HOME score is an average of 59 items related to: parental responsibility, encouragement, emotional climate, learning materials and opportunities, active stimulation, family participation, parental involvement, and physical environment.

2.3. Biomarker measurement

Greater detail of collection procedures for blood samples can be found in a previous publication (Kordas et al., 2018b). For blood Pb, a phlebotomist collected samples from the children between 8–11 am after an overnight fast. Blood Pb was measured using Atomic Absorption Spectrometry (AAS, VARIAN SpectrAA-55B) with either graphite furnace or flame ionization, depending on sample volume. Detection limits for graphite and flame AAS were 0.7 μg/dL and 1.8 μg/dL, respectively. Throughout enrollment, both methods were used except in the final year, when graphite furnace was used exclusively. In total, 87 (28%) of children had flame ionization and 228 (72%) had graphite furnace. The blood Pb assay method was accounted for in statistical modeling. To measure hair manganese (Mn), samples were taken using stainless steel scissors, cutting from the base of the occipital region of the skull. The hair was deposited in a clean envelope and stored at room temperature. For the analysis, one centimeter of hair was cut from the collected strand, placed in a vial and cleaned by sonication for 15 min in 10 mL of 1% Triton X-100; it was rinsed with distilled water. After drying, one milliliter of nitric acid (HNO_3) was added to the vial for 11-hour digestion at 80 °C; the digested sample was diluted in 20 ml of double distilled water. The Material Characterization Laboratory at the Pennsylvania State University analyzed the samples using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) with Collision Cell Technology (Thermo Scientific XSERIES 2, Bremen, Germany). The detection limit for hair Mn was 0.01 ng/ml.

2.4. Cognitive measures

All cognitive measures were individually administered by trained testers—psychologists or psychology students from the Catholic University of Uruguay. The children underwent two cognitive testing sessions. Administration was performed at the school, each lasting approximately 1.5 h. The testing was carried out in a separate room provided by the school.

To elucidate the meaning of our cognitive profiles, each cognitive test (described below) was conceptualized within the Cattell-Horn-Carroll (CHC) framework (Flanagan, 2008; McGrew, 2005; Schneider and McGrew, 2012). The CHC framework divides general cognitive functioning into ten domains, each meant to represent a conceptually unidimensional ability. Taub and McGrew (Taub and McGrew, 2004) further condensed these domains into seven abilities measured with the Woodcock-Johnson battery (Woodcock et al., 2001). These seven domains included: fluid intelligence (Gf), crystallized intelligence (Gc), short-term memory (Gsm), visual processing (Ga), auditory processing (Ga), long-term retrieval (Glr), and processing speed (Gs).

2.4.1. Woodcock-Muñoz tests of cognitive abilities (W-M)

The Woodcock-Muñoz is the Spanish translation of the Woodcock-Johnson test battery (Muñoz-Sandoval et al., 2009; Woodcock et al.,

2001) and is commonly used to evaluate cognitive impairments in clinical settings (Dumont et al., 2016; Ledbetter et al., 2017; Scantlebury et al., 2016; Taub and McGrew, 2004). All seven tests were administered: verbal comprehension, visual-auditory learning, spatial relations, sound blending, concept formation, visual matching and numbers reversed.

The verbal comprehension scale (VCS) includes four subtests: vocabulary, synonyms, antonyms and analogies. In the verbal subtest, children are presented with commonly recognized images (e.g. animals, fruits, household items), and are asked to say the word for each image. In the synonym subtest, children say an alternative word for a series of visually presented and spoken words. In the antonym subtest, children speak an opposite word for a series of visually presented and spoken words. Lastly, in the analogies subtest, children complete an analogy presented by the examiner (e.g. finish what I say – a bird flies and a fish…). Scores from each of the four subtests of the VCS were included in the analysis to capture task-specific cognitive performance. Median internal consistency reliability for the full verbal comprehension scale is 0.95 for all ages (Schrank et al., 2005).

In the visual auditory learning task, children are prompted to associate words with symbols. In the first trial, four symbols are presented. Afterwards, the testing sequence forms a story with the symbols. The child must repeat the learned words for each symbol, creating a word story. Subsequent trials introduce four new symbol/word associations. Trials increase in difficulty with all previous symbols included in the testing sequence. A total of 28 symbols is presented and seven trials are completed. Scoring is based on the number of errors committed. Median internal consistency reliability for the visual auditory learning task is 0.83 across all ages (Schrank et al., 2005).

The spatial relations task asks children to identify components of a shape. Children are presented with a completed shape and a series of smaller shapes. Children must identify which of the smaller shapes form the completed shape. Scoring is based on the correct number of smaller shapes chosen. The median internal consistency reliability for the spatial relations task across all ages is 0.81 (Schrank and McGrew, 2001).

The sound blending test requires children to speak a completed word from two delayed auditory segments (e.g. 'fi' and 'sh' are presented with 'fish' as the correct response). Children are scored on the number of correctly completed words. Median internal consistency across all ages is 0.98 (Schrank et al., 2005).

The concept formation test requires children to identify differences between shapes, presented as either circles or squares. All shapes can be red or yellow, big or small or come in pairs. Each test item contains an unknown rule. If a shape in the series follows the rule, it is presented again in a separate box. The child must identify the unknown rule and repeat it to the examiner (e.g. red and small shapes go in the box). Scoring is based on the number of correct responses during testing. Median internal consistency across all ages is 0.94 (Schrank and McGrew, 2001).

The timed, visual matching test requires children to identify two identical shapes in a row of shapes presented on a page. The measure is scored according to the number of rows the child completes correctly in two minutes. Median internal consistency across all ages is 0.91 (Schrank et al., 2005).

Lastly, the number inversion task (digit span backwards) requires children to repeat a series of numbers in reverse order (e.g. for numbers two & five, the correct response is five & two). Difficulty of the task increases with each trial, progressing from two to eight numbers. Children are scored on the number of correct trials. Median internal consistency reliability across all ages is 0.87 (Schrank and McGrew, 2001).

2.4.2. Cambridge neuropsychological test automated battery (CANTAB)

The CANTAB test battery is a set of computerized cognitive assessments administered on a touch-screen (Fray and Robbins, 1996; Luciana and Nelson, 2002; Pase and Stough, 2014). All CANTAB

instructions were provided in Spanish by the tester. No translation of the computerized assessment was necessary as the CANTAB uses only non-lingual, visual and auditory stimuli. Three CANTAB tests were used: Stockings of Cambridge (SOC), Intra-dimensional-extra-dimensional shift task (IED), and Spatial Span (SSP). Among adults, reliability measures have been found to be good for the IED (~0.80) and moderate for the SOC and SSP tasks (0.60 and 0.68, respectively) (Lowe and Rabbitt, 1998). Test-retest reliability assessments among children have not been conducted with large samples. However, intra-class correlations for the IED among 11–17 year olds with ADHD are relatively high for the IED (ICC = 0.78–1.0) (Gau and Shang, 2010).

The SOC is based on the Tower of London task which is used to measure higher order planning and executive function (Anderson et al., 1996). The child is asked to shift positions of colored circles to match a completed example. Four problems were presented, each with increasing difficulty and number of moves (two to five minimum moves). The primary outcome was the number of problems solved using the minimum number of moves.

The IED is a measure of attention and set shifting similar to the Wisconsin Card Sorting Task (Heaton et al., 1993). During each trial, the child is required to discover which of two geometric shapes is the correct choice, according to an implicit rule. After each choice, the computer indicates by sound and color if the choice was correct. The stage continues until the correct shape is chosen in six consecutive trials. Nine test stages are administered. Stages one and two introduce shapes only, with stages three, four and five introducing a white line pattern overlaid on the shape. During stages six and seven, new shapes and white line patterns are introduced. This is considered the intra-dimensional shift. During stages eight & nine, the white line pattern becomes the discriminating selection factor instead of the geometric shape. This is considered the extra-dimensional shift. The outcomes of interest are the number of errors during the pre-extra-dimensional shift trials and the number of errors in the trials after the extra-dimensional shift. Five children failed the IED task before stage four. An additional two children received zero errors after failing stage nine, after which there are no errors to accumulate. Data for these seven children were set to missing on the pre-extra-dimensional shift errors and the extra-dimensional shift errors.

The SSP is a spatial span task based on the Corsi Block Tapping test (Berch et al., 1998). SSP assesses short term visual memory for a sequence of spatial locations. During the task, ten, randomly placed, white squares are displayed on the computer screen. One by one, the white squares randomly change color and return to white. After the sequence is finished, the child is prompted to press the squares in the order indicated by the color change. The task begins with a sequence of two and ends with nine squares. Each sequence is repeated three times before increasing the number of squares by one, resulting in a potential for 24 sequences if the child successfully completes each trial. The outcome of interest is the longest sequence correctly recalled (maximum span length). In our study, 27 children (8%) did not succeed in correctly repeating the pattern during the two-square sequence after three attempts. These children were assigned a maximum span length of one.

2.4.3. Bender-gestalt test

The Bender-Gestalt Test (BGT) assesses visual-spatial processing and visual-motor coordination. During this task, children were shown a complex figure and asked to reproduce it on paper. Reproduction accuracy was scored using the Koppitz method (Koppitz, 1964). The BGT has been used in clinical settings to evaluate developmental delay in young children, learning disabilities, and traumatic brain injury (Memisevic and Djordjevic, 2018; Ozkan et al., 2018; Rezaie et al., 2014). Test retest reliability for the Koppitz scoring method of the BGT is 0.80 (Hustak et al., 1976).

2.4.4. Wechsler intelligence scale for children III (WISC-IV) - block design subtest

The WISC-IV block design test is a timed task designed to measure pattern recognition, problem solving and perceptual reasoning (Wechsler, 2003). During the task, children are provided with bi-colored or mono-colored blocks. They are shown a pattern and then given 90 s to reproduce the pattern with their blocks. Internal consistency for the perceptual reasoning index is estimated to be 0.92 (Kaufman et al., 2006).

2.5. Statistical methods

2.5.1. Analysis plan and missing data

A latent profile analysis (LPA) was performed on all children who had undergone neuropsychological testing (details below). Of the 357 children in the study, twelve did not complete any neuropsychological assessments, resulting in a final sample of 345. In total, 16 cognitive tests were administered. Of the children with incomplete testing, 32 (9%) were missing between one and four cognitive tests, and 17 (5%) were missing between six and 12 tests. Missing data among the cognitive variables were handled using robust maximum likelihood estimation in Mplus. RML is useful for producing non-biased estimates with both normal and non-normal indicators while simultaneously improving identification of model misfit (Maydeu-Olivares, 2017; Roesch, Villodas, & Villodas, 2010; Finney and DiStefano, 2006).

Latent profiles on the 345 children were compared on biomarkers and demographic characteristics using ANOVA and chi-squared tests. These initial, descriptive, tests of profile differences did not impute missing data. A multivariate ordinal regression was conducted to predict the association between blood Pb, hair Mn and cognitive profile membership, adjusting for relevant covariates. To utilize the full sample of 345 children, multiple imputation (MI) was performed in STATA. Children were missing an average of 22 (range 1–74) data points. MI models included demographic characteristics, blood Pb, hair Mn, as well as all cognitive indicators used in the LPA. Fifty imputation cycles by chained equations were used to account for missingness. Stata imputation commands specified for each variable depended on its distribution and included *regress*, *nbreg*, *poisson*, *pmm* and *truncreg*. The *truncreg* option was used to constrain estimated values to fall within the range of observed data. The *mi estimate* command was used to create model estimates (beta coefficients and 95% confidence intervals) using Rubin's rules (White et al., 2011; Royston, 2004). Imputed and non-imputed models were both tested for significant differences in model estimates. Overall, there was no difference in model interpretation. Therefore, only imputed results are presented for the ordinal regression analysis.

2.5.2. Latent profile analysis

LPA (Collins and Lanza, 2013) was used to identify subpopulations of children with differing profiles of cognitive performance. Latent profile analysis is similar to clustering techniques (e.g. K-Means clustering) in which similar groups are identified for a given set of exogenous indicators. LPA assumes that the true number of groups is unobserved (latent). Participants are assigned group membership based on their highest likelihood of profile membership. Starting with two, profiles were added to the model, and model fit was assessed. Model fit and final solution were evaluated using the Bayesian Information Criterion (BIC), Lo-Mendell Rubin Test (LMR), entropy, number of individuals in each profile, and plausibility.

LPA assumes that all indicators are normally distributed and are not highly correlated. Normality was assessed for all cognitive indicators using a skewness statistic; if it falls between -1 and 1, it indicates minimal data skewness (Bulmer, 1979; Groeneveld and Meeden, 1984; Hoyle, 1995). All indicators fell between -0.93 and 0.49 except for the block design subtest (skewness = 1.42) and the IED pre-extradimensional shift errors (skewness = 2.62). Both were log-transformed. The resulting skewness statistic was satisfactory (-0.16 & 0.70 respectively). Pearson correlations between cognitive indicators were checked and ranged from -0.25 to 0.49, indicating no high collinearity.

Statistical power of our LPA for 345 subjects and 16 indicators was adequate, as suggested by published simulations with similar numbers of participants and indicators. Tein et al. (2013) found that the statistical power of the BIC to detect the correct number of profiles was close to 1.0 for a sample of 250 with up to 15 indicators (similar to our analysis). At this sample size and number of indicators, the effect size difference between indicators was greater than 0.80 using Cohen's d statistic (Tein et al., 2013).

To aid interpretation of the latent profile means, all cognitive indicators were standardized to a mean of zero and a standard deviation of one. For each model, age of the child was added as a covariate of profile membership. Mplus7 (Muthén and Muthén, 2004) was used for LPA. Qualitative labels were given to each profile based on the relative means for each indicator variable within the profile.

2.5.3. Differences in profile demographics and biomarkers

After obtaining the latent profiles, univariate differences between profiles with respect to cognitive, demographic, and biomarker variables were assessed using univariate ANOVA and Chi-Squared, as appropriate. Subsequently, a multivariate ordinal regression analysis was used to quantify the association of blood Pb with the likelihood of belonging to a higher cognitive profile, after adjusting for confounders (Model 1). A directed acyclic graph was used to select confounders, with the following variables determined to be minimally sufficient for adjustment: the child's age in months, sex, hemoglobin concentration, hair manganese concentration, the household's HOME inventory score, crowding, possessions of wealth, and mother's education in years. Additionally, we adjusted for recruitment year to account for the secular trend of decreasing blood Pb over time, tester, and method of blood Pb measurement (graphite vs. flame AAS). We considered conducting analyses stratified by Pb method but decided against it because of the relatively small number of samples analyzed by flame AAS (n = 87, 28%), and additional tests (phi coefficient of 0.11) that indicated small effect of this variable in the model. Finally, sample clustering by school was accounted for using the STATA *cluster* command.

The proportional odds assumption was tested for the ordinal regression model in both imputed and non-imputed data. Quadratic associations of blood Pb and hair Mn with profile membership were tested in a fully adjusted model. If either quadratic term was significant, it was retained in Model 1. A linear interaction term between hair Mn and blood Pb was tested in Model 2. Lastly, we tested the interaction between quadratic terms for hair Mn and blood Pb. Interactions with a p-value < 0.1 were retained.

Interpretation of an ordinal regression is based on the change in the proportional odds of ordinal group membership for any given predictor. One unit change in a predictor indicates a proportional change in odds of group membership to a higher ordinal group relative to an equal or lower ordinal group.

Predicted probabilities were calculated to help interpret the probability of LPA profile membership across levels of blood Pb, and the interaction between blood Pb and hair Mn. When obtaining model estimated values, predicted probabilities can be generated for variable levels using STATA's *mimrgns* for imputed data (Klein, 2017). Predicted probabilities in an ordinal regression represent the likelihood of membership in a specified ordinal category. The predicted probability is presented with all predictors centered at the mean.

3. Results

The study sample consisted of 345 children from poor to lower middle class families. The children were aged 81 ± 5 months (6–8 years of age) and 55% were boys. Half (53%) of the children had one or
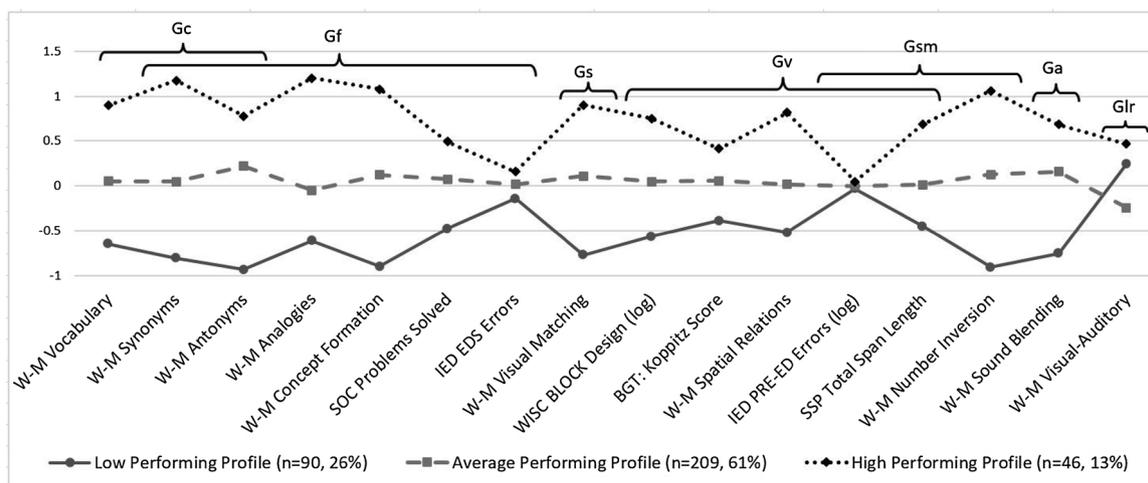
Fig. 1. Standardized Test Means by Cognitive Profile (n = 345).
Latent profiles are plotted with cognitive testing variables on the X-axis, and grouped according to CHC cognitive domains. All variables were standardized in Mplus to ease with interpretation across tests. Mean differences demonstrate a three tiered level of cognitive performance, generally with low, average and high performers.
Note: log – log-transformed variable; SSP – Spatial Span Task; IED – Intra-dimensional Extra-dimensional Shift Task; SOC – Stockings of Cambridge Task; W−M – Woodcock-Muñoz; BGT – Bender-Gestalt Test; Gsm – Short term memory; Gf – Fluid Intelligence; Gv – Visual spatial processing; Gc – Crystalized Intelligence; Glr – Long-Term Retrieval; Ga – Auditory Processing; Gs – Processing Speed.

both parents who smoked. The households averaged 1.9 ± 0.7 persons per room and 3.5 ± 1.7 possessions of wealth (i.e. TV, car, dish-washer). Mothers were 33.5 ± 6.4 years old, with 9.1 ± 2.7 years of education. Blood Pb was 4.1 ± 2.0 μg/dL, with approximately 35% of the sample having blood Pb above 5 μg/dL. Hair Mn median [5%, 95%] was 0.82 [0.27, 4.12] ppb.

3.1. Latent profile analysis

Three cognitive subgroups were identified using LPA. Information criterion (BIC) indicated model improvement from the 2-profile solution to the 3-profile solution, but the LMR test did not (p = 0.18). BIC and the LMR test both indicated no improvement in model fit for the 4-profile solution over the 3-profile solution (3-profile BIC: 14563.08 vs. 4-profile BIC: 14570.83; LMR p = 0.59). Because the BIC is understood to be the best criterion for determining the number of profiles (Nylund, Asparouhov, & Muthén, 2007), the 3-profile solution was retained. Entropy for the 3-profile solution was 0.80 indicating acceptable assignment of individuals into latent profiles.

Standardized means of each cognitive indicator by profile membership are plotted in Fig. 1. Three groups were identified, separated generally by high, average and low performance across all neuropsychological tests. The 3-profiles were classified as high (n = 46, 13%), average (n = 209, 61%) and low performing profiles (n = 90, 26%). The profiles differed on all cognitive variables except IED extra-dimensional shift errors and pre-extra-dimensional shift errors (Table 1).

3.2. Profile differences in demographic characteristics and biomarkers

Cognitive profiles were compared on socio-demographic variables and biomarkers (Table 2). These differences were assessed using complete data for each variable and multiple imputation was not performed at this point. In contrast with the low and average-performing profiles, children in the high profile had more possessions of wealth, higher HOME Index scores, and mothers with greater education levels. Compared to the low-performing profile, average-performing children also had higher HOME Index scores and mothers with greater education levels. Average performing and low performing children did not differ on the number of possessions. The three profiles did not differ on blood Pb. Compared to the high-performing profile, the low profile had

approximately 2% more children with blood Pb ≥ 5 μg/dL and the average profile approximately 4% more; these differences were not statistically significant.

3.3. Ordinal regression analysis

The association between blood Pb and profile membership was estimated using a multivariate ordinal regression model. Quadratic term for blood Pb was significant (p = 0.005), while the quadratic term for hair Mn was not (p = 0.61), indicating a non-linear association with blood Pb and profile membership (Table 3). In model 1, the linear Pb term indicated a 28% greater likelihood of membership in a poorer performance profile for each one μg/dL of blood Pb (p = 0.04). However, the quadratic term indicated that at higher blood Pb levels, the negative relationship between blood Pb and higher profile membership was attenuated (p = 0.03).

Because of the non-linear association, the predicted probabilities of membership in each cognitive profile across blood Pb levels are presented in Fig. 2. Predicted probabilities are presented at average levels of other model predictors. Predicted probability of membership in the highest profile was lowest between 0–5 μg/dL Pb, and did not change above 5 μg/dL Pb. Predicted probability of membership in the average performing class did not differ across levels of blood Pb. Lastly, the probability of membership in the lowest profile was higher between 0–5 μg/dL Pb, and did not change above 5 μg/dL Pb. For hair Mn, each unit the biomarker was associated with a 12% lower likelihood of membership in a poorer cognitive performance profile. Probability obtained from prediction models depends on the predictors used and the levels of the covariates, as well as the amount of variance explained by the model. Due to limited ability to obtain variance for models based on imputed data, McFadden's pseudo R^2 for the non-imputed model was obtained. Typically, levels of 0.2-0.4 indicate excellent fit; in this case, 0.15 suggested moderate fit (McFadden, 1974).

An interaction was estimated between the linear terms of blood Pb and hair Mn (Model 2) and was retained (p = 0.06). An interaction between quadratic terms for blood Pb and hair Mn was also tested, but was not significant (p = 0.66), and not retained in Model 2. Given a p-value of 0.06, we further explored to interaction between blood Pb and hair Mn by plotting predicted probabilities at high (5 μg/dL blood Pb and 0.82 ppb hair Mn) and low (1.8 μg/dL blood Pb and 0.01 ppb hair Mn) values of each biomarker (Fig. 3). We chose the median (0.82 ppb)

Table 1

Test Characteristics by Cognitive Profile among 6–8 Year-Old Children, using non-imputed data.

Cognitive Measures (Mean, Standard Deviation)	n	Low Performing	n	Average Performing	n	High Performing	F-Stat.	P-Value
SSP: Maximum Span Length	83	2.98 (0.95) [a]	204	3.53 (1.09) [b]	52	4.31 (1.12) [c]	25.01	< .0001
IED: Pre Extra-Dimensional Shift Errors	80	9.01 (6.25)	205	8.26 (4.23)	51	8.53 (5.97)	0.63	0.531
IED: Extra-dimensional Shift Errors	79	35.24(21.70)	204	30.13 (22.12)	51	27.69 (22.02)	2.20	0.112
SOC: Problems Solved in Min. Moves	82	4.05 (1.89) [a]	206	5.17 (1.69) [b]	51	5.94 (1.55) [c]	21.01	< .0001
W-M: VCS Vocabulary Total Correct	84	10.60 (1.95) [a]	195	12.02 (1.50) [b]	50	13.68 (1.32) [c]	59.24	< .0001
W-M: VCS Synonyms Total Correct	84	0.95 (1.14) [a]	195	2.57 (1.53) [b]	50	4.94 (1.30) [c]	126.06	< .0001
W-M: VCS Antonyms Total Correct	84	3.13 (2.29) [a]	195	6.13 (2.18) [b]	50	7.72 (1.48) [c]	88.13	< .0001
W-M: VCS Analogies Total Correct	84	2.34 (1.52) [a]	195	3.52 (1.61) [b]	50	6.18 (1.89) [c]	86.60	< .0001
W-M: Visual-Auditory Learning Total Errors	84	28.32 (11.52) [a]	195	33.48 (7.85) [b]	50	26.08 (9.33) [a]	18.01	< .0001
W-M: Spatial Relations Total Correct	84	41.43 (12.78) [a]	194	48.01 (10.16) [b]	49	57.71 (7.07) [c]	37.21	< .0001
W-M: Sound Blending Total Correct	84	11.42 (5.37) [a]	195	16.89 (4.89) [b]	50	19.92 (3.83) [c]	55.26	< .0001
W-M: Concept Formation Total Correct	84	4.68 (3.30) [a]	195	12.26 (5.54) [b]	50	19.16 (6.36) [c]	127.44	< .0001
W-M: Visual Matching Total Correct	83	12.86 (4.96) [a]	192	17.78 (4.80) [b]	48	22.21 (4.45) [c]	61.81	< .0001
W-M: Numbers Reversed Total Correct	83	3.33 (2.70) [a]	191	6.47 (2.00) [b]	49	9.10 (1.97) [c]	114.64	< .0001
BGT: Koppitz Score	81	95.42 (15.59)[a]	198	101.46 (11.37)[b]	51	106.12 (11.40)[c]	12.30	< .0001
WISC: Block Design Subtest Score	83	6.60 (2.12) [a]	205	8.56 (3.24) [b]	50	11.38 (4.29) [c]	35.15	< .0001

Post−LPA mean testing of cognitive variables using ANOVA. All means were significantly different except for the IED EDS Errors and IED PRE−ED Errors. Post−hoc t−test differences are indicated using the superscripts: a, b & c. Each indicate means that differ significantly from one another (p < 0.05).

Note: SSP – Spatial Span Task; IED – Intra-dimensional Extra-Dimensional Shift Task; SOC – Stockings of Cambridge Task; W−M – Woodcock-Muñoz; BGT – Bender-Gestalt Test; VCS – Verbal Comprehension Scale; Gsm – Short term memory; Gf – Fluid Intelligence; Gv – Visual spatial processing; Gc – Crystalized Intelligence; Glr – Long-Term Retrieval; Ga – Auditory Processing; Gs – Processing Speed.

as a conservative estimate for 'high' hair Mn; a choice of a higher hair Mn level would have given a limited sample size with which to predict a 3-level outcome. The cut-off at 5 µg/dL blood Pb reflects the current toxic dose set by the CDC (CDC, 2013). The predicted probabilities overlapped at lower and higher Mn levels, indicating lack of a meaningful interaction effect.

Other predictors of profile membership included HOME score and mother's education (Table 3). Each higher year of maternal education was associated with 18% lower likelihood of membership in a poorer cognitive performance profile. Each unit of the HOME score was associated with a 12% lower likelihood of membership in a poorer cognitive performance profile.

4. Discussion and conclusions

While levels of lead are decreasing globally, exposure to low levels of Pb is still a major concern, especially in low and middle-income countries (Kordas et al., 2018a). Children exposed to Pb have lower cognitive and academic achievement, but the effect of Pb on specific cognitive domains is unclear. Compounding the complexity of the Pb

effect, children are often co-exposed to other metals, like Mn. Our study elucidates three key findings: 1) a specific behavioral signature of low-level Pb or Mn exposure was not identified, 2) blood Pb was non-linearly associated with profile membership, such that lower levels of Pb predicted the greatest changes in the likelihood of profile membership, 3) hair Mn did not modify the effect of blood Pb.

Our inability to detect a behavioral signature of Pb is consistent with early reports of detrimental effects of Pb on global measures of IQ and cognition (Stiles and Bellinger, 1993). Numerous cross-sectional studies of low-level Pb exposure and IQ have demonstrated persistent detrimental effects (Canfield et al., 2003; Lanphear et al., 2000; Needleman and Gatsonis, 1990). Unlike other studies, we used 16 measures of cognitive domains and a latent profile analysis in an attempt to identify specific domains or abilities that might be affected by Pb. If a behavioral signature was ubiquitous among Pb exposed children, latent profile analysis should have detected this subpopulation. It is noteworthy that our analysis still separated children into subpopulations of general cognitive ability. Membership in these profiles was modestly associated with Pb exposure, particularly blood Pb below 5 µg/dL, providing additional support to a lack of threshold effect. On

Table 2

Demographic and Biomarker Characteristics by Cognitive Profile among 6–8 Year-Old Children, using non-imputed data.

Measures (Mean, Standard Deviation)	n	Low Performing	n	Average Performing	n	High Performing	F-Statistic / Chi-Square	P-Value
Sociodemographic factors								
% Male	86	57%	208	55%	51	49%	0.87	0.65
Age in Months	86	81.14 (7.02)	208	81.16 (6.42)	51	81.80 (5.44)	0.22	0.80
Child Height for Age Z-Score	83	0.55 (1.12)	192	0.42 (1.05)	44	0.59 (1.05)	0.68	0.51
Household Density Score	76	2.06 (0.75)	186	1.93 (0.75)	41	1.76 (0.61)	2.47	0.09
HOME Score	65	43.67 (3.25)[a]	164	44.94 (3.08)[b]	42	45.93 (3.52)[c]	6.86	0.001
Possessions of Wealth	78	3.28 (1.24) [a]	185	3.44 (1.17) [a]	43	3.98 (0.86) [b]	5.29	0.006
Mother's Education in Years	84	8.23 (2.39)[a]	197	8.94 (2.48)[a]	49	11.10 (3.07)[b]	20.39	< .0001
% Parents Smoking	76	53%	184	56%	43	40%	3.78	0.15
Biomarkers								
Hemoglobin g/dL	82	13.02 (1.04)	192	13.20 (1.04)	43	13.39 (1.23)	1.67	0.19
Blood Pb µg/dL	81	3.89 (1.75)	185	4.31 (2.20)	44	3.86 (1.93)	1.64	0.20
Hair Mn ppb	83	1.36 (1.41)	192	1.48 (2.42)	42	1.30 (1.35)	0.20	0.82
% Blood Pb ≥ 5µg/dL	81	23.46%	185	24.32%	44	20.45%	0.30	0.86

Post−LPA comparisons of cognitive profiles by socio−demographic measures and biomarkers. Differences were found for indicators of socioeconomic status only. Post−hoc t−test differences are indicated using the superscripts: a, b & c. Each indicate means that differ significantly from one another (p < 0.05).

Note: Pb – lead, Mn – manganese, g/dL = grams per deciliter, µg/dL = micrograms per deciliter, ppb = parts per billion.

Table 3

Ordinal Regression Predicting Likelihood of Poorer Performing Cognitive Profiles among 6–8 Year-Old Children (n = 345).

Variables	Model 1 OR (95% CI)	Model 2 OR (95% CI)
Tester		
Tester 1 vs Tester 3	0.75 (0.38, 1.51)	0.74 (0.39, 1.40)
Tester 2 vs Tester 3	0.71 (0.40, 1.27)	0.73 (0.40, 1.33)
Year of Enrollment	1.57 (1.26, 1.95)***	1.57 (1.26, 1.97)***
Pb Method	1.80 (1.10, 1.97)*	1.96 (1.11, 3.46)*
Age in Months	1.00 (0.97, 1.02)	1.00 (0.97, 1.02)
Gender (1 = Male)	1.16 (0.83, 1.61)	1.19 (0.84, 1.67)
HOME Score	0.88 (0.82, 0.95)**	0.88 (0.82, 0.95)**
Household Density Score	1.13 (0.90, 1.43)	1.15 (0.92, 1.44)
Possessions of Wealth	0.91 (0.77, 1.07)	0.91 (0.77, 1.07)
Mother's Education in Years	0.82 (0.77, 0.88)***	0.82 (0.77, 0.87)***
Hemoglobin (g/dL)	0.88 (0.76, 1.02)#	0.88 (0.76, 1.01)#
Blood Pb (µg/dL)	1.28 (1.01, 1.61)*	1.38 (1.08, 1.76)**
Blood Pb^2 (µg/dL)	0.98 (0.97, 1.00)*	0.98 (0.97, 1.00)*
Hair Mn (ppb)	0.88 (0.82, 0.96)**	1.19 (0.88, 1.61)
Blood Pb by Hair Mn	–	0.94 (0.88, 1.00)#

Ordinal regression models predicting likelihood of membership in a poorer cognitive performance profile. Important predictors included blood Pb, hair Mn, HOME score and mother's education in years. The quadratic term for blood Pb was also significant. A marginal interaction was found between linear blood Pb and hair Mn. Multiple imputation performed to account for missing data.

Note: # p < .10, * p < .05, **p < .01, **p < .001.

Pb = lead, Mn = manganese, OR = odds ratio, g/dL = grams per deciliter, µg/dL = micrograms per deciliter, ppb = parts per billion.

the other hand, some studies demonstrated a behavioral signature of Pb, pointing to unique deficits in fine motor, social behavior, attention, and executive functioning (Chiodo et al., 2004; Wasserman et al., 1997). Given the declines in blood Pb globally, it would be useful to understand which cognitive domains are particularly affected by low-level Pb exposure to provide more targeted intervention to children starting school or even earlier. Our results indicate that a global approach to improve a broad range of cognitive outcomes may be most appropriate.

The non-linear association between Pb exposure and cognition supports previous findings that there is no safe level of lead. In our study, the likelihood of profile membership changed the most at lower blood Pb levels. For example, the predicted probability of membership in the low performing group changed by 6% between 1 and 4 µg/dL of blood Pb, whereas between 4 and 8 µg/dL the probability changed by 2%. In a study of three and five-year-old children from Rochester NY, the loss of IQ points was most pronounced (7.4 points) at blood Pb below 10 µg/dL, compared to 2.5 points between 10–30 µg/dL (Canfield et al., 2003). The highest level of blood Pb in the Rochester sample was assessed at two years of age. At 9.7 µg/dL, that level was much higher than the mean Pb in our sample (4.1 µg/dL). Similarly, in a pooled analysis of international cohorts, an increase in blood Pb from 2.4 to 10.0 µg/dL was associated with a 3.9-point loss in IQ, compared to a 1.9-point loss between 10 and 20 µg/dL of Pb (Lanphear et al., 2005). In a sample where 65% of children had Pb ≤ 5 µg/dL, the effect size we observed was comparatively small, but nonetheless consistent with previous reports.

We tested, but found little evidence for an effect modification between hair Mn and blood Pb on cognitive performance. Other studies have reported mixed findings, potentially due to differences in biomarkers used to measure Mn. A gold standard biomarker for Mn exposure has not been determined, but the choice of Mn biomarker may affect the ability to detect interactive effects. For example, a study of 7–12 year olds from Brazil found no interaction between hair Mn (median 0.74 ppb) and blood Pb (median 1.2 µg/dL) on children's IQ. Analyses with toenail Mn, however, did reveal a stronger detrimental effect of blood Pb on IQ levels with higher Mn exposure (Menezes-Filho et al., 2018). Similarly, in a sample of adolescents and adults from

Brazil, a measure of cognitive flexibility (not IQ) was associated with nail but not hair Mn (Viana et al., 2014). Further research is needed to understand how differences in assessment of Mn exposure may affect inferences about neurotoxicity.

Another consideration is that manganese is an essential micronutrient which is important for child growth (Rivera et al., 2003). Therefore, the association between low levels of Mn in the body and cognitive performance may be quite different from associations with high levels of Mn. In fact, our study revealed a positive association between hair Mn and cognitive profile membership. Other studies have also shown differential effects at low vs. high levels of Mn. For example, among newborns from Oklahoma, Mn in maternal blood had a non-linear relationship with birthweight (Zota et al., 2009). In a study of 12–24 month old Mexican children, measures of infant development were highest at average levels of blood Mn (˜2.4 µg/dL), while lower scores were noted at both the lowest and highest quintiles of blood Mn (Claus Henn et al., 2011). Standard reference levels for hair manganese have not been determined (Harkins and Susten, 2003). However, reference intervals for hair Mn has been suggested between 0.15–1.2 µg/g for adults in Rio de Janeiro (Miekeley et al., 1998). For reference, children between 6–12 years living near a ferro-manganese alloy plant had average hair Mn levels of 5.83 µg/g (Menezes-Filho et al., 2011). Canadian children and adolescents (6–15 years) living near public water with high levels of manganese had average hair Mn levels of 6.2 µg/g (Bouchard et al., 2006). It is possible, therefore, that at median 0.82 ppb, our hair Mn levels may not be reflective of neurotoxicity.

In addition to blood Pb, we identified mother's education and HOME Inventory as salient predictors of cognitive performance. These predictors were associated with lower odds of membership in a poorer performance profile (18% and 12%, respectively). Our sample was drawn primarily from households of average to low SES. Possessions of luxury items (i.e. television, car, dishwasher, cellphone) were relatively uncommon, with nearly 45% possessing 3 or less of these items. Furthermore, approximately 19% of mothers only had elementary school education.

The relationship between SES, achievement (Lanphear et al., 2000; Sirin, 2005; White, 1980) and cognitive performance (Blums et al., 2017; Duncan and Magnuson, 2012; Hackman and Farah, 2009; Hackman et al., 2010; Sarsour et al., 2011) is well documented. Specific domains that are negatively affected by low SES include executive functioning (Lawson et al., 2018; Noble et al., 2005), language and vocabulary (Fernald et al., 2013), and crystalized intelligence (Schmidt and Crano, 1974). However, the relationship between SES and cognition is complicated by the fact that individuals of lower SES have disproportionate Pb exposure. For example, in a study of children (1–16 years) from Detroit, neighborhoods of low SES and high racial segregation had disproportionately higher blood Pb (Moody et al., 2015, 2016). Similarly, in Latin America, a disproportionate number of children of low SES are more likely to be exposed to environmental Pb. In Venezuela, ˜6 year old children with blood Pb above 10 µg/dL were 7.3 times more likely to live in a geographic region of low SES (Rojas et al., 2003). In Montevideo, we had fairly low-level Pb exposure and low variability in the SES measures, and likely for this reason, did not see differences in the effects of Pb on cognitive performance by SES status (data not shown).

The interrelationship between SES, Pb exposure and cognition can be difficult to disentangle. Bellinger highlights the challenge of assessing the impact of Pb on cognition in the context of SES, which has been conceptualized as a confounder for Pb exposure, an effect modifier, and a mediator along the causal pathway (Bellinger, 2008a). All of these effects have been reflected in the literature. For example, among Mexican first-graders, the negative effect of Pb on cognition was stronger among students with fewer resources, mothers with less than a high-school degree and lower parental school involvement (Kordas et al., 2006). However, utilizing the same cross-sectional dataset, a structural equation modeling approach found that SES did not modify
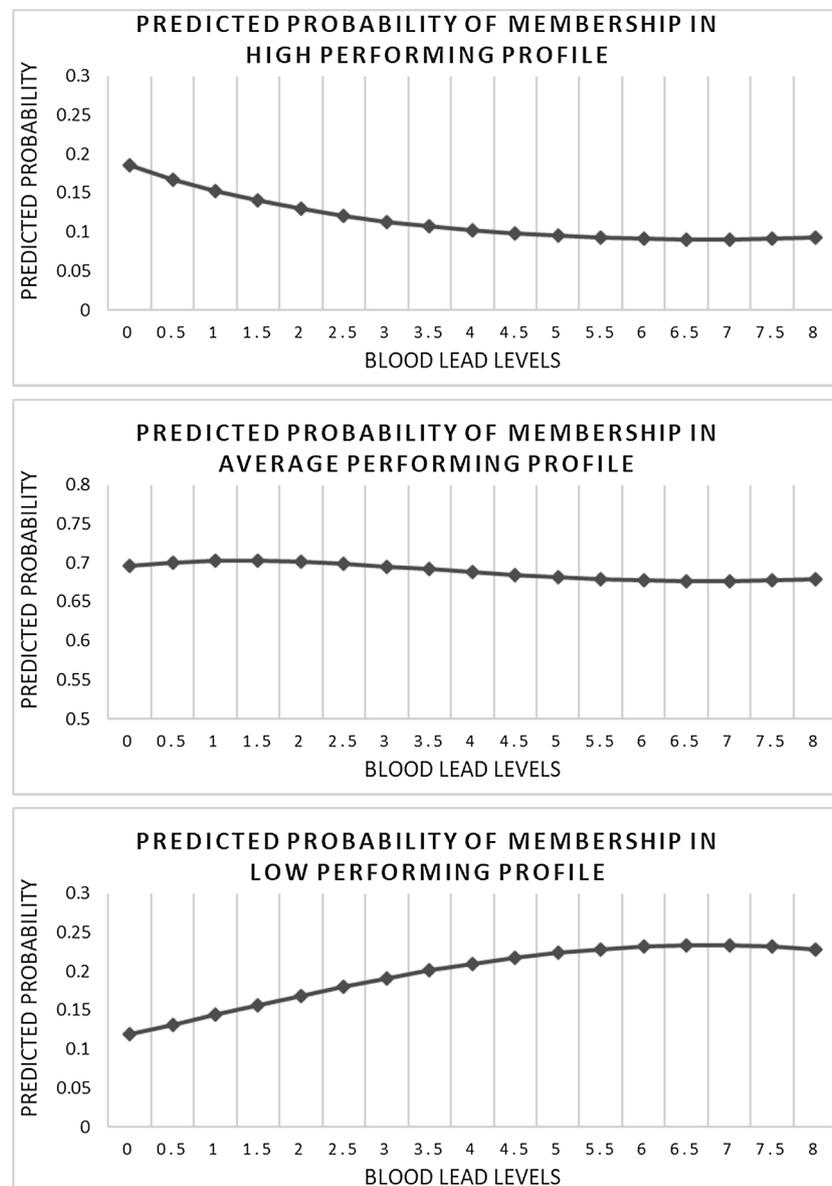
PREDICTED PROBABILITY OF MEMBERSHIP IN HIGH PERFORMING PROFILE

PREDICTED PROBABILITY OF MEMBERSHIP IN AVERAGE PERFORMING PROFILE

PREDICTED PROBABILITY OF MEMBERSHIP IN LOW PERFORMING PROFILE

Fig. 2. Model Predicted Probabilities of Membership in Each Cognitive Profile among 6–8 Year-Old Children.

the effect of lead on cognitive outcomes. Instead, SES mediated the relationship between lead and cognition, in that lead was negatively related with the home environment, which in turn was positively related to cognition (Moodie et al., 2013).

Our study should be interpreted in light of some limitations. LPA is useful when attempting data reduction. Without this data reduction step, we would have tested the relationship between Pb exposure and 16 different cognitive tests. Increasing the number of statistical tests can inflate Type I error; this is a common critique for Pb-cognition studies (Kaufman, 2001). One limitation with our use of LPA is that while we achieved an acceptable entropy (0.80) (Celeux and Soromenho, 1996), levels above 0.90 would have been optimal. Furthermore, it is possible that we left out a relevant cognitive domain, which if included, might have allowed us to identify a behavioral signature of Pb or Mn. A large number of our tests were related to fluid intelligence (Gf) or visual spatial processing (Gv) (11 out of 16 measures). On the other hand, some domains, like processing speed (Gs), were only represented by one measure. Lastly, because this is a cross-sectional study, we did not track changes in cognitive development over time. It is possible that trajectories of cognitive development more closely reflect a behavioral signature of Pb or Mn. While a single

'snapshot' of cognitive performance may not differentiate between exposed and non-exposed groups, it is possible that children with low Pb or Mn exposure develop abilities at slower rates than non-exposed children. Prior history of Pb or Mn exposure (i.e. prenatal exposure) is unknown for our sample, but may be critical if occurring during vulnerable windows of development (Braun et al., 2012; Selevan et al., 2000). Lastly, as this was a cross-sectional study, our study is primarily descriptive in nature and is limited in ability to infer causal associations. Thus, we are unable to quantify risk of poor cognitive performance due to multiple metal exposure.

Our study did have a number of strengths. Our sample reflects the low range of Pb exposure reported globally. Our study considered the effects of more than one metal: we elucidated the conjoint relationship between low-level hair Mn and blood Pb on cognitive performance. In another report, we showed that low-level arsenic exposure was not associated with general cognitive abilities in this sample of children (Desai et al., 2018). Our participants underwent an extensive battery of cognitive tests allowing us to examine the effect of Pb and Mn across multiple cognitive domains. This was the first study to utilize a latent subgroup selection technique to identify profiles of cognitive performance among children exposed to metals. Finally, school-entry is an
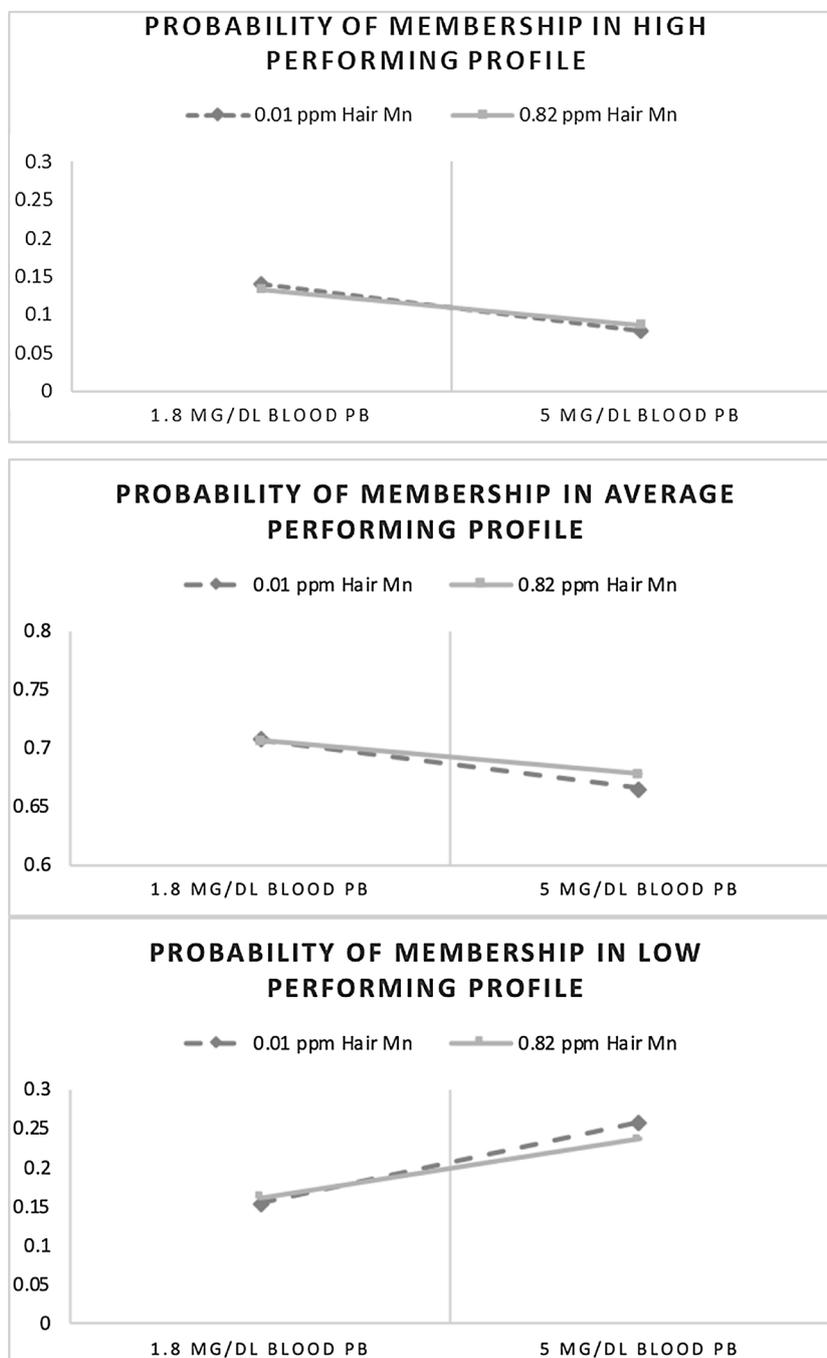
Fig. 3. Model Predicted Probabilities of Membership in Each Cognitive Profile by High and Low Levels of Blood Pb and Hair Mn.

important period to study the effects of metal exposures on cognitive development. Children's cognitive and behavioral readiness at school entry strongly predicts later academic achievement (Duncan et al., 2007; Pagani et al., 2010). Randomized controlled trials demonstrate that early childhood educational and cognitive interventions reduce juvenile arrests and school dropout while increasing educational attainment (Reynolds et al., 2001). This sensitive window of development should be protected against metal exposures.

To conclude, using an extensive battery of cognitive tests, we were unable to detect a behavioral signature of Pb or Mn, but identified high, average and low levels of general cognitive ability. The likelihood of profile membership was negatively associated with blood Pb, particularly below 5 μg/dL. SES indicators such as maternal education and home environment also predicted cognitive performance. Eliminating

exposure to Pb and improving developmental resources in children's homes are important pathways to overcoming cognitive performance gaps in school-age children.

Conflict of interest

No competing interests to declare.

Acknowledgments

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