Latent childhood exposure to mixtures of metals and neurodevelopmental outcomes in 4–5-year-old children living in Spain.

Notario-Barandiaran, L[1], Díaz-Coto, S.[1], Jimenez-Redondo, N.[2], Guxens, M.[3,4,5,6], Vrijheid, M.[4], Andiarena, A.[7,8], Irizar, A.[7], Riaño-Galan, I.[3,9,10,11], Fernández-Somoano, A.[3,10,11], Llop, S.[3,12], Lozano, M.[12,13], Karagas, M.R.[1], Meharg, A.[14], Carey, M.[14], Meharg, C.[14], Ralphs, K.[14], McCreanor, C.[14], Vioque, J.[3,15,16], Martinez-Camblor, P.[17,18], Signes-Pastor, A. J.[3,15,16,(†)]

[1]Department of Epidemiology, Geisel School of Medicine, Dartmouth College, NH, USA. [2]Universidad Miguel Hernández de Elche, Centro de Investigación e Innovación Agroalimentario y Agroambiental (CIAGRO-UMH, Desamparados-Orihuela, Spain. [3]CIBER de Epidemiología y Salud Pública (CIBERESP), Instituto de Salud Carlos III (ISCIII), Madrid, Spain. [4]ISGlobal, Barcelona, Spain. [5]Universitat Pompeu Fabra, Spain. [6]Department of Child and Adolescence Psychiatry/Psychology, Erasmus University Medical Center, Rotterdam, The Netherlands. [7]Health Research Institute, Biodonostia, Donostia-San Sebastian, Spain. [8]Faculty of Psychology, University of the Basque Country, UPV/EHU, Donostia/San Sebastian, Spain. [9]Servicio de Pediatría. Endocrinología pediátrica. HUCA. Oviedo. Asturias, Spain. [10]Instituto de Investigación Sanitaria del Principado de Asturias (ISPA), Oviedo, Asturias, Spain. [11]IUOPA – Department of Medicine, University of Oviedo, Oviedo, Asturias, Spain. [12]Epidemiology and Environmental Health Joint Research Unit, FISABIO–Universitat Jaume I-Universitat de València. Valencia, Spain. [13]Preventive Medicine and Public Health, Food Sciences, Toxicology and Forensic Medicine Department, Universitat de València, Valencia, Spain. [14]Biological Sciences, Institute for Global Food Security, Queen's University Belfast, 19 Chlorine Gardens, Belfast BT9 5DL, Northern Ireland, UK. [15]Unidad de Epidemiología de la Nutrición. Universidad Miguel Hernández, Alicante, Spain. [16]Instituto de Investigación Sanitaria y Biomédica de Alicante (ISABIAL), Spain. [17]Biomedical Data Science Department, Geisel School of Medicine, Dartmouth College, Lebanon, NH, USA. [18]Faculty of Health Sciences, Universidad Autonoma de Chile, Chile.

[(†)]**Corresponding author**: Antonio j. Signes-Pastor (asignes@umh.es)

Abstract

Neurodevelopmental disorders are increasing globally, and metal exposure may play a significant role as an environmental factor. This cross-sectional study aimed to identify metal mixture patterns and assess their impact on children's neurodevelopment. Data from 962 children (aged 4-5) participating in the Spanish INMA cohort study were analysed. Urinary metal concentrations (cobalt (Co), copper (Cu), molybdenum (Mo), selenium (Se), lead (Pb), zinc (Zn), and arsenic speciation) were used as exposure biomarkers. Principal component analysis (PCA) revealed four latent exposure variables representing uncorrelated metal mixture patterns. Linear regression analyses examined the associations between these variables and children's neuropsychological functions assessed through the McCarthy Scales of Children's Abilities. The first latent exposure variable (Cu, Se, Pb, Zn) and the second (inorganic arsenic, monomethylarsonic acid) showed negative associations with verbal executive function (ß = -1.88, 95% confidence interval (CI) = -3.17 to -0.59) and gross motor function (ß = -1.41, 95% CI = -2.36 to -0.46), respectively. Conversely, the third variable (Mo, Co) and the fourth (arsenobetaine) exhibited positive associations with visual and verbal span functions (ß = 1.14, 95% CI = 0.16 to 2.12) and fine motor function (ß = 1.01, 95% CI = 0.11 to 1.92), respectively. This study suggests that even relatively low levels of metal latent exposures, notably inorganic arsenic and a mixture of metals including Pb, adversely affect children's neuropsychological development function scores, while exposure to AsB and a mixture of Co and Mo has a positive impact.

Keywords: mixture; heavy metals; children's environmental health; neurodevelopmental outcomes; biomarkers of exposure; neuropsychological functions.

1. Introduction

The prevalence of neurodevelopmental disorders appears to be increasing worldwide. While genetic factors play an important role in the development of these disorders, they likely account for only around 35% of all cases (Grandjean and Landrigan, 2014). Childhood represents a critical period for the development and maturation of neurodevelopmental domains, such as psychomotor, cognitive, and socio-emotional skills (NRCM, 2000). Consequently, concerns have been raised regarding childhood exposure to environmental pollutants and its potential impact on cognitive neurodevelopment (Au, 2002; Davis et al., 2019; Landrigan and Goldman, 2017; Vermeir et al., 2005). Myelination and synaptogenesis are processes that occur during childhood and adolescence and are crucial for proper cognitive development (Deoni et al., 2018; Rice and Barone, 2000). Environmental exposures during this stage of life can disrupt the rapid ontogenetic events in cognitive development and have an adverse impact on learning and memory skills, as well as the development of executive functions. These disruptions can contribute to neurodevelopmental disorders such as autism and attention-deficit hyperactivity disorder (ADHD) (Grantham-McGregor et al., 2007).

Recent studies suggest that children's cognitive neurodevelopment can be adversely affected by exposure to certain levels of metals and metalloids (hereafter, referred to as "metals") present in air, soil, water, and food (Dack et al., 2022; Liu et al., 2019; Schildroth et al., 2022). Children are particularly vulnerable to metal exposure due to their increased rates of inhalation, ingestion, and hand-to-mouth behaviours, as well as their immature detoxification mechanisms, which may also vary based on sex (Au, 2002; Davis et al., 2019; Landrigan et al., 2004; Rodríguez-Barranco et al., 2013). A systematic review found that lead (Pb) and manganese (Mn) exposure had the strongest negative effects on children's neurodevelopment among all metals studied in low and middle-income countries (Heng et al., 2022). In a separate study conducted in China, positive associations were found between neurological development and tin (Sn), selenium (Se), and iron (Fe), while negative associations were observed for cadmium (Cd), nickel (Ni), and cobalt (Co) in a group of 703 children aged 2-3 years. The study used urine metal concentrations as a biomarker of metal exposure (C. Liu et al., 2022). In epidemiology studies, urine metal concentrations are widely used to assess metal exposures due to its ease of collection (Heng et al., 2022).

Ensuring a balanced intake of elements with biological functions while minimizing exposure to toxic elements is essential for healthy growth and development. Indeed, an increasing body of evidence highlights the potential adverse health effects of even relatively low toxic levels of

chronic metal exposures (Freire et al., 2018; García-Villarino et al., 2022; Signes-Pastor et al., 2019). Furthermore, it's important to highlight that children in real-life scenarios are simultaneously exposed to various metals through ingestion, inhalation, and dermal contact. The potential additive, synergistic, or antagonistic effects of these metal exposures, influenced by mechanisms involving absorption and distribution in the body and elimination, are not yet fully elucidated (Au, 2002; Claus Henn et al., 2014). This uncertainty is further compromised by the lack of standardized methodologies to directly measure environmental the mixture exposure patterns or latent variables with potential health effects (Henn et al., 2014). Therefore, further research is needed to clarify the complex association between different metals at different levels and their combined effects on children's neurodevelopment. This understanding is needed to support and implement effective protective measures that promote children's health. Recently, several valuable statistical procedures for studying the combined effect of environmental exposures have been satisfactorily implemented. Among them, perhaps highlight the Bayesian Kernel Machine Regression (BKMR) (Bobb et al., 2015) or the Quantile g-computation approach (Keil et al., 2014). These are regression models focused on determining the combine effect of a set of exposures on a particular outcome. However, the metal exposures frequently have common sources. For instance, food intake does not provide isolate exposures to individual metal but mixtures of them. Therefore, it is difficult to intervene on the exposure of a single metal in an individual way. Therefore, here we identify latent metal exposures (not related with particular outcomes), and its effects on children's neuropsychological development.

In short, in this study, our aim was to identify latent metal exposures and investigate their association with child neuropsychological development. To accomplish this, we conducted a cross-sectional investigation to explore the relationship between metal mixture exposure patterns or latent exposure variables, identified through Principal Component Analysis (PCA) using urinary metal concentrations (Co, copper (Cu), molybdenum (Mo), Se, Pb, zinc (Zn), and arsenic speciation), and childhood neuropsychological functions among children aged 4-5 years living in Spain.

2. Methods

2.1. Study population

The present study is based on a population from INMA (*INfancia y Medio Ambiente*, meaning Environment and Childhood), which is a multicentre and population-based birth cohort study located in several areas in Spain (www.proyectoinma.org). In this study we included data from the following INMA sub-cohorts: Asturias, Gipuzkoa, Sabadell and Valencia. Between 2003 and 2008, 2,644 singleton pregnant women were included in the study during their first trimester of pregnancy, of which 2,506 delivered a live infant (Guxens et al., 2012). At 4-5 years follow-up assessment, a total of 2,139 children completed the paediatric interview. Of these children, 1,006 and 1,879 had available data on urine metal concentrations and neuropsychological development assessment, respectively, with a total sample of 978 children with data for both variables. After excluding 16 participants with no information on the covariates of interest, we had a final sample of 962 children (**Figure S1**). All the participant parents provided informed consent, and the ethical committees of the centres involved in the study approved the protocol, which includes the Hospital La Fe, Valencia; Hospital Parc Taulí, Sabadell; San Agustín University Hospital, Avilés, Asturias; and Zumarraga Hospital, Gipuzkoa.

2.2. Laboratory analysis

Spot urine samples were collected during the 4-5 years follow-up paediatric interview and stored at or below -20°C until analysis in 100 mL polyethylene containers. Inductively coupled plasma mass spectrometry (ICP-MS) in direct solution acquisition mode using a Cetax ASX-520 Auto Sampler was used to analyse urinary metal concentrations of Co, Cu, Se, Mo, Pb, and Zn. The mean limit of detection (LOD) across batches for each metal was 0.10 µg/L for Co, 1.46 µg/L for Cu, 1.24 µg/L for Se, 14.36 µg/L for Mo, 0.19 µg/L for Pb, and 4.71 µg/L for Zn. No values below the LOD were observed for urinary Se, Mo, and Zn concentrations. However, a percentage of values below LOD were observed for Co (2%), Cu (17%), and Pb (38%) concentrations. Values below the LOD were imputed by the LOD divided by the square root of two and included in the statistical analyses. Each analytic batch included blank and replicate samples of Clinchek® lyophilized urine samples for quality control. The average recovery based on 18 Clinchek urine samples was 88.9% (Co), 84.2% (Cu), 75.0% (Se), 114.0% (Mo), 78.5% (Pb), and 84.2% (Zn).

We used a Thermo Scientific IC5000 ion chromatography system, with a Thermo AS7, 2 x 250 mm column and a Thermo AG7, 2 x 50 mm guard column interfaced with a Thermo ICAP Q ICP-MS to analyse arsenic speciation including arsenobetaine (AsB), dimethylarsinic acid (DMA), monomethylarsonic acid (MMA) and inorganic arsenic (iAs), which comprises the sum of arsenite and arsenate. To ensure quality control, we included blank and replicate samples of

the National Institute of Standards and Technology (NIST) human urine standard reference material 2669 – level I or ClinChek® Control level I in each analytical batch. The average recovery, based on 28 SRM 2669 and 33 ClinChek® Control level I samples, was 96.8% including AsB, DMA, MMA, and iAs. The mean limit of detection (LOD) was determined using DMA and the mean value across batches was 0.008 µg/L. A percentage of values below LOD were observed for AsB (4%), DMA (12%), MMA (27%) and iAs (9%) concentrations. Values below the LOD were imputed by the LOD divided by the square root of two.

The urine metals included in the study were selected to maximize the sample size, excluding metals with large number of values <LOD that lacked evidence of neurotoxicity or certified reference values for analysis quality control.

All urine metal concentrations were adjusted for urine dilution using $E_{sg} = E_0 \; x \; \frac{SG_{median}-1}{SG_0-1}$ where E_{sg} is the specific gravity-standardized exposure biomarker concentration, E_0 is the observed exposure biomarker concentration, SG_{median} is the median of specific gravity value in the study sample, and SG_0 is the observed specific gravity value (Kuiper et al., 2022).

2.3. Neuropsychological functions assessment

The neuropsychological function of the children was evaluated by trained psychologist using a standardized version of the McCarthy Scales of Children's Abilities (MSCA) adapted to the Spanish population (McCarthy, 2009). MSCA tests were performed at a median age of 4.4 ranging from 4.0 to 5.3 years for Asturias, Gipuzkoa and Sabadell children, while for Valencia the tests were administered later, with a median age of 5.7 ranging from 5.6 to 6.4 years. The MSCA was selected based on its established reliability, validity, and extensive use in environmental health and neuropsychological development research, including previous investigations conducted by INMA (Andiarena et al., 2017; Forns et al., 2012; Nagle, 1979; Signes-Pastor et al., 2019). The original MSCA is a psychological instrument for young children that measure cognitive ability in six domains areas: general cognition, global verbal, global perceptive-performance, global quantitative, general cognitive, global memory, and global motor function. Each domain scale contains a different number of individual subtests with a total of 18 (McCarthy, 2009). For this study, we additionally used new scales created by the reorganization of these 18 subtests according to tasks highly correlated with specific neurocognitive function (Baron, 2004; Lezak et al., 2004). These new subarea scores were

executive functions, visual and verbal span, working memory, verbal memory, gross and fine motor functions, and cognitive functions of the posterior cortex (Julvez et al., 2011).

2.4. Covariates

During the first trimester of pregnancy, a structured questionnaire was used to collect information about socio-demographic and socioeconomic characteristics of the parents. Based on prior studies, we selected as potential confounders the following maternal covariates: maternal education (primary, secondary, or university), maternal number of previous live births (0, 1 or >1) and maternal social class (I-II (highest), III, or IV-V (lowest)) (OLI, 2012). The first category (I-II) included managers or professionals, the second category (III) included technicians and associate professionals, clerical support workers, skilled agricultural, forestry and fishery workers and, the third category (IV-V) included craft and related trades workers, plant and machine operators and assemblers. For children, we included the following covariates: age at the MSCA test (years), sex (male or female), body mass index (BMI) (kg/m^2). Trained personnel followed standard protocols to measure children's weight (kg) and height (m) while urine samples were collected (Viet and Verschuren, 2008).

2.5. Statistical analysis

Continuous variables including metal concentrations were described by their median and interquartile range (IQR) values. Categorical variables were described through their absolute and relative frequencies. We used these descriptive statistics to summarize the characteristics of the participants and their urine metal concentrations. Before performing any further analysis, we log-transformed the urine metal concentrations to address the skewness distributions.

Urinary iAs, MMA, and DMA are the major metabolites that result from exposure to iAs. However, it should be noted that DMA can be susceptible to exposure misclassification because it can arise from direct exposure or the metabolism of complex organoarsenical compounds (Aylward et al., 2014; Hata et al., 2012). To ensure a cautious and accurate analysis, we adopted a conservative approach by excluding DMA from the primary statistical analysis.

We calculated Pearson's correlation matrix to examine the associations among urinary metal concentrations. We determine the most relevant patterns of metal mixture exposure or latent variables by conducting PCA on the log-transformed concentrations of urinary metals (Co, Cu,

Mo, Se, Pb, Zn, AsB, MMA, and iAs), using their correlation matrix (equivalent to perform the PCA on the typified variables). PCA is an exploratory method for reducing data dimensionality, it is applied to evaluate the association between indirect measurements of metal mixture exposure patterns or latent variables in childhood neurodevelopment. This expands its conventional use by providing insights into the relationships between these variables (Chu et al., 2018; Pozo et al., 2012). PCA projects each data point on a smaller number of variables, preserving as much as possible the data variability. PCA helps to understand the internal relationship among the original variables and, in this context, to determine latent variables of exposure. The transforming and loading matrix used to generate the latent variables of exposure through PCA can be found in the Supplemental Information.

We found no graphical evidence of non-linear association between each exposure latent variables and children's MSCA scores in local regression (loess) analysis. Thus, we performed unadjusted and adjusted multiple linear regression analyses, using the MSCA scores standardized to a mean of 100 points and a standard deviation of 15 (Signes-Pastor et al., 2019; Soler-Blasco et al., 2021). The adjusted models included the covariates, chosen based on prior studies (Rodríguez-Barranco et al., 2013; Signes-Pastor et al., 2019): cohort (Asturias, Gipuzkoa, Sabadell, or Valencia), children's BMI (kg/m^2, continuous), maternal social class (I-II , III, or IV-V, categorical), maternal level of education (primary, secondary, or university, categorical), maternal number of previous live births (0, 1 or >1, categorical), age at the MSCA test (years, continuous), and sex (male or female, categorical).

We conducted sensitivity analyses, excluding the Valencia cohort, which had an average age slightly different from the rest of the cohorts, and including sex-stratified linear regression and regression analysis to assess the association between urinary Pb concentrations in the first PC with the MSCA scores to facilitate the interpretation as it gathers essential (Cu, Se, Zn) and a non-essential toxic (Pb) element. The latter analyses were adjusted for the selected confounding factors and adding the remaining metals of the first PC.

We used a *p*-value <0.05 to define associations as statistically significant. All statistical analyses and graphics were performed using R version 4.1.2 (R Core Team, 2021).

3. Results

A total of 460 participants out of 962 (47.8%) were females. The sub-cohort distribution across the different regions of Asturias, Gipuzkoa, Sabadell, and Valencia was as follows: Asturias (221; 23.0%), Gipuzkoa (273; 28.4%), Sabadell (374; 38.9%), and Valencia (94; 9.7%). Children urine concentrations (median) include AsB (12.3 µg/L), iAs (1.1 µg/L), MMA (0.4 µg/L), DMA (3.2 µg/L), Co (0.8 µg/L), Pb (0.5 µg/L), Se (24.9 µg/L), Zn (379.0 µg/L), Mo (83.7 µg/L), and Cu (8.9 µg/L). The MSCA was performed at the median age of 4.5 years ranging from 4.0 to 6.4 years. More information on the selected characteristics of the entire study population and stratified by sex is provided in **Table 1**. No major differences in the sociodemographic variables of our study population were observed when compared to the excluded participants (**Table S1**).

The urinary metal concentrations were correlated, including Pb and Cu, Se and Zn, and MMA and iAs, with Pearson's coefficients of 0.52, 0.56, and 0.51, respectively. More modest correlations were 0.44 between Pb and Se, 0.34 between Cu and Zn, and 0.31 between Cu and Se (**Figure 1**).

Figure 2, **Table S2**, and **Table S3** provide the resulting principal components (PCs), along with the specific weights assigned to the original (log-transformed) metals. To achieve a cumulative explained variance of approximately 70%, we identified four principal components or latent variables (the final cumulative explained variance reached 68.6%). The first principal component (PC1) had the highest loading weights, indicating the most relevant contribution to this component, for Pb (0.83), Cu (0.72), Se (0.72), and Zn (0.61). That is, higher values of this component are associated with higher values of those metals. The second principal component PC2 showed the highest loading weights for MMA (0.86) and iAs (0.87), while for the third principal component (PC3) were for Co (0.64) and Mo (0.86). In the fourth principal component (PC4), AsB (0.92) had the highest loading weight. In terms of interpreting the findings based on the transformation matrix (**Table S2**), an increase of one point in PC1 can be achieved through various methods. For example, one approach is to multiply Se levels by 4 and double Pb levels while keeping the other variables constant. Another approach is to double Se and Pb levels and multiply Zn levels by 4 while keeping the rest of the variables constant.

The multiple linear regression indicated several statistically significant associations between the PCs and the MSCA scores. Specifically, PC1 had inverse associations with global verbal (ß = -1.44, 95% CI = -2.76 to -0.11), executive (ß = -1.46, 95% confidence interval (CI) = -2.72 to -0.20), verbal executive (ß = -1.88, 95% CI = -3.17 to -0.59), and working memory (ß = -1.74,

95% CI = -3.07 to -0.40) function scores (**Figure 3** and **Table S4**). The observed inverse association in PC1 is primarily driven by the concentrations of Pb, as indicated by the loading weights from the PCA and sensitivity analysis. This was done by examining the association between urinary Pb concentrations and the MSCA scores while adjusting for the selected confounding factors and then adding the remaining metals of the PC1 (**Table S5** and **Table S6**). PC2 was associated with lower scores in global motor (ß = -0.94 95% CI =-1.88 to 0.00) and gross motor function (ß = -1.41, 95% CI = -2.36 to -0.46). In contrast, PC3 was positively associated with function scores in visual and verbal span (ß = 1.14, 95% CI = 0.16 to 2.12), as well as global motor function (ß = 1.02, 95% CI = 0.03 to 2.01), indicating that higher scores on this component were related to better performance in these areas. PC4 was associated with increased fine motor function (ß = 1.01, 95% CI = 0.11 to 1.92) (**Figure 3** and **Table S4**). The unadjusted associations between the PCs derived from children's urinary metals and the MSCA scores are shown in **Table S7**. Consistent with the overall findings, significant reductions in gross motor with PC2 (ß = -1.22, 95% CI = -2.41 to -0.03) and increases in visual and verbal span with PC3 (ß = 1.52, 95% CI = 0.06 to 2.98) were observed among female children (**Table S8**). Among male children, PC1 was associated with reduced verbal executive (ß = -2.30, 95% CI = -4.20 to -0.41) and working memory (ß = -2.85, 95% CI = -4.73 to -0.97) scores (**Table S9**). The unadjusted associations between the PCs derived from children's urinary metals and the MSCA scores stratified by sex are shown in **Table S10** and **Table S11**.

4. Discussion

This study applies a latent exposure approach to investigate the cross-sectional association between children's neurodevelopmental outcomes and their exposure to mixture of metals. We observe that the metal mixture of Pb, Cu, Zn and Se exposures was associated with a decrease in cognitive function scores, including global verbal, executive, verbal executive, and working memory function. Exposure of iAs and MMA as a mixture related to reduced global motor and gross motor scores. Contrary, exposure to Mo and Co as a mixture was associated with increased function scores in visual and verbal span, as well as global motor performance. Lastly, exposure to AsB was associated with an increase in fine motor function.

This study focused on children with relatively low toxic level of metal exposures relevant in Spain and other comparable regions. In a study conducted in China with 1,220 children aged 2 to 6 years, Pb and Zn concentrations were 2-fold and 1.2-fold higher than those found in our study, respectively (Y. Liu et al., 2022). Another study with schoolchildren from Sweden showed 1.3-fold and 1.2-fold higher concentrations of DMA and MMA, respectively, than the ones in

our study (Mattisson et al., 2020). The findings of this study provide evidence supporting the hypothesis that assessing the effects of metal exposures on neurodevelopment as a combination of metals or latent exposures enhances our understanding of the intricate associations involved (Forns et al., 2014; Freire et al., 2018; Heng et al., 2022).

In this population, diet is considered the primary source of metal exposure, and the metals grouped into the four latent variables are expected to have common dietary sources of exposure. In the first latent variable, all constituent metals may share common sources of dietary exposure, with organ meat and shellfish expected to be the primary contributors (Martinez-Morata et al., 2023). The second latent variable, comprising inorganic arsenic species, is also expected to share dietary sources of exposure, such as rice (Signes-Pastor et al., 2017). As for Mo and Co, the metals forming the third latent variable, the main source of exposure is expected to be leafy vegetables and grains (Leyssens et al., 2017; Martinez-Morata et al., 2023). Lastly, the fourth latent variable is composed of AsB, which is associated with fish and seafood consumption (Martinez-Morata et al., 2023; Signes-Pastor et al., 2017).

Our analysis reveals a consistent negative association between exposure to the first latent variable, primarily led by Pb, and multiple cognitive scales. This finding is supported by several studies. In the USA, a study of 1,009 mother-child pairs from the Project Viva showed that increasing metal mixture exposure of Pb, mercury, manganese, and Se, measured in erythrocytes, were associated with worsening neurobehavioral scores, where Pb and Se were the major contributors (Fruh et al., 2021). Similarly, a study of 726 adolescents aged 14-16 years in Bangladesh found a negative association between blood levels of Pb and working memory and verbal comprehension scores (Wasserman et al., 2018). Another study of 635 Italian adolescents aged 10-14 years found that higher levels of metal mixtures, measured in multiple biomarkers, driven by Pb, chromium, and manganese were associated with lower verbal intelligence quotient scores, especially at low Cu levels (Bauer et al., 2020). A study of 490 mother-child pairs in the INMA Valencia cohort observed an inverted U-shaped association between serum Se concentrations and executive function scales (Amorós et al., 2018). Additionally, a case-control study of 136 children aged 4-9 years in Russia found that elevated Cu-Zn ratio serum levels were significantly associated with higher odds of ADHD (Skalny et al., 2020). The cognitive scales impacted by exposure to the first latent variable include executive function, verbal executive, verbal global function, and working memory. These cognitive functions are interrelated, as working memory is a key component of executive function, which involves the mental process of storing, managing, and using information for subsequent planning, decision making, and problem solving. Thus, it is consistent with our findings of a

negative association between the first latent exposure variable and these cognitive functions. While the mechanisms by which these metals have a neurotoxic effect are not completely understood, it is believed that they can induce changes in myelination, oxidative stress, cellular signaling, and multiple neurotransmitter pathways related to behaviour and executive function. These changes are particularly sensitive in young children, as suggested in the case of Pb exposure (Roy et al., 2009).

The second latent exposure variable we identified primarily reflects overall iAs exposure, as it combines iAs and MMA. Urinary DMA is also a metabolite of iAs exposure; however, it is prone to exposure misclassification due to direct exposure or the metabolism of complex organoarsenical compounds, particularly among individuals who are high consumers of fish and seafood, such as the population in our study (Aylward et al., 2014; Hata et al., 2012; Signes-Pastor et al., 2017). The second latent exposure was found to be negatively associated with global motor and gross motor scores. Consistent with our findings, a study conducted in Bangladesh with 304 children aged 8-11 years showed that drinking water, blood, and urinary total arsenic levels were inversely associated with motor function scores (Parvez et al., 2011). In Taiwan, another study with 98 children aged 4-6 years found a positive association between the risk of developmental delay and urinary total arsenic and MMA levels (Hsieh et al., 2014). Similarly, results from our previous study about the association between iAs exposure and children's neurodevelopment in the same population showed a negative association between iAs exposure, as single metal exposure, and motor function (Signes-Pastor et al., 2019). Inorganic arsenic is a potent neurotoxicant that can cross the blood-brain barrier, leading to a wide range of effects on the white matter in the brain (Rosado et al., 2007). Animal studies have shown that iAs accumulates and biomethylates in the brain, producing intermediate and final products that are more toxic and reactive (Stýblo et al., 2002). Inorganic arsenic accumulates in the cerebral cortex, where the frontal and parietal lobes are located. These lobes contain the main motor centre responsible for the initiation, coordination, and sequencing of body movements, so changes in this brain region may contribute to detrimental motor function (Choo et al., 2021).

Our analysis identified a third latent exposure variable that included Mo and Co and was positively associated with higher scores in global motor function as well as visual and verbal span functions. Few studies have investigated the association between exposure to metals such as Mo and Co and child neurodevelopment, with most of the research focusing on prenatal exposures and yielding inconsistent results, which emphasize the need for further investigations including an assessment of their potential interactions at varying exposure levels

(Forns et al., 2014; C. Liu et al., 2022; Nozadi et al., 2022; Vázquez-Salas et al., 2014). In Mexico, a study of children aged 10-13 months found a negative association between higher prenatal urinary Mo exposure and children's communication scores and psychomotor development indices (Vázquez-Salas et al., 2014). The INMA sub-cohort of Sabadell found no statistically significant association between prenatal urinary Co exposure and the MSCA in children aged 4-5 years (Forns et al., 2014). However, consistent with our study, C. Liu *et al*. found that higher prenatal serum Mo concentrations were associated with better social developmental quotient in children aged 2-3 years (C. Liu et al., 2022). The inconsistent results across studies may be due to differences in the analysis of prenatal versus postnatal exposures and the use of different scales to measure neurodevelopment. In this study, as a tool for the evaluation of neuropsychological functions we used the MSCA, which has been the most widely used tool in the studies mentioned above (Amorós et al., 2018; Forns et al., 2014; Mendez et al., 2009; Signes-Pastor et al., 2019), followed by the Weschler's Intelligence Scale for Children (WISC) (Bauer et al., 2020; Wasserman et al., 2018).

A fourth exposure latent variable was identified in our analysis, which primarily reflects exposure to the organic arsenic form arsenobetaine (AsB). It was found to be positively associated with higher scores in fine motor function. AsB is the predominant arsenic species in fish and seafood and is typically considered non-toxic (Luvonga et al., 2020; Taylor et al., 2017). Previous studies have shown a positive association between moderate to high fish intake and greater neurodevelopment in children and adolescents (Butler et al., 2017; Mendez et al., 2009). A study conducted in Japan found that higher maternal fish intake was associated with a lower risk of fine motor delay in one-year-old infants (Hamazaki et al., 2020). Another study carried out with 7,421 British children, showed a positive association between fish intake and neurodevelopmental test scores (Daniels et al., 2004). Fish is a food rich in essential nutrients related to brain development such as, proteins, vitamins, and omega 3 long-chain polyunsaturated fatty acids (LCPUFA), particularly the docosahexaenoic acid (DHA) (Nevins et al., 2021). This omega 3 fatty acid is the predominant within the brain, developing several functions as cell-survival, neuroinflammation, and its protective function in maintaining blood-brain barrier integrity (Basak et al., 2020). Because of this, our results, which suggest a relationship between AsB and fine motor function, are consistent with these previous studies where fish consumption, whose main arsenic species is AsB, has a positive impact on children's neurodevelopment.

In our sex-stratified analyses, the results suggest that females may be more susceptible than males to impaired gross motor functions due to iAs toxicity. Additionally, exposure to the third

latent exposure (Mo and Co) in females had a stronger positive association on visual and verbal functions than males. Similar results were found in a study carried out in Bangladesh, where urinary iAs was inversely associated with neurodevelopmental function in females but not in males (Hamadani et al., 2011). Moreover, a negative association between the first exposure latent (primary led by Pb) and verbal executive and working memory functions was observed only for males. Previous studies support these findings, a study in Cincinnati with adolescents suggest a worse attention function due to exposure to Pb in males but not in females (Ris et al., 2004). Results from the Boston Birth Cohort study showed a strong association between blood Pb concentrations and ADHD only in early childhood males (Ji et al., 2018). These results suggest a different effect between sex in neurodevelopment. Apart from genetics, the observed differences in the neurodevelopmental outcomes due to metal exposure may be due to the slower brain development in males than in females, which increases the period of vulnerability to the toxic effects of metals (Bale, 2016). In addition, the different concentrations of enzymes and hormones between males and females related to neurodevelopment, such as the O-linked N-acetylglucosamine transferase (OGT), may be responsible for these differences. The OGT have an important role in neurodevelopmental programming and metabolic regulation and its levels are higher in females. A reduction in OGT levels in both males and females would have a higher detrimental effect on males increasing the risk of neurodevelopmental disorders (May et al., 2019). The sensitivity analyses, excluding the Valencia cohort due to its slightly different average age, align with the main findings (data not shown).

It is important to acknowledge the limitations of the study. Firstly, the use of a one-time spot urine sample as a biomarker of metal exposure, which may lead to a higher risk of exposure misclassification. The cross-sectional design used in this study limits our ability to establish causality since we cannot assure the temporality between the exposure and outcome. Additionally, the modest sample size may limit the statistical power of the analyses; however, several significant associations emerged from our statistical analysis. Although we adjusted for a wide range of potential confounding factors, residual confounding factors remain a possibility. Urine is commonly used as an exposure matrix in numerous epidemiological studies and is generally accepted as a suitable biomarker for evaluating exposure to arsenic, Mo, Cd, Se, and Co. However, for Pb, Zn, and Cu, other matrices besides urine may be more suitable biomarkers. In addition, the excretion rate varies across metals, for arsenic, Mo and Co, urine reflects short-term exposures, while for Cd, urine is an exposure biomarker of long-term (Fort et al., 2014). The findings of this study are specific to a child population in Spain who were exposed at relatively low toxic levels, including lead (Pb), which had several urine

concentrations <LOD. We need to bear in mind the limitations of imputing a substantial portion of values <LOD with a fixed value (i.e., LOD divided by the square root of two), which can introduce bias by reducing the estimate variability. To mitigate this concern, stochastic imputation has been suggested (Lubin et al., 2004). However, while this procedure is practical and supported by at least two R packages, it involves multiple repetitions, potentially impacting the construction of the PC and thereby making very unclear the final interpretation of our results. Therefore, it is important to be cautious when extrapolating the results to other populations with varying levels of metal exposure. Despite these limitations, the study has strengths, including the analysis of arsenic speciation. The toxicity levels of arsenic are primarily dependent on its oxidation state, with the inorganic forms being the most toxic. On the other hand, organic forms of arsenic, such as AsB, are rapidly excreted in urine unchanged (Cullen, 2014; Dopp et al., 2004; Moe et al., 2016). In this study, the authors applied a conservative biomarker approach to assess internal exposure to iAs from all sources by excluding urinary DMA, which can be derived from direct exposure. Specifically, we excluded urinary DMA from their analysis due to its susceptibility to exposure misclassification (Aylward et al., 2014; Hata et al., 2012). We also used PCA, a metal mixture approach that allows us to assessed exposure in a more realistic way, being able to assess their additive, synergistic or antagonistic effects. Regarding the MSCA assessment, several quality controls were introduced, and the psychologist received extensive training to reduce the possible biases. Finally, the INMA project is an ongoing birth cohort in which we will be able to continue studying these associations at different ages, and even carry out prospective studies.

5. Conclusion

This study provides compelling evidence supporting the application of PCA in evaluating metal exposures as a mixture of metals or latent exposure variables in childhood neuropsychological development, achieved by reducing dimensionality in a multivariate dataset while preserving its underlying structure. The findings suggest that even low toxic levels of metal exposures, which are relevant to the Spanish population and others, impact children's neurodevelopment. Specifically, increased exposure to iAs and a mixture of metals including Pb were found to be associated with a decrease in certain domains of neurological function scores. Conversely, exposure to AsB and a mixture of Co and Mo enhanced children's neuropsychological development scores. However, it is crucial to minimize exposure to toxic metals such as Pb, especially during critical periods of early life. Further investigation is necessary to identify the primary sources of toxic metal exposure in childhood.

Credit authorship contribution statement

LN-B contributed to methodology and writing; AJS-P contributed to conceptualization, methodology, statistical analysis, visualization, reviewing of manuscript and obtaining funding; PMC and SDC contributed to conceptualization, formal statistical analysis and reviewing of manuscript; NJR, MG, MV, AA, AI, IRG, AFS, SLL, ML, MRK, ES and JV were responsible for the acquisition of data and made a critical revision of the manuscript for intellectual content and approved the final manuscript; AM, MC, CM, KR, and CM measured urinary metal concentrations and reviewing of the manuscript.

Funding

This study was funded by CIDEGENT/2020/050; by grants from Instituto de Salud Carlos III: Red INMA G03/176; CB06/02/0041; PI041436; PI081151, PI11/01007, PI07/0314, PI04/2018, PI09/02311, PI13/02429, PI18/00909 co-funded by the European Regional Development Fund (FEDER), "A way to make Europe"/"Investing in your future"; Fundación Cajastur, and Universidad de Oviedo.; FIS-FEDER: 13/1944, 16/1288, 19/1338; ISGlobal acknowledges support from the grant CEX2018-000806-S funded by MCIN/AEI/ 10.13039/501100011033, and support from the Generalitat de Catalunya through the CERCA Program; Miguel Servet-FSE: MS15/0025, MS20/0006, H2020-EU No 874583 (ATHLETE project), Generalitat Valenciana (CIAICO/2021/132, BEST/2020/059 and AICO 2020/285. Ministry of Universities (CAS21/00008, NextGenerationEU). Generalitat de Catalunya-CIRIT 1999SGR 00241, Fundació La marató de TV3 (090430). We acknowledge support from the grant CEX2018-000806-S funded by MCIN/AEI/ 10.13039/501100011033, and support from the Generalitat de Catalunya through the CERCA Program. Mònica Guxens is funded by a Miguel Servet II fellowship (CPII18/00018) awarded by the Spanish Institute of Health Carlos III.

Declaration of Competing Interest

The authors declare no conflict of interest.

Data Availability Statement

The data presented in this study are available on request from the corresponding author. The data are not publicly available due to confidentiality and ethical reasons.

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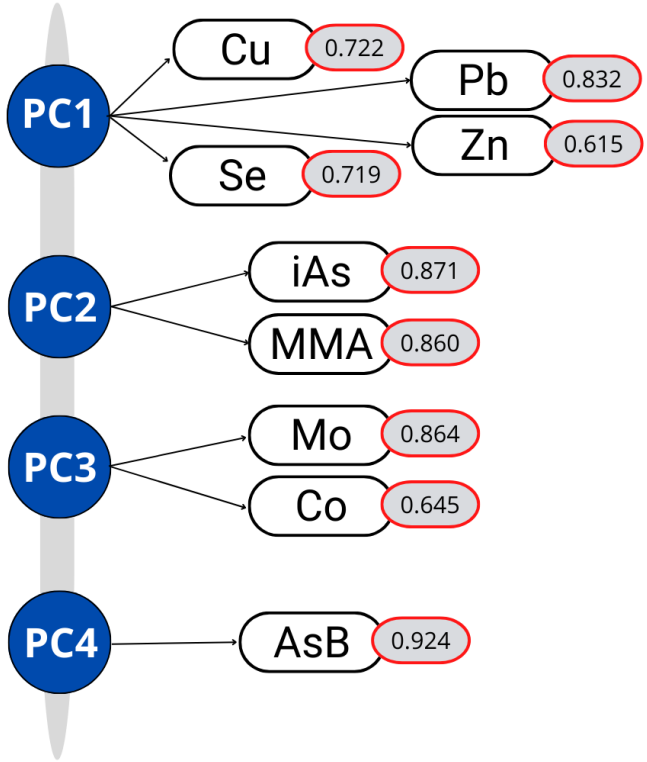
Figure 1. Pearson's correlation matrix

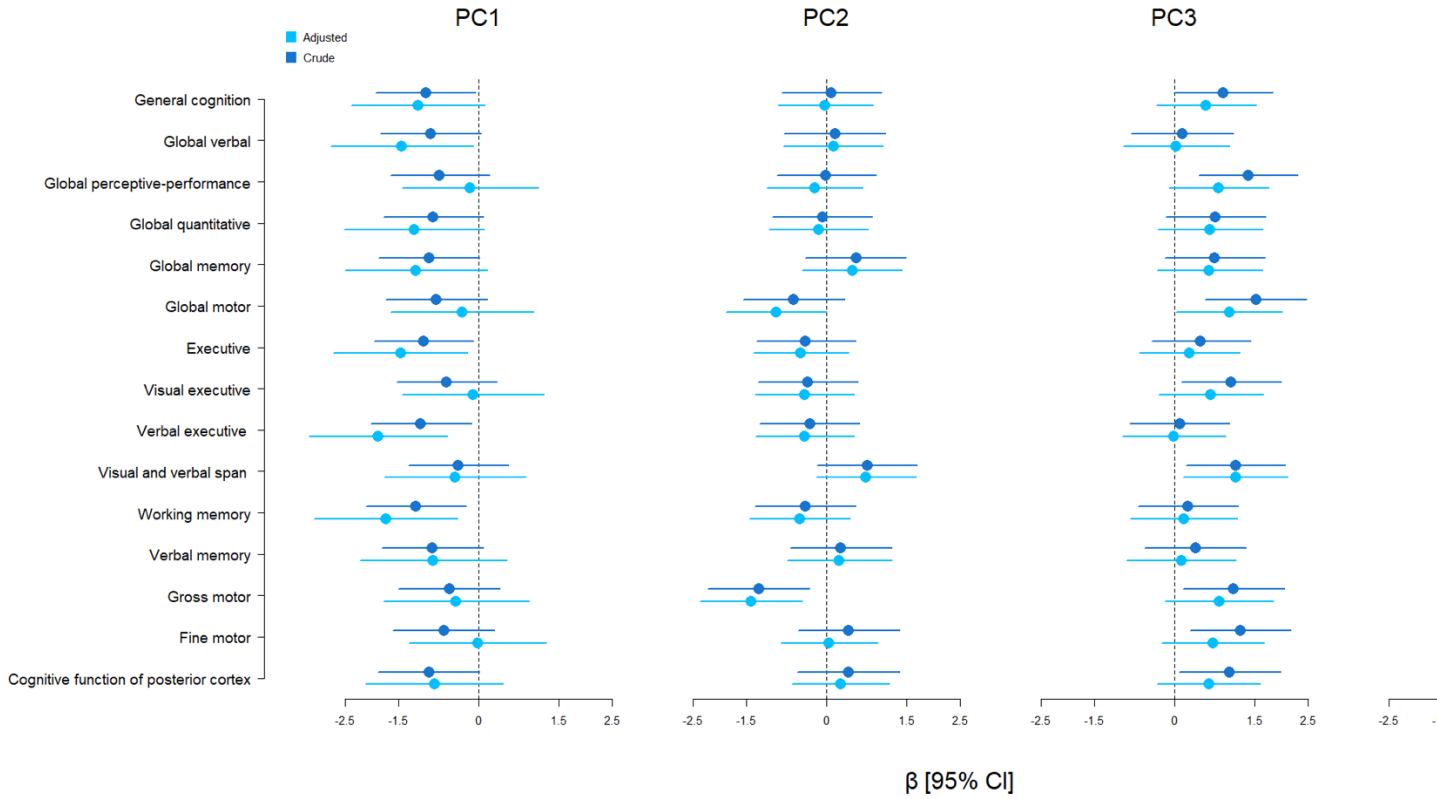
Values below the limit of detection (LOD) are imputed using LOD/$\sqrt{2}$. Urine concentrations are adjusted for specific gravity following the method described by Kuiper et al. (2022).

Figure 2: Principal components (PC) and PC loadings generated from urinary metal concentrations.



n = 962. The cumulative explained variance for PC1 to PC4 was 24.7%, 41.6%, 57.0%, and 68.6%, respectively.

Figure 3: Associations between Principal Components (PC) Derived from Children's Urinary Metal Concentrations and the McCarthy Scales of Children's Ability Scores.



Principal Component 1 (PC1) includes Pb, Cu, Se, and Zn; PC2 includes iAs and MMA; PC3 includes Mo and Co; PC4 includes AsB. All models were adjusted for cohort (Asturias, Gipuzkoa, Sabadell, or Valencia), children's BMI (kg/m^2), maternal social class (I-II, III, or IV-V), maternal highest attained level of education (primary, secondary, or university), maternal number of previous live births (0, 1 or >1), age at the MSCA test (years), and sex (male or female). Dark blue indicates confounding-adjusted models, while light blue indicates crude models.

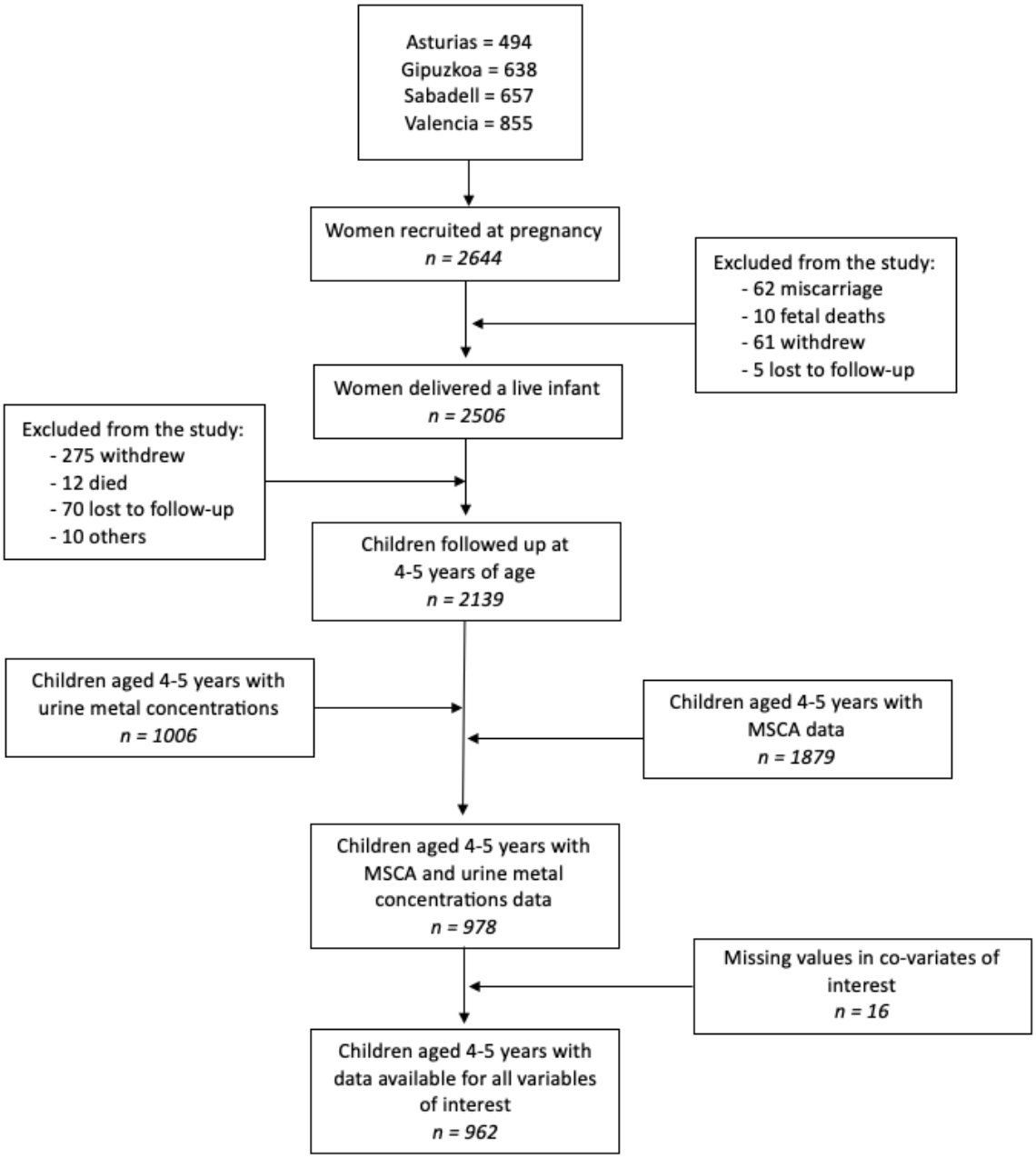
Table 1: Selected characteristics of the study population, both for the entire dataset and stratified by sex.

Selected characteristics	All	Females	Males
	n = 962	*n* = 460 (47.8%)	*n* = 502 (52.2%)
Sub-cohort - *n* (%)			
Asturias	221 (23.0%)	91 (19.8%)	130 (25.9%)
Gipuzkoa	273 (28.4%)	140 (30.4%)	133 (26.5%)
Sabadell	374 (38.9%)	180 (39.1%)	194 (38.6%)
Valencia	94 (9.77%)	49 (10.7%)	45 (8.96%)
Children:			
Urine concentrations - µg/L			
AsB	12.30 [3.19;42.3]	12.30 [3.36;38.1]	12.20 [3.09;48.5]
iAs	1.09 [0.39;2.15]	1.17 [0.42;2.15]	1.01 [0.34;2.13]
MMA	0.41 [0.01;0.81]	0.46 [0.02;0.92]	0.36 [0.01;0.74]
DMA	3.21 [0.38;6.56]	3.29 [0.58;6.69]	3.15 [0.33;6.06]
Co	0.77 [0.47;1.30]	0.79 [0.50;1.33]	0.75 [0.45;1.26]
Pb	0.49 [0.21;1.08]	0.50 [0.21;1.11]	0.49 [0.21;1.05]
Se	24.90 [18.1;35.8]	25.40 [18.4;35.8]	24.20 [17.9;36.0]
Zn	379.00 [252;578]	410.00 [246;623]	355.00 [253;537]
Mo	83.70 [50.4;124]	81.90 [46.8;129]	87.60 [52.8;122]
Cu	8.97 [4.64;13.2]	8.90 [4.22;13.3]	9.03 [5.06;13.2]
BMI - kg/m^2	16.00 [15.2;17.0]	15.80 [15.1;17.0]	16.10 [15.3;17.1]
Age MSCA - years	4.45 [4.37;4.58]	4.45 [4.38;4.56]	4.45 [4.37;4.59]
Maternal:			
Number of previous life births			
0	521 (56.8%)	252 (57.3%)	269 (56.3%)
1	347 (37.8%)	160 (36.4%)	187 (39.1%)
>1	50 (5.45%)	28 (6.36%)	22 (4.60%)
Social class			
Upper - I+II	241 (26.2%)	111 (25.2%)	130 (27.1%)
Middle - III	263 (28.6%)	127 (28.9%)	136 (28.4%)
Lower - IV+V	415 (45.2%)	202 (45.9%)	213 (44.5%)
Highest attained level of education			
Primary	175 (18.2%)	81 (17.6%)	94 (18.7%)
Secondary	391 (40.6%)	195 (42.4%)	196 (39.0%)
University	396 (41.2%)	184 (40.0%)	212 (42.2%)

AsB, arsenobetaine; iAs, inorganic arsenic; MMA, monomethylarsonic acid; DMA, dimethylarsinic acid; Co, cobalt; Pb, lead; Se, selenium; Zn, zinc; Mo, molybdenum; Cu, copper; BMI, body mass index; MSCA, McCarthy Scales of Children's Abilities. Categorical variables are presented as n (%) and continuous variables are presented as median [Q1; Q3]. Values below the limit of detection (LOD) are imputed using LOD/$\sqrt{2}$. Urine concentrations are adjusted for specific gravity following the method described by Kuiper et al. (2022).

Supplemental information

Figure S1: Study population flowchart.



MSCA, McCarthy Scales of Children's Abilities

1 **Table S1**: Differences in sociodemographic variables between participants included and
2 excluded from the study.

3

Selected characteristics	Included (n=962)	Excluded*
Sub-cohort - *n* (%)		
Asturias	221 (23.0%)	232 (19.7%)
Gipuzkoa	273 (28.4%)	258 (21.9%)
Sabadell	374 (38.9%)	225 (19.1%)
Valencia	94 (9.77%)	462 (39.3%)
Children:		
BMI - kg/m^2	16.00 [15.2; 17.0]	16.21 [15.1; 17.0] (*n=956*)
Maternal:		
Number of previous life births		
0	521 (56.8%)	650 (57.1%)
1	347 (37.8%)	412 (36.2%)
>1	50 (5.45%)	76 (6.7%)
Social class		
Upper - I+II	241 (26.2%)	248 (21.8%)
Middle - III	263 (28.6%)	295 (25.9%)
Lower - IV+V	415 (45.2%)	595 (52.3%)
Highest attained level of education		
Primary	175 (18.2%)	269 (23.4%)
Secondary	391 (40.6%)	498 (43.3%)
University	396 (41.2%)	384 (33.4%)

* The sample size of the excluded population depends on the variable described. BMI, body mass index; MSCA, McCarthy Scales of Children's Abilities. Categorical variables are presented as n (%) and continuous variables are presented as median [Q1; Q3].

4

Table S2: Principal component transformation matrix.

Terms	PC1	PC2	PC3	PC4
Intercept	-2.265	1.299	-5.658	-3.258
Co	0.018	0.015	0.349	-0.026
Pb	0.273	0.04	-0.154	-0.084
Cu	0.147	0.016	-0.016	-0.092
Se	0.393	-0.037	-0.017	0.354
Mo	-0.191	-0.046	0.668	-0.012
Zn	0.199	-0.018	0.175	0.113
MMA	0.018	0.177	-0.02	0.007
iAs	0.005	0.216	0.003	-0.052
AsB	-0.014	-0.016	-0.005	0.257

Values below the limit of detection (LOD) are imputed using LOD/$\sqrt{2}$. Urine concentrations are adjusted for specific gravity following the method described by Kuiper et al. (2022).

Table S3: Principal component loading matrix.

Terms	PC1	PC2	PC3	PC4
Co	0.320	0.059	0.645	-0.016
Pb	0.832	-0.032	-0.080	-0.087
Cu	0.722	-0.056	0.156	-0.180
Se	0.719	-0.042	0.174	0.322
Mo	-0.014	0.025	0.864	-0.027
Zn	0.615	-0.025	0.398	0.155
MMA	-0.010	0.86	-0.001	0.152
iAs	-0.086	0.871	0.070	-0.021
AsB	-0.004	0.116	-0.035	0.924

Values below the limit of detection (LOD) are imputed using LOD/$\sqrt{2}$. Urine concentrations are adjusted for specific gravity following the method described by Kuiper et al. (2022).

Table S4: Confounding-adjusted associations between principal components (PCs) derived from children's urinary metals and children's McCarthy scales scores.

McCarthy Scales of Children's Abilities		PC1 (ß 95%CI)	PC2 (ß 95%CI)	PC3(ß 95%CI)	PC4 (ß 95%CI)
Original functions	General cognition	-1.13 (-2.38; 0.12)	-0.03 (-0.91; 0.86)	0.59 (-0.34; 1.52)	0.71 (-0.18; 1.59)
	Global verbal	-1.44 (-2.76; -0.11)	0.13 (-0.81; 1.06)	0.03 (-0.96; 1.02)	0.36 (-0.58; 1.30)
	Global perceptive - performance	-0.16 (-1.42; 1.11)	-0.22 (-1.11; 0.67)	0.82 (-0.11; 1.76)	0.75 (-0.14; 1.65)
	Global quantitative	-1.21 (-2.51; 0.10)	-0.15 (-1.07; 0.77)	0.66 (-0.31; 1.64)	0.46 (-0.47; 1.38)
	Global memory	-1.17 (-2.49; 0.16)	0.48 (-0.45; 1.41)	0.65 (-0.33; 1.64)	0.37 (-0.57; 1.30)
	Global motor	-0.31 (-1.64; 1.02)	-0.94 (-1.88; 0.00)	1.02 (0.03; 2.01)	0.40 (-0.54; 1.34)
New functions	Executive	-1.46 (-2.72; -0.20)	-0.48 (-1.37; 0.41)	0.28 (-0.66; 1.22)	0.50 (-0.39; 1.40)
	Visual executive	-0.10 (-1.42; 1.22)	-0.41 (-1.34; 0.52)	0.68 (-0.30; 1.66)	0.42 (-0.51; 1.35)
	Verbal executive	-1.88 (-3.17; -0.59)	-0.41 (-1.32; 0.51)	-0.01 (-0.97; 0.96)	0.43 (-0.48; 1.35)
	Visual and verbal span	-0.44 (-1.76; 0.88)	0.74 (-0.19; 1.67)	1.14 (0.16; 2.12)	0.34 (-0.59; 1.28)
	Working memory	-1.74 (-3.07; -0.40)	-0.50 (-1.44; 0.44)	0.18 (-0.82; 1.17)	0.22 (-0.73; 1.16)
	Verbal memory	-0.85 (-2.22; 0.52)	0.24 (-0.73; 1.21)	0.13 (-0.90; 1.15)	0.13 (-0.84; 1.10)
	Gross motor	-0.42 (-1.78; 0.94)	-1.41 (-2.36; -0.46)	0.84 (-0.17; 1.85)	-0.37 (-1.33; 0.59)
	Fine motor	-0.02 (-1.31; 1.26)	0.05 (-0.85; 0.96)	0.72 (-0.23; 1.67)	1.01 (0.11; 1.92)
	Cognitive function of posterior cortex	-0.83 (-2.12; 0.46)	0.26 (-0.65; 1.17)	0.64 (-0.33; 1.60)	0.66 (-0.26; 1.57)

The first principal component (PC1) includes Pb, Cu, Se, and Zn, while the second principal component (PC2) includes iAs and MMA. The third principal component (PC3) includes Mo and Co, and the fourth principal component (PC4) includes AsB. All statistical models were adjusted for potential confounding factors, including cohort (Asturias, Gipuzkoa, Sabadell, or Valencia), children's body mass index (BMI) in kg/m^2, maternal social class (I-II, III, or IV-V), maternal highest attained level of education (primary, secondary, or university), maternal number of previous live births (0, 1 or >1), age at the McCarthy Scales of Children's Abilities (MSCA) test in years, and sex (male or female).

Table S5: Associations between urinary lead concentrations and the McCarthy Scales of Children's Ability scores adjusted by selected confounding factors (model 1) and adding the remaining metals of the first principal component (model 2).

McCarthy Scales of Children's Abilities		Model 1 - ß (95% CI)	Model 2 - ß (95% CI)
Original functions	General cognition	-0.81 (-1.77; 0.15)	-0.89 (-1.93; 0.15)
	Global verbal	-0.73 (-1.75; 0.29)	-0.48 (-1.59; 0.62)
	Global perceptive - performance	-0.48 (-1.45; 0.49)	-0.89 (-1.94; 0.16)
	Global quantitative	-0.99 (-1.99; 0.02)	-1.13 (-2.22; -0.04)
	Global memory	-1.37 (-2.38; -0.36)	-1.64 (-2.74; -0.54)
	Global motor	-0.36 (-1.38; 0.66)	-0.67 (-1.78; 0.44)
New functions	Executive	-0.86 (-1.83; 0.10)	-0.79 (-1.85; 0.26)
	Visual executive	-0.40 (-1.4; 0.61)	-0.74 (-1.84; 0.36)
	Verbal executive	-0.96 (-1.95; 0.04)	-0.69 (-1.77; 0.39)
	Visual and verbal span	-0.9 (-1.91; 0.11)	-1.32 (-2.42; -0.22)
	Working memory	-1.19 (-2.21; -0.17)	-1.17 (-2.29; -0.06)
	Verbal memory	-1.04 (-2.09; 0.01)	-1.16 (-2.30; -0.02)
	Gross motor	0.08 (-0.96; 1.12)	0.12 (-1.01; 1.26)
	Fine motor	-0.63 (-1.61; 0.35)	-1.16 (-2.22; -0.09)
	Cognitive function of posterior cortex	-0.85 (-1.84; 0.14)	-1.08 (-2.16; -0.01)

All statistical model 1 were adjusted for potential confounding factors, including cohort (Asturias, Gipuzkoa, Sabadell, or Valencia), children's body mass index (BMI) in kg/m^2, maternal social class (I-II, III, or IV-V), maternal highest attained level of education (primary, secondary, or university), maternal number of previous live births (0, 1 or >1), age at the McCarthy Scales of Children's Abilities (MSCA) test in years, and sex (male or female). In model 2 children urinary Cu, Se, and Zn concentrations were included as potential confounding factors.

Table S6: Associations between urinary lead concentrations and the McCarthy Scales of Children's Ability scores unadjusted/crude (Model 1) and adjusting by the remaining metals of the first principal component (model 2).

McCarthy Scales of Children's Abilities		Model 1 - ß (95% CI)	Model 2 - ß (95% CI)
Original functions	General cognition	-0.90 (-1.7; -0.10)	-1.21 (-2.21; -0.22)
	Global verbal	-0.67 (-1.49; 0.14)	-0.71 (-1.72; 0.30)
	Global perceptive - performance	-0.84 (-1.64; -0.05)	-1.34 (-2.33; -0.35)
	Global quantitative	-0.80 (-1.6; 0.00)	-1.09 (-2.09; -0.09)
	Global memory	-1.09 (-1.89; -1.89)	-1.58 (-2.58; -0.59)
	Global motor	-0.71 (-1.53; 0.11)	-1.06 (-2.08; -0.04)
New functions	Executive	-0.87 (-1.66; -0.08)	-1.04 (-2.03; -0.06)
	Visual executive	-0.71 (-1.51; 0.09)	-1.13 (-2.13; -0.13)
	Verbal executive	-0.80 (-1.6; 0.00)	-0.81 (-1.81; 0.19)
	Visual and verbal span	-0.73 (-1.53; 0.06)	-1.44 (-2.43; -0.46)
	Working memory	-0.95 (-1.75; -0.14)	-1.02 (-2.02; -0.01)
	Verbal memory	-0.9 (-1.72; -0.08)	-1.19 (-2.2; -0.17)
	Gross motor	-0.22 (-1.03; 0.60)	-0.13 (-1.15; 0.89)
	Fine motor	-0.88 (-1.69; -0.07)	-1.52 (-2.53; -0.52)
	Cognitive function of posterior cortex	-0.94 (-1.75; -0.12)	-1.37 (-2.38; -0.36)

In model 2 children urinary Cu, Se, and Zn concentrations were included as potential confounding factors.

Table S7: Associations between principal components (PCs) derived from children's urinary metals and the McCarthy Scales of Children's Ability scores: Unadjusted/Crude models.

McCarthy Scales of Children's Abilities		PC1	PC2	PC3	PC4
Original functions	General cognition	-0.99 (-1.92; -0.06)	0.09 (-0.84; 1.02)	0.91 (-0.02; 1.84)	0.97 (0.04; 1.90)
	Global verbal	-0.90 (-1.84; 0.05)	0.16 (-0.79; 1.10)	0.15 (-0.80; 1.10)	0.63 (-0.32; 1.57)
	Global perceptive - performance	-0.73 (-1.65; 0.20)	-0.01 (-0.93; 0.92)	1.38 (0.45; 2.30)	0.94 (0.01; 1.86)
	Global quantitative	-0.85 (-1.78; 0.08)	-0.08 (-1.01; 0.85)	0.77 (-0.16; 1.70)	0.60 (-0.33; 1.54)
	Global memory	-0.92 (-1.86; 0.01)	0.55 (-0.39; 1.48)	0.75 (-0.18; 1.69)	0.50 (-0.43; 1.43)
	Global motor	-0.79 (-1.74; 0.16)	-0.61 (-1.56; 0.34)	1.52 (0.57; 2.47)	0.25 (-0.70; 1.21)
New functions	Executive	-1.03 (-1.96; -0.11)	-0.39 (-1.31; 0.54)	0.49 (-0.43; 1.42)	0.81 (-0.11; 1.74)
	Visual executive	-0.60 (-1.53; 0.34)	-0.35 (-1.28; 0.58)	1.06 (0.13; 1.99)	0.65 (-0.29; 1.58)
	Verbal executive	-1.08 (-2.01; -0.14)	-0.31 (-1.25; 0.62)	0.10 (-0.84; 1.03)	0.73 (-0.21; 1.66)
	Visual and verbal span	-0.38 (-1.3; 0.55)	0.76 (-0.17; 1.68)	1.14 (0.22; 2.07)	0.44 (-0.49; 1.36)
	Working memory	-1.17 (-2.10; -0.23)	-0.40 (-1.34; 0.54)	0.25 (-0.68; 1.19)	0.40 (-0.54; 1.34)
	Verbal memory	-0.86 (-1.81; 0.09)	0.27 (-0.68; 1.22)	0.39 (-0.56; 1.34)	0.19 (-0.76; 1.14)
	Gross motor	-0.55 (-1.50; 0.40)	-1.27 (-2.21; -0.32)	1.10 (0.16; 2.05)	-0.64 (-1.59; 0.31)
	Fine motor	-0.65 (-1.60; 0.29)	0.41 (-0.53; 1.36)	1.23 (0.29; 2.17)	1.05 (0.11; 1.99)
	Cognitive function of posterior cortex	-0.93 (-1.88; 0.02)	0.41 (-0.54; 1.36)	1.03 (0.09; 1.98)	0.86 (-0.08; 1.81)

The first four principal components (PCs) are as follows: PC1 includes Pb, Cu, Se, and Zn. PC2 includes iAs and MMA. PC3 includes Mo and Co. PC4 includes AsB.

Table S8: Confounding-adjusted associations between principal components (PCs) derived from female children's urinary metals and the McCarthy Scales of Children's Ability scores.

McCarthy Scales of Children's Abilities		PC1	PC2	PC3	PC3
Original functions	General cognition	-1.39 (-3.11; 0.33)	-0.47 (-1.74; 0.79)	0.64 (-0.68; 1.97)	0.50 (-0.81; 1.8)
	Global verbal	-1.73 (-3.50; 0.05)	-0.27 (-1.57; 1.04)	0.08 (-1.28; 1.45)	-0.29 (-1.64; 1.06)
	Global perceptive - performance	-0.55 (-2.28; 1.18)	-0.52 (-1.79; 0.76)	0.96 (-0.36; 2.29)	1.25 (-0.05; 2.56)
	Global quantitative	-0.53 (-2.36; 1.31)	-0.43 (-1.78; 0.92)	0.55 (-0.86; 1.96)	0.42 (-0.97; 1.81)
	Global memory	-1.09 (-2.98; 0.80)	0.19 (-1.2; 1.58)	1.01 (-0.44; 2.46)	0.00 (-1.44; 1.43)
	Global motor	-1.08 (-2.80; 0.65)	-0.87 (-2.14; 0.39)	0.60 (-0.72; 1.93)	0.27 (-1.04; 1.58)
New functions	Executive	-1.28 (-2.99; 0.43)	-1.11 (-2.37; 0.15)	-0.08 (-1.40; 1.23)	0.56 (-0.74; 1.85)
	Visual executive	-0.10 (-1.88; 1.69)	-0.84 (-2.15; 0.47)	0.68 (-0.69; 2.05)	1.13 (-0.22; 2.48)
	Verbal executive	-1.62 (-3.40; 0.15)	-1.01 (-2.32; 0.3)	-0.47 (-1.84; 0.9)	0.11 (-1.25; 1.46)
	Visual and verbal span	-0.97 (-2.88; 0.94)	0.61 (-0.79; 2.01)	1.52 (0.06; 2.98)	0.61 (-0.84; 2.05)
	Working memory	-0.73 (-2.63; 1.17)	-1.20 (-2.59; 0.19)	0.07 (-1.39; 1.53)	0.00 (-1.44; 1.44)
	Verbal memory	-1.11 (-2.99; 0.77)	0.35 (-1.03; 1.73)	0.20 (-1.25; 1.64)	-0.74 (-2.17; 0.68)
	Gross motor	-1.10 (-2.72; 0.53)	-1.22 (-2.41; -0.03)	0.06 (-1.19; 1.31)	-0.84 (-2.07; 0.39)
	Fine motor	-0.49 (-2.31; 1.32)	-0.08 (-1.42; 1.25)	0.85 (-0.54; 2.25)	1.25 (-0.12; 2.62)
	Cognitive function of posterior cortex	-1.15 (-2.91; 0.62)	0.04 (-1.26; 1.33)	1.10 (-0.25; 2.45)	0.26 (-1.08; 1.60)

The first principal component (PC1) includes Pb, Cu, Se, and Zn, while the second principal component (PC2) includes iAs and MMA. The third principal component (PC3) includes Mo and Co, and the fourth principal component (PC4) includes AsB. All statistical models presented in the table were adjusted for the following confounding variables: cohort (Asturias, Gipuzkoa, Sabadell, or Valencia), body mass index (BMI) of the children (in kg/m^2), maternal social class (I-II, III, or IV-V), maternal education level (primary, secondary, or university), maternal number of previous live births (0, 1 or >1), and age at which the children were tested using the McCarthy Scales of Children's Ability (in years).

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Table S9: Confounding-adjusted associations between principal components (PCs) derived from male children's urinary metals and the McCarthy Scales of Children's Ability scores.

McCarthy Scales of Children's Abilities		PC1	PC2	PC3	PC4
Original functions	General cognition	-1.03 (-2.83; 0.78)	0.3 (-0.95; 1.54)	0.58 (-0.73; 1.90)	0.80 (-0.41; 2.01)
	Global verbal	-1.47 (-3.44; 0.49)	0.41 (-0.95; 1.76)	-0.11 (-1.54; 1.33)	0.94 (-0.37; 2.26)
	Global perceptive - performance	0.31 (-1.53; 2.15)	0.05 (-1.22; 1.32)	0.83 (-0.51; 2.17)	0.23 (-1.01; 1.46)
	Global quantitative	-1.96 (-3.82; -0.11)	0.01 (-1.28; 1.29)	0.90 (-0.46; 2.26)	0.30 (-0.95; 1.55)
	Global memory	-1.49 (-3.33; 0.36)	0.59 (-0.68; 1.87)	0.44 (-0.91; 1.79)	0.56 (-0.68; 1.80)
	Global motor	0.55 (-1.48; 2.58)	-0.93 (-2.32; 0.47)	1.44 (-0.03; 2.91)	0.49 (-0.87; 1.85)
New functions	Executive	-1.75 (-3.61; 0.11)	0.06 (-1.22; 1.35)	0.60 (-0.75; 1.96)	0.41 (-0.84; 1.66)
	Visual executive	-0.05 (-2.00; 1.9)	-0.07 (-1.41; 1.28)	0.75 (-0.67; 2.17)	-0.28 (-1.58; 1.03)
	Verbal executive	-2.30 (-4.2; -0.41)	0.12 (-1.19; 1.44)	0.39 (-1.00; 1.77)	0.70 (-0.57; 1.98)
	Visual and verbal span	-0.06 (-1.88; 1.76)	0.65 (-0.60; 1.90)	0.90 (-0.43; 2.22)	-0.03 (-1.25; 1.19)
	Working memory	-2.85 (-4.73; -0.97)	0.03 (-1.28; 1.33)	0.35 (-1.03; 1.73)	0.26 (-1.01; 1.53)
	Verbal memory	-0.94 (-2.94; 1.06)	0.10 (-1.27; 1.48)	-0.03 (-1.48; 1.43)	0.91 (-0.43; 2.24)
	Gross motor	0.41 (-1.77; 2.60)	-1.40 (-2.90; 0.10)	1.55 (-0.03; 3.13)	0.10 (-1.36; 1.56)
	Fine motor	0.45 (-1.36; 2.27)	0.11 (-1.14; 1.37)	0.65 (-0.67; 1.97)	0.68 (-0.54; 1.90)
	Cognitive function of posterior cortex	-0.79 (-2.66; 1.07)	0.33 (-0.96; 1.61)	0.18 (-1.18; 1.54)	0.89 (-0.36; 2.14)

The first principal component (PC1) includes Pb, Cu, Se, and Zn, while the second principal component (PC2) includes iAs and MMA. The third principal component (PC3) includes Mo and Co, and the fourth principal component (PC4) includes AsB. All statistical models presented in the table were adjusted for the following confounding variables: cohort (Asturias, Gipuzkoa, Sabadell, or Valencia), body mass index (BMI) of the children (in kg/m^2), maternal social class (I-II, III, or IV-V), maternal education level (primary, secondary, or university), maternal number of previous live births (0, 1 or >1), and age at which the children were tested using the McCarthy Scales of Children's Ability (in years).

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Table S10: Associations between principal components (PCs) derived from female children's urinary metals and the McCarthy Scales of Children's Ability scores: Unadjusted/Crude models.

McCarthy Scales of Children's Abilities		PC1	PC2	PC3	PC4
Original functions	General cognition	-1.63 (-2.88; -0.38)	-0.31 (-1.56; 0.94)	1.26 (0.03; 2.50)	1.05 (-0.22; 2.33)
	Global verbal	-1.84 (-3.10; -0.58)	-0.15 (-1.41; 1.12)	0.74 (-0.51; 1.99)	0.16 (-1.14; 1.45)
	Global perceptive - performance	-1.19 (-2.45; 0.06)	-0.29 (-1.55; 0.96)	1.63 (0.39; 2.86)	1.73 (0.45; 3.00)
	Global quantitative	-0.38 (-1.69; 0.94)	-0.46 (-1.77; 0.85)	0.55 (-0.75; 1.85)	0.84 (-0.5; 2.17)
	Global memory	-1.05 (-2.37; 0.28)	0.32 (-1.00; 1.64)	1.21 (-0.09; 2.52)	0.28 (-1.06; 1.63)
	Global motor	-1.60 (-2.84; -0.37)	-0.32 (-1.56; 0.92)	1.53 (0.31; 2.75)	0.47 (-0.79; 1.73)
New functions	Executive	-1.18 (-2.43; 0.06)	-1.01 (-2.26; 0.23)	0.32 (-0.92; 1.55)	1.12 (-0.15; 2.39)
	Visual executive	-0.52 (-1.79; 0.76)	-0.66 (-1.93; 0.61)	1.01 (-0.25; 2.26)	1.51 (0.22; 2.80)
	Verbal executive	-1.3 (-2.58; -0.02)	-0.97 (-2.25; 0.31)	-0.10 (-1.37; 1.17)	0.64 (-0.66; 1.95)
	Visual and verbal span	-0.60 (-1.93; 0.73)	0.71 (-0.61; 2.04)	1.47 (0.16; 2.78)	0.77 (-0.58; 2.12)
	Working memory	-0.65 (-2.00; 0.70)	-1.11 (-2.45; 0.23)	0.11 (-1.22; 1.45)	0.41 (-0.96; 1.78)
	Verbal memory	-1.80 (-3.13; -0.46)	0.58 (-0.76; 1.92)	1.10 (-0.22; 2.43)	-0.59 (-1.95; 0.78)
	Gross motor	-1.11 (-2.27; 0.04)	-0.73 (-1.88; 0.42)	0.74 (-0.40; 1.89)	-0.90 (-2.07; 0.28)
	Fine motor	-1.22 (-2.52; 0.08)	0.27 (-1.03; 1.57)	1.56 (0.28; 2.84)	1.63 (0.31; 2.95)
	Cognitive function of posterior cortex	-1.92 (-3.19; -0.64)	0.30 (-0.98; 1.58)	1.99 (0.74; 3.25)	0.78 (-0.53; 2.08)

The first four principal components (PCs) are as follows: PC1 includes Pb, Cu, Se, and Zn. PC2 includes iAs and MMA. PC3 includes Mo and Co. PC4 includes AsB.

Table S11: Associations between principal components (PCs) derived from male children's urinary metals and the McCarthy Scales of Children's Ability scores: Unadjusted/crude models.

McCarthy Scales of Children's Abilities		PC1	PC2	PC3	PC4
Original functions	General cognition	-0.52 (-1.87; 0.83)	0.31 (-1.04; 1.66)	0.49 (-0.87; 1.85)	0.84 (-0.48; 2.16)
	Global verbal	-0.10 (-1.50; 1.29)	0.34 (-1.05; 1.73)	-0.47 (-1.87; 0.93)	1.00 (-0.36; 2.37)
	Global perceptive - performance	-0.46 (-1.79; 0.87)	0.07 (-1.26; 1.39)	1.05 (-0.28; 2.38)	0.19 (-1.11; 1.49)
	Global quantitative	-1.38 (-2.69; -0.06)	0.18 (-1.14; 1.5)	0.94 (-0.39; 2.27)	0.37 (-0.92; 1.67)
	Global memory	-0.88 (-2.19; 0.44)	0.68 (-0.63; 2.00)	0.27 (-1.06; 1.60)	0.66 (-0.63; 1.95)
	Global motor	-0.13 (-1.55; 1.30)	-0.99 (-2.41; 0.43)	1.47 (0.04; 2.9)	0.03 (-1.37; 1.43)
New functions	Executive	-0.98 (-2.33; 0.36)	0.10 (-1.25; 1.45)	0.62 (-0.74; 1.98)	0.51 (-0.81; 1.83)
	Visual executive	-0.73 (-2.09; 0.63)	-0.12 (-1.48; 1.24)	1.09 (-0.28; 2.46)	-0.12 (-1.46; 1.21)
	Verbal executive	-0.96 (-2.30; 0.39)	0.20 (-1.15; 1.55)	0.24 (-1.12; 1.60)	0.76 (-0.56; 2.09)
	Visual and verbal span	-0.24 (-1.53; 1.05)	0.72 (-0.56; 2.01)	0.79 (-0.5; 2.09)	0.12 (-1.14; 1.39)
	Working memory	-1.71 (-3.01; -0.40)	0.21 (-1.10; 1.52)	0.36 (-0.96; 1.68)	0.37 (-0.91; 1.65)
	Verbal memory	-0.03 (-1.38; 1.32)	-0.07 (-1.42; 1.28)	-0.33 (-1.7; 1.03)	0.85 (-0.47; 2.17)
	Gross motor	0.10 (-1.37; 1.57)	-1.63 (-3.09; -0.17)	1.52 (0.04; 2.99)	-0.37 (-1.81; 1.07)
	Fine motor	-0.39 (-1.67; 0.89)	0.23 (-1.05; 1.51)	0.78 (-0.51; 2.07)	0.44 (-0.81; 1.70)
	Cognitive function of posterior cortex	-0.18 (-1.54; 1.18)	0.31 (-1.06; 1.67)	0.01 (-1.37; 1.38)	0.87 (-0.46; 2.21)

The first four principal components (PCs) are as follows: PC1 includes Pb, Cu, Se, and Zn. PC2 includes iAs and MMA. PC3 includes Mo and Co. PC4 includes AsB.

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