Exposure and Health
https://doi.org/10.1007/s12403-023-00585-6

ORIGINAL PAPER

Prenatal Metals Exposure and pre-adolescents' Emotional and Behavioral Problems

Manuel Lozano[1,2] · Karin Broberg[3,4] · Raquel Soler-Blasco[2,5] · Gabriel Riutort-Mayol[6] · Ferran Ballester[2,5,7] · Llúcia González[2,5,6] · Mario Murcia[2,8] · Joan O. Grimalt[9] · Fernando Gil[10] · Pablo Olmedo[10] · Simone Braeuer[11] · Maribel Casas[5,12,13] · Mònica Guxens[5,12,13,14] · Amaia Irizar[15,16] · Nerea Lertxundi[15,17] · Miren Begoña Zubero[15,16] · Loreto Santa Marina[5,15,18] · Sabrina Llop[2,5]

Received: 5 November 2022 / Revised: 28 June 2023 / Accepted: 29 June 2023

Abstract
Emotional and behavioral problems during childhood raise the risk of subsequent developmental of mental disorders. Our aim was to study the association between maternal metal and trace element concentrations during gestation and these problems in 9 year-old children. The study sample comprised Spanish mother-child pairs in the INMA project (n = 1003). Metals and trace elements (As, Cd, Co, Cu, Mo, Ni, Pb, Sb, Se, Tl and Zn) were measured in urine samples collected during pregnancy. Inorganic As metabolites were speciated in a subsample (n = 729). Emotional and behavioral problems were assessed using the Child Behavior Checklist (CBCL) composed of three scales: internalizing, externalizing and total problems. Sociodemographic, dietary and exposure to other environmental pollutants were obtained through questionnaires. Single nucleotide polymorphisms in brain- and metabolism-related genes *APOE*, *BDNF*, *GSTP1*, and *PON1* were determined in cord blood. Multivariate negative binomial models were used. The interaction with sex and genotypes was evaluated including interaction terms. A multi-element analysis was carried out by a principal component analysis. Higher concentrations of Cu, monomethylarsonic acid, and Pb during pregnancy were associated with an increased incidence ratio risk (IRR) between 4.6 and 7.5% for internalizing and externalizing problems for all three CBCL scales in the children. Increasing Mo, Ni and Co concentrations were associated with higher IRR for internalizing problems (up to 8%), and Cd for externalizing problems (6.7%). Modifications by sex and genotypes were found for several associations. Multi-element analysis associated multiple metals and trace elements (Ni, Cu, Se, Cd and Pb) with higher internalizing problems.

Keywords Metals · Arsenic speciation · Trace elements · Emotional and behavioral problems · Prenatal exposure

Introduction

Mental disorders among children and adolescents, including anxiety or depression, behavioral disorders, and attention-deficit/hyperactivity disorder (ADHD) (Fonseca-Pedrero et al. 2020) are a worldwide health challenge. Their pooled prevalence reached 20% during the last decade (Vasileva et al. 2021). Emotional and behavioral symptoms at the subclinical level raise the risk of subsequent development of mental disorders. These behavioral symptoms can be assessed during pre-adolescence through internalizing and externalizing problems (González et al. 2021). Internalizing or emotional problems are inward-directed symptoms (including behaviors related to anxiety, depression, somatic complaints and withdrawal) associated with changes in eating or sleeping habits, social withdrawal, violence, drug abuse, changes in personality, and teenage suicide (Liu et al. 2011). Externalizing, or behavioral, problems (including aggressive and oppositional behaviors, inattention/hyperactivity and emotion dysregulation are outward-directed symptoms (Jaspers et al. 2012) associated with poorer educational achievement, work incapacity in young adulthood and antisocial personality disorder (Narusyte et al. 2017).

The prevalence of internalizing and externalizing problems in Spanish adolescents was reported to be 22.6% and 14.6%, respectively (Ortuño-Sierra et al. 2014). Pre- and postnatal environmental exposures, including outdoor, indoor, chemical and lifestyle exposures, have been related to internalizing and behavioral problems (Maitre et al. 2021;

Extended author information available on the last page of the article

González et al. 2021). The European HELIX project (Maitre et al. 2018) based on six longitudinal population-based birth cohorts (N = 1287 6–11 year-old children), found that higher pre- and postnatal lead (Pb) and copper (Cu) blood concentrations, indoor air pollution, and unhealthy diet, were related to more internalizing and externalizing problems, whereas longer sleep duration, healthy diet and higher socioeconomic status were associated with fewer problems (Maitre et al. 2021). This study did not assess interaction effects. However, sex, age, and genetics may modify these associations during pre-adolescence and their persistence later in life. Behavioral problems appear to be more common and more persistent in boys than girls, whereas internalizing problems are more common in girls and increases with age (Jaspers et al. 2012; Klipker et al. 2018; Supke et al. 2021).

Some metals, metalloids and trace elements (abbreviated further on as metals) have been associated with mental disorders. In the general population, exposure to metals mainly occurs via diet, drinking water, air pollution, dust ingestion, cigarette smoking, medication or dietary supplements (17). Most metals are able to cross the placenta and the blood brain barrier (Iyengar and Rapp 2001). The foetus is thus particularly susceptible to exposure and, due to the development of the nervous system, to neurotoxicity. However, the relationship between metal exposure during the prenatal period and risk of internalizing and behavioral problems during childhood remains unclear. Most of this research has addressed exposure to lead (Pb), which has been associated with inattention (Xu et al. 2015) and impaired cognitive development (Yorifuji et al. 2011; Liu et al. 2014; Vigeh et al. 2014). Other metals, or combinations of them, have been linked to internalizing and behavioral problems. Combined prenatal exposure to manganese (Mn), zinc (Zn), and Pb have been associated with more internalizing problems in 8–11 year old children (Yousef et al. 2011; Horton et al. 2018). Further, increased cord blood cadmium (Cd) concentrations were related to more internalizing problems in 7–8 year old boys (Sioen et al. 2013), and cord blood methylmercury (MeHg) concentrations were associated with increased ADHD symptoms in 11 year old children (Boucher et al. 2012). Moreover, sex and genetic polymorphisms have shown to be modifiers of the toxicity of these metals (Napier et al. 2016; Broberg et al. 2019; Lozano et al. 2021). Therefore, further research is needed to assess the relationships between internalizing and behavioral problems and metals, as well as potential effect modifiers.

Some single nucleotide polymorphisms (SNPs) in *Apolipoprotein E (APOE), Brain Derived Neurotrophic Factor (BDNF), Glutathione S-Transferase Pi 1 (GSTP1)* and *Paraoxonase* 1 (*PON1) genes*, have shown a possible role in pathways related with metal neurotoxicity (Gaynor et al. 2009; Benke et al. 2014; Trucco et al. 2018; Della Torre et al. 2018; Paes et al. 2018; Cimino et al. 2020; Lozano et al. 2021; Treble-Barna et al. 2022). For instance, a Taiwanese study (Ng et al. 2015) observed that children's carriers of APOE ε4 showed a higher risk of internalizing and externalizing problems with increasing mercury at the age of two years. Additionally, we reported in a previous study a significant modification of the association between mercury and externalising problems scores according to genotypes for *BDNF* SNPs rs1519480 and rs7934165 (Lozano et al. 2021). Other SNPs are able to modify metals neurotoxicity by influencing their toxicokinetics; for instance, those in glutathione transferase related genes, considered one of the main mechanisms of metals elimination from the body. Thus, children from mother's homozygote for the rare allele (G) in the *GSTP1* rs1695 SNP presented **worse** mental development with increasing Hg in a prospective study of Seychellois population (Wahlberg et al. 2018).

The INMA, INfancia y Medio Ambiente (Childhood and Environment), project is a seven birth cohorts study in Spain that aims to study the association of the exposure to environmental pollutants during pregnancy with children and adolescent growth and development. Previous INMA studies have already assessed the associated factors to the exposure to these metals measured in urine (Lozano et al. 2022), as well as the relationship of some of them with neuropsychological scales measured in children during the study follow up (Soler-Blasco et al. 2020, 2021).

The objective of this study is to relate the prenatal exposure to metals with pre-adolescents' internalizing, externalizing and total problems assessed at 9 years. We also consider the effect modification of children's sex and genetic polymorphisms in *Apolipoprotein E* (*APOE*), *Brain Derived Neurotrophic Factor* (*BDNF*), *Glutathione S-Transferase Pi 1* (*GSTP1*), and *Paraoxonase 1* (*PON1*), which showed associations with behavioral problems in a previous INMA project (Lozano et al. 2021).

Methods

Study Population

The sample was composed by participants in the Valencia (east, n = 855), Gipuzkoa (north, n = 638) and Sabadell cohorts (north-east, n = 777) of the INMA Project with available metals measures. Recruitment took place by consecutive sampling of those women who met the inclusion criteria: ≥16 years, singleton pregnancy, non-assisted conception, delivery scheduled at the reference hospital, and no impairment hindering communication. The study protocol has been published elsewhere (Guxens et al. 2012). The study sample (n = 1003, 45.2%) comprised mother-child pairs for whom at least one the following metals: total arsenic (tAs), Cd, cobalt (Co), Cu, molybdenum (Mo), nickel (Ni), Pb, antimony (Sb), selenium (Se), thallium (Tl) and zinc (Zn) had been determined in urine at both the first and the third trimesters of gestation. Children's internalizing, externalizing and total problems were reported by mothers at 9 years old as well as the covariates included in the study. As metabolites (unmetabolized inorganic As (iAs), monomethylarsonic acid (MMA), dimethylarsinic acid (DMA) and arsenobetaine (AB) were determined in the first trimester only in Gipuzkoa and Valencia cohorts (n = 704). Characteristics for the study groups included and not included in the present study are shown in Supplemental Table S1.

Exposure Assessment

Metals and creatinine (to control for differences of urine dilution) were determined in urine following a protocol reported elsewhere (Lozano et al. 2022).

Urinary concentrations of total As (tAs), Cd, Co, Cu, Mo, Ni, Pb, Sb, Se, Tl, Zn and creatinine were available in the three INMA cohorts. Detailed description of methods, limits of detection and references can be found in the Supplementary Methods file. Metals concentrations and associated factors have been reported previously (Soler-Blasco et al. 2021; Lozano et al. 2022). The As speciation details are described in a previous INMA study (Soler-Blasco et al. 2021) and have been included in the Supplementary Methods file. A new variable of total inorganic As (tiAs: MMA + DMA + iAs) was created to be used in the statistical analysis. Urinary metal concentrations were expressed in µg/g of creatinine for descriptive purposes.

Internalizing, Externalizing and Total Problems Assessment

The presence of internalizing, externalizing and total problems in the children were assessed when they were 9 years of age (mean [standard deviation (SD)] = 9.33 (0.23) by using the Child Behavior Checklist test (CBCL) (Achenbach and Ruffle 2000). The test has been validated for the Spanish population (Rubio-Stipec et al. 1990) and the scores were used in its discrete form (natural numbers).

The CBCL provides eight syndrome scales (anxious/depressed, withdrawn/depressed, somatic complaints, social problems, thought problems, attention problems, rule-breaking behavior, and aggressive behavior) grouped by three composite scales: (1) internalizing problems (emotional scales: anxiety, depression, somatic complaints); (2) externalizing problems (behavior scales: rule-breaking and aggressive behavior); and (3) total problems, which is the sum of internalizing, externalizing, and three other scales which are considered mixed-syndrome scales that do not belong to either domain: social, thought, and attention problems (Achenbach and Rescorla 2001). The total problems composite scale finally quantifies general impairment and corresponds to the sum of scores from all eight syndrome scales, together with a group of 17 "other problems" items that do not belong to any specific syndrome scale. The CBCL is a one hundred and twelve-item parent-report questionnaire which refers to problems that might have occurred in the preceding two months and are rated on a three-point scale (0 = not true, 1 = somewhat or sometimes true and 2 = very or often true). Higher scores mean more behavioral problems in all scales. Parents assessed the behavioral functioning of pre-adolescents under the supervision of a trained psychologist blinded to the metals exposure status of the children.

Sociodemographic Covariates

Sociodemographic, environmental and lifestyle information was collected by trained interviewers using questionnaires at the first trimester (mean [SD] = 13.21 [1.58] weeks of gestation) and at the third trimester (mean [SD] = 32.3 [2.21] weeks of gestation). The maternal covariates used in this study were age (years), body mass index (BMI) (kg/m^2), country of birth (Spain, Latin America, other), education level (up to primary, secondary, university), parity (0, 1, ≥ 2), type of zone of residence (urban, semi-urban, rural), working during pregnancy (yes/no), smoking during pregnancy (yes/no), and passive exposure to tobacco smoke (yes/no). Children's sex (male/female) and their age at the time of the test (years) were also collected. Family social class was defined from the maternal or paternal occupation during pregnancy with the highest social class, according to a widely used Spanish adaptation of the International Standard Classification of Occupations, approved in 1988 (ISCO88) (Class I + II: managerial jobs, senior technical staff and commercial managers; class III: skilled non-manual workers; and class IV + V: manual and unskilled workers) (Domingo-Salvany et al. 2013).

Dietary Covariates

Information on diet during pregnancy was obtained from a validated food frequency questionnaire (FFQ) completed at the time of sampling (Vioque et al. 2013). We obtained data (expressed as the mean of the daily servings registered in both trimesters) on the consumption of dairy products, eggs, meat, seafood, fruit, vegetables, legumes, nuts, potatoes, cereals and pasta, bread, sweets, beverages with and without alcohol, coffee, tea and other infusions, animal and vegetal fats, processed food and dressings. Estimates on daily caloric intake were derived from the FFQ.

Environmental Covariates

Exposure to environmental pollutants was assessed through questionnaires at the third trimester of pregnancy according to the participants' perception and included the proximity of the house to a street with traffic (metres), the frequency with which cars pass near the house (constantly, frequently, rarely, never), the frequency with which heavy traffic passes near the house (constantly, frequently, rarely, never), and the proximity to a greenhouse (yes/no), to a crop field (yes/no) or to industrial activity (yes/no).

Percentages of agricultural areas, semi-natural areas and wetlands in use, urban fabric land use and green urban areas, and sports and outdoor leisure facilities near the house were obtained within a buffer area of 300 m for each geocode of the house (Lozano et al. 2022).

Prenatal exposure to air pollutants including particulate matter (<2.5 µm in diameter, $PM_{2.5,}$ µg/m^3) and nitrogen dioxide (NO_2) was also measured following the methodology explained in previous INMA studies (Iñiguez et al. 2009; Lertxundi et al. 2019) and detailed in the Supplementary Methods file.

Genetic Analysis

Cord blood DNA was used for genotyping in all three cohorts (n = 1221) and available for 716 (69.7%) of the samples included in the present study: Gipuzkoa (n = 295), Sabadell (n = 195), and Valencia (n = 226). Detailed information about genotyping and quality control is available in the Supplementary Methods file. We assessed several single nucleotide polymorphisms (SNPs) in genes previously related to metal toxicity and behavioral problems: rs429358, rs769449, and rs7412 (*APOE*); rs11030104, rs10835210, rs2049045, rs11030119, rs7934165, rs6265 and rs962369 (*BDNF*), rs1138272 and rs1695 (*GSTP1*); and rs662 (*PON1*) (Supplemental Table S2).

Statistical Analysis

Descriptive statistics were performed for CBCL behavioural tests (mean and standard deviation (SD)). Kruskal-Wallis tests were carried out between CBCL scores and categorical covariates for contrasting the scores between socio-demographic, environmental, dietary and genetic characteristics of the study population. Spearman tests adjusted for cohort were performed to evaluate correlations between CBCL scores and continuous covariates. Metal levels measured in the first and third trimesters were averaged to smooth the variance and better estimate the exposure during all the pregnancy. These averaged metals exposure were described calculating their geometric mean (GM) and confidence intervals (CI). Due to their skewed distribution, they were log2-transformed before their inclusion in statistical models. In order to identify possible susceptibility windows, we also did analysis separately for first and third trimester.

To eliminate the influence of seafood arsenicals, we calibrated the methylated and non-methylated species concentrations using a mathematical method proposed by Jones et al. (Jones et al. 2016). See Supplemental Methods file for details.

To assess the relation between prenatal exposure to metals during pregnancy and the scores on the CBCL behavioral tests, multivariate negative binomial models were built following a two steps procedure. Firstly, three outcome multivariate models were built for the CBCL scales in their discrete form (internalizing, externalizing and total problems). To do this, bivariate and multivariate negative binomial models adjusted for sex, age at test and cohort were assessed. Each multivariate model was built as follows: (1) Obtaining a sociodemographic multivariate basal model by using all the sociodemographic covariates previously associated with a p-value < 0.2 in the bivariate analyses. Following a backward elimination procedure, all the sociodemographic covariates associated with the outcome at a p-value level < 0.1 in the likelihood ratio test were retained in the model; (2) Dietary covariates were added to this sociodemographic basal model individually and those with a p-value < 0.2 were candidates to enter in the model. Following a backward elimination procedure, all the dietary candidate covariates associated with the outcome at a p-value level < 0.1 were also retained in the model; (3) The same procedure was repeated on this new sociodemographic and dietary basal model using environmental covariates in order to obtain the final outcome multivariate model. Models with dietary covariates were also adjusted for caloric intake.

Secondly, each log2-averaged metal variable was introduced into each outcome multivariate model obtaining forty-five multivariate models. The log2-averaged metal concentrations (unadjusted for creatinine) were included in the models with urinary creatinine added as a separate independent variable (Barr et al. 2005). All covariates associated with each metal exposure described in a previous INMA study carried out on the same sample were considered to be potential confounders (Lozano et al. 2022). This procedure was repeated using each metal individually measured at first and third trimesters in order to assess the difference in associations between metals in early and late pregnancy. The coefficients of these negative binomial models are presented as Incidence Risk Ratios (IRR) and they mean the risk of a one-point of increase in the score with one unit of the exposure increase. The associations were considered statistically significant in the final multivariate models at the p-values < 0.05 and marginally significant at the p-values < 0.1.

Generalized additive negative binomial models were also built to evaluate non-linear patterns. Linear and non-linear models were compared through Akaike (AIC) scores. Then, the model with the lowest AIC was chosen and checked with graphical examination. Segmented models were used to determine possible break-points in the linear association between each outcome and metals concentrations (Muggeo 2003).

Effect modifications by children's sex and SNPs were evaluated through inclusion of an interaction term in the models. Metals coefficients according to each sex and genotype were graphically evaluated. Multi testing correction by false discovery rate (FDR) was applied for the SNPs effect assessing.

Sensitivity analyses were performed excluding the participants from Sabadell cohort due to its different analytical methodology for metal analysis. See Supplemental Methods file for details.

Additionally, a multi-element analysis was carried out by means of a principal component analysis (PCA) with varimax normalized rotation (Kaiser 1958), using only the urinary metal concentrations (as the first- and third-trimester average concentrations) adjusted for creatinine. PCA was used in order to assess correlation patterns and identify clusters among metals. These clusters may indicate which urinary metals are associated with each other based on sources of exposure and the influence of associated sociodemographic, environmental and dietary factors (Bommarito et al. 2019). The main principal components were included in the multivariable adjusted models for each CBCL outcome to identify clusters associated with CBCL scales.

The validity of the regression models was tested by residual analysis. No influential data was identified by graphical representation. Collinearity diagnostics were conducted on the final models.

Statistical analysis was performed with the R statistical package (v. 4.1.1) (R Core Team 2017).

Results

Children's CBCL scores according to sociodemographic, dietary and environmental characteristics are shown in Supplemental Table S3. The mean age of the mothers at the time of conception was 30.6 years, around 92% of them were born in Spain, around 35% had a university degree, more than 40% belonged to the lowest social class and almost 80% lived in urban and semi-urban areas. Around 85% of the women worked and did not smoke during pregnancy. Children from the Gipuzkoa cohort showed better behavioral test results for all three scales, i.e. the lowest CBCL scores. Children showed higher scores (worse test results) for all scales when living near a greenhouse (Supplemental Table S3). Children also showed higher scores when their mothers were born abroad, had higher BMI, a low educational or social class level, were non-workers, active or passive smokers, or had a higher consumption of potatoes, bread or meat (especially red and processed meat) during pregnancy (Supplemental Table S3). Boys showed higher scores than girls, but only for externalizing problems. Statistically significant positive correlations were found between MMA concentrations and internalizing and total problems. Details, discussion about metals concentrations and their contextualization with respect to other studies, and metals correlations have been shown in a previous study with the same population (Lozano et al. 2022). Regarding the differences of the study participants not included in the present study, we found higher proximity to traffic, agricultural areas and crop fields, and lower fish and cereals consumption (Supplemental Table S1).

Variables included in the multivariate models for the internalizing, externalizing and total problems scales are shown in Figure S1. Figure 1 shows the Incidence Rate Ratios (IRR) for the averaged log2-transformed maternal urinary metal concentrations in relation to the three CBCL scales scores in the final adjusted multivariate models (effect estimates are presented in Supplemental Table S4). Higher maternal urinary concentrations of Cu, MMA and Pb were associated with a higher incidence ratio risk (IRR 4.6–7.5% of increase) for all three CBCL scores in the children. Co, Mo, and Ni were associated with higher risk for internalizing problems (up to 8% of increase), and Cd with higher risk for externalizing problems (6.1%). The arsenic metabolism efficiency (by adding percentages of the individual As metabolites to the adjusted multivariable models; data not shown) only showed marginal significance for %MMA for internalizing problems (1.3% of increase in IRR, p-value = 0.066). Detailed information regarding IRR coefficients for each adjusted multivariable model, effect modification by sex, and sensitivity models can be found in Supplemental Table S4. Sensitivity models excluding Sabadell (n = 729) did not show discrepancies compared with multivariable models including all three cohorts (n = 1003).

Some of the associations between maternal metals concentrations and children's scores showed a sex-dependent pattern with interaction p-values statistically significant for Ni and Se. (Supplemental Table S4). Ni concentrations were positively associated with internalizing problems only in boys (IRR increased in 10.4%, p-value = 0.006), and Se

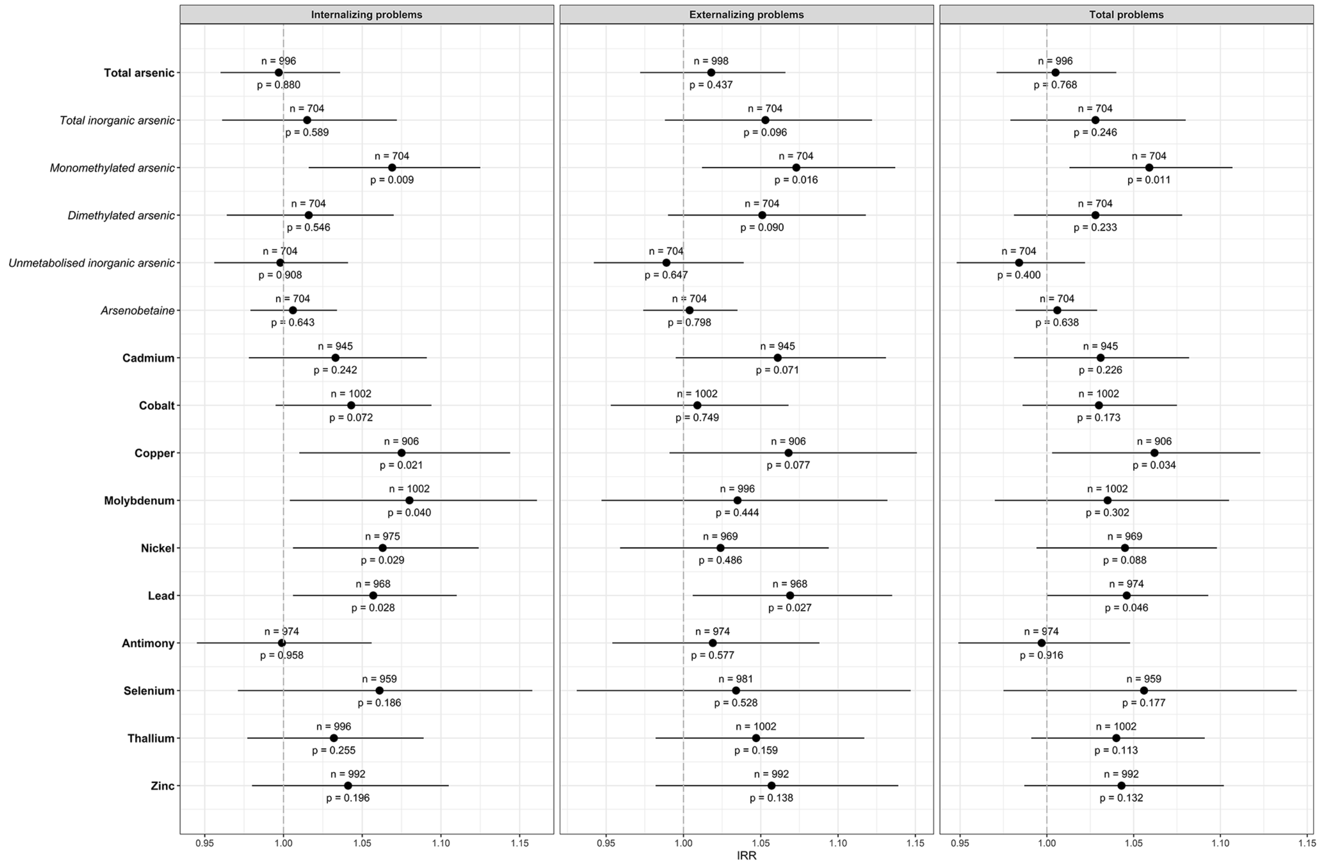

Fig. 1 Maternal log2-metal concentrations in urine and incidence risk ratios (IRR) for internalizing, externalizing and total problems
Only metals coefficients adjusted for covariates are showed. All models were adjusted for smoking, parity, maternal education, BMI, cohort, sex, age at the time of the test and creatinine. Confounders from each metal basal model modifying more than 10% of the metal coefficient in each multivariable model were also incorporated (see supplemental material). Metals in bold were assessed in all three cohorts (Gipuzkoa, Sabadell and Valencia) and As metabolites only in Gipuzkoa and Valencia cohorts. IRRs mean the risk of a one-point of increase in the score with one unit of metals concentration increase.

was positively related to total problems in girls (IRR 9.5% of increase, p-value = 0.048). Other metals' coefficients showed a non-significant sex-dependent effect. Pb concentrations were positively associated with all three CBCL scales only in boys (an IRR increase of 7.3%, 9.6% and 5.1% for the internalizing, externalizing and total problems scales, respectively) and Zn concentrations were positively associated with the three scales only in girls (7.6%, 9.4% and 6.4%) (Supplemental Figure S2). Boys only showed associations with maternal total iAs (10.2% and 6.1% higher IRR for externalizing and total problems, respectively), MMA (8.2% for internalizing problems), DMA (9.3% and 5.6% for externalizing and total problems, respectively) and Ni (7.9% for total problems) (Supplemental Figure S3). Girls only showed associations with maternal Co (6.0% and 5.1% for internalizing and total problems, respectively), Cu (7.9.% and 6.5% for externalizing and total problems, respectively), Se (12.9% for internalizing problems), and Tl (9.6% and 7.0% for externalizing and total problems, respectively) (Supplemental Figure S4).

Most significant relationships between metals concentrations and CBCL scales were linear (Supplemental Table S4). There were some exceptions with non-linear relationships showing inverted U shapes (with specific concentration break-points in the linear relationships) according to the plotting and AIC criteria: MMA concentrations showed an estimated break-point at 0.95 µg/L (p-value of the smooth term 0.019) for internalizing problems scale, 0.72 µg/L (0.006) for externalizing problems scale, and 0.92 µg/L (0.023) for the total problems scale); DMA showed estimated break-points at 5.2 µg/L (0.005) for externalizing problems and 5.6 µg/L (0.018) for total problems; Co showed a break-point at 0.16 µg/L (0.076) for internalizing problems; and Mo a break-point at 15.8 µg/L (0.085) for internalizing problems and 27.2 µg/L (0.086) for total problems. No differences in associations between metals in early and late pregnancy were found excepting in the case of Ni, which only showed significance at the first trimester (4.8%). Coefficients details for all multivariable models are shown in Supplemental Table S4. These inverted U-shape nonlinear relationships indicate higher scores with increasing metal concentrations until reaching the linearity break-point. For increasing metal concentrations above the breakpoint, the scores no longer correlate with metal levels or even decrease, showing a potential dose-effect association.

PCA with varimax normalized rotation pointed to three optimal principal rotated components (RC) by using non-graphical solutions for Cattell's scree test (Raîche et al. 2013), which explained 57% of the total variance (Supplemental Figure S5). PCA clustered the metals to each RC as follows: (1) RC1: Ni + Cu + Se + Cd + Pb (24% of explained variance); (2) RC2: Co + Zn + Mo + Sb (18%); and (3) RC3: AB + DMA + MMA + iAs (15%) (Supplemental Table S5). These RC were assessed as exposures in the multivariable adjusted models for each outcome. RC1 showed marginal significance for internalizing and total problems (9.2% and 8.3% increased IRR, respectively).

Several SNPs modified the associations between maternal metal concentrations and CBCL scores in the children after correction for multiple testing (FDR < 0.1, Fig. 2). The association between Co and internalizing problems differed according to *APOE* rs429358 (unadjusted interaction term p-value = 0.024), *BDNF* rs11030119 (0.029) and rs962369 (0.025) genotypes. Only children homozygous for common alleles of rs429358, rs11030119, and rs962369 showed increased IRR of internalizing problems with increasing Co concentrations (17.4%, 6.2% and 6.3%, respectively), whereas children being variant homozygotes of rs11030119 or rs962369 had lower scores (IRR −21.9% and −22.7%, respectively). The associations between Pb and MMA and total problems were modified according to *BDNF* rs11030104 (0.010 and 0.004, respectively) genotype. For total problems, children being homozygous for the common allele of rs11030104 showed higher risk with increasing Pb (6.7% increase) and children homozygous or heterozygous for rs11030104 showed higher risk with increasing MMA concentrations (11.3% and 11.2%, respectively). For both Pb and MMA, homozygous carriers of the variant allele of

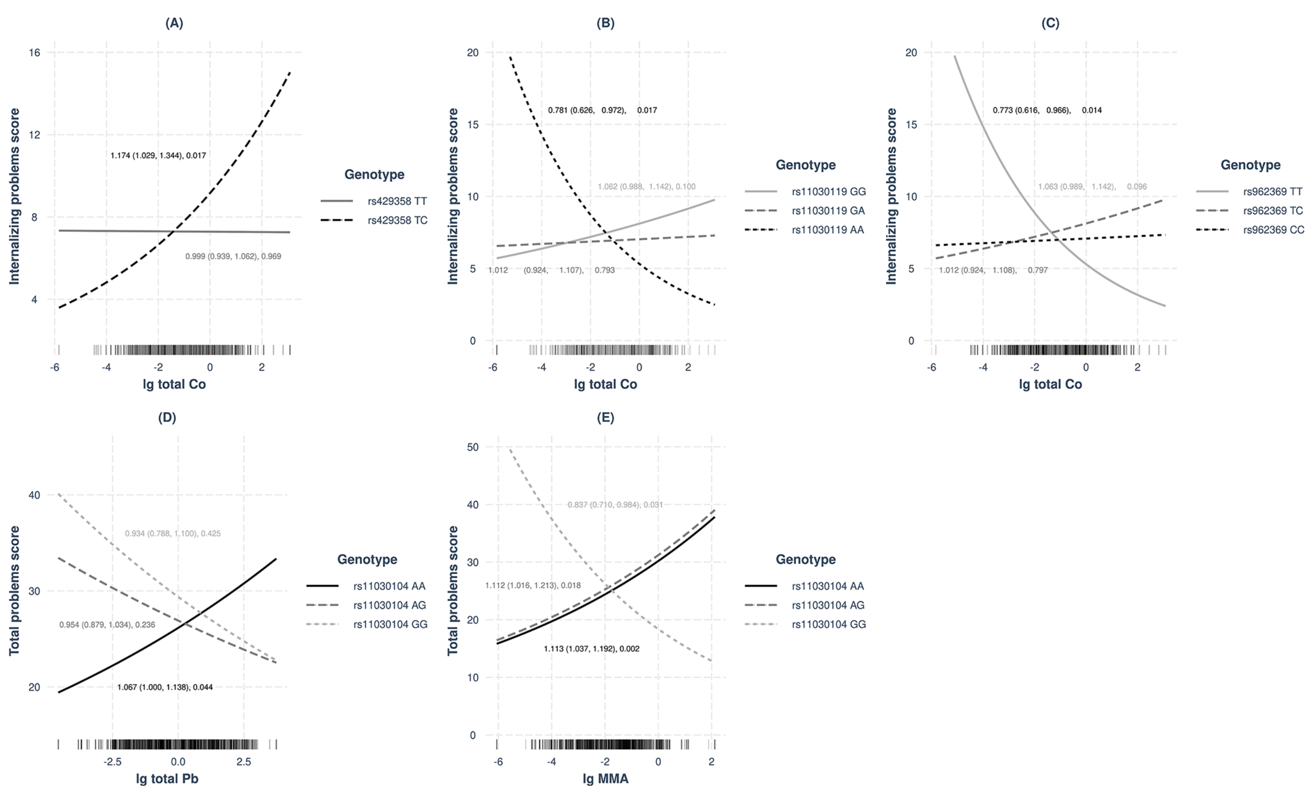

Fig. 2 Incidence risk ratios (IRR) for cobalt (Co) with modified effect on the internalizing problems scores according to genotypes of *APOE* rs429358 (A), *BDNF* rs11030119 (B) and *BDNF* rs962369 (C); and for lead (Pb) and monomethylated arsenic (MMA) on total problems scores depending on *BDNF* rs11030104 (D and E, respectively)
Only models with corrected interaction p-values with FDR < 0.1 are shown. IRR (Confident Interval 95%) and p-values included in the plots below to the metal exposure for each genotype of reference. IRRs mean the risk of a one-point of increase in the score with one unit of metals concentration increase

rs11030104 showed decreasing scores (16.3% decrease). No interactions were found for *PON1* and *GSTP1*.

Discussion

The aim of the study was to study the relationship between prenatal urinary concentrations of metals and internalizing, externalizing and total problems assessed during pre-adolescence, taking multiple covariates and confounders into account. We observed that increasing concentrations of some metals in maternal urine during pregnancy increased the risk of presenting internalizing and externalizing problems among children at 9 years of age. Specifically, increasing prenatal concentrations of Cu, MMA and Pb were associated with an increased risk for all three problem scales. Increasing prenatal concentrations of Mo, Ni and Co were associated with higher risk for internalizing problems, and Cd with externalizing problems. We also performed a multi-element analysis and we found that the mixture of Ni, Cu, Cd, Se and Pb increased the risk of internalizing and total problems, although, the association was only marginally significant. *BDNF* seem to be the most important susceptibility gene for several metals. Boys are more sensitive to heavy metals whereas girls to trace elements.

Early life exposure to Pb has previously been associated with different behavioral outcomes during pre-adolescence; for instance, blood Pb levels measured during late pregnancy increased the risk of behavioral problems in 5 year-old boys from Korea (n = 1751) (Joo et al. 2018), and worse executive functions (n = 549) in 6–7 year-old Mexican children (Merced-Nieves et al. 2022a). Additionally, in both studies sexually dimorphic differences on the prenatal Pb neurobehavioral function effects were observed, similar to our study; boys obtained worse scores with increasing Pb for all three scales. The relationship between prenatal exposure to a mixture of Pb, Mn, Hg and Se (measured in maternal erythrocytes) and children's social, emotional, and self-regulatory behaviors at 7 years was evaluated in mother-child pair participants in the VIVA Project in the USA (n = 1006) (Fruh et al. 2019). In this study, a trend of worsening performance with increasing concentrations of the mixture was observed, with Pb and Se as the largest contributors. A combined effect of particulate matter, Pb, Mn, and Ni airborne exposure during pregnancy was associated with increased risk of autism in 3–18 years old children from the USA (n = 325 cases and 22,101 controls) (Roberts Andrea et al. 2013).

In our study, prenatal exposure to other toxic metals, such as Cd, Ni and MMA, also increased the risk of children's behavioral problems. The literature regarding behavioral effects associated with prenatal exposure to these metals is more limited and the results are heterogeneous. A prospective study of Belgian children highlighted an increased risk for internalizing problems in 7- to 8-year old boys, but not in girls, with higher cord blood Cd concentrations (n = 270) (Sioen et al. 2013). Similarly, in a Chinese study, maternal blood Cd concentrations were negatively associated with the social domain among 12 months old children (n = 300) (Wang et al. 2016). Contrarily, Cd measured in maternal blood did not correlate with problematic behavior among 3–5 years old children from Greenland (n = 102) (Kornvig et al. 2021). In a smaller study conducted on pregnant women of Arab-Bedouin origin (Karakis et al. 2021), a link was observed between maternal concentrations of several metals, including Li, Co, Ni, Sr, V, As, Mo, and Cd and behavioral outcomes in the children at an age of 6 years (n = 111). In a recent study conducted in Mexico, the behavioral effects of a mixture of maternal blood Mn and Pb and urinary Cd and total As (which may include the non-toxic organic arsenic) were evaluated among 322 6–7 year-old children (Merced-Nieves et al. 2022b). This mixture was associated with a significant decrease in several task performance measures (related with learning behavior), with Pb, Cd and As being those which contributed the greatest weight to the mixture effect. Regarding prenatal exposure to arsenic, in a mother-child cohort from rural Bangladesh (n = 1523) prenatal exposure to total As (which in this cohort was shown to mainly reflect inorganic arsenic) was associated with several outcomes related with behavior, especially in girls (Hamadani et al. 2011; Vahter et al. 2020). It should be noted that a previous publication conducted on the same population as the present study reported an inverse association between maternal urinary MMA concentrations and the scores obtained by the children in the McCarthy Scales of Children Abilities at 5 years of age (Soler-Blasco et al. 2022).

Associations between maternal levels of Cu, Mo and Co, and children's behavioral problems have been scarcely reported in the literature. A study conducted with Polish children at an early school age evaluated the association between maternal blood (and hair for Hg) concentrations of Pb, Cd, Hg, Se, Zn and Cu and neurodevelopmental outcomes including externalizing and internalizing problems (n = 436) (Garí et al. 2022). Authors reported some long-term deleterious effects of two neurotoxic metals (Hg and Pb) and two micronutrients (Zn and Se) on child behavior and IQ. A recent multi-element analysis performed in the US Rhode Island Child Health Study (n = 192) found a positive association (worse tests results) between increased levels of a mixture of placental metals (Cd, Co, Cu, Fe, Mn, Mo, Se and Zn) and atypical behavior in newborns (Tung et al. 2022), with Cd having the largest weight in the mixture effect.

Human exposures to metals and metalloids frequently occur as mixtures, and hence it is important to consider the joint action of these elements, which may produce additive, synergistic/potentiating, or antagonistic effects (Nordberg et al. 2014). In the present study, we observed an increased risk of internalizing and total problems related to the mixture of Ni, Cu, Cd, Se and Pb. If we consider their action individually, Cd was not associated with the CBCL scores and Se only for females. This fact could be indicating that toxicity of Cd or Se seems to be enhanced by the action of other metals, such as Cu, Ni or Pb. At present, there is much to be learned about the joint action of both toxic and trace elements, and this is clearly a critical area of research. There is a pressing need for an in-depth discussion of the public health implications of exposure to mixtures of metals and the role of modified dose-response relationships in the risk assessment of such exposures.

Comparability between the different studies evaluating the influence of prenatal levels of metals on children's behavior is challenging, since the exposure assessment, the tests for internalizing and externalizing problems, and age of the children tested differ between studies. Different biological samples (urine, serum, plasma, erythrocytes, total blood, placenta) taken at different time points during the prenatal period have been used. Urinary metals have been frequently used as an indicator of recent exposure and it is considered as an appropriate biomarker for some metals, such as As, Cd or Co (Nordberg et al. 2014). However, for other metals, such as Pb, concentrations in blood are considered more suitable for biomonitoring than urine because it reflects the combination of exposure during the previous months and exposure over the past few years (Nordberg et al. 2014). Blood, plasma, serum, or urinary Ni, Se, Zn and Mo measurements can be useful monitors of environmental or occupational exposures (Nordberg et al. 2014). Most metals evaluated in this study are able to cross the placenta and blood-brain barriers and provoke changes, such as neurotransmitter impairment or brain cell death and degeneration, which seem to increase the susceptibility to develop negative effects in the neuropsychological development throughout childhood (Grandjean and Landrigan 2006; Heindel et al. 2015).

The children's genetic background could be also influencing the heterogeneity of the results observed in the different studies. The main genetic effect modification was found for the *BDNF* gene. *BDNF* is coding for a polypeptide known to play an important role in the survival, differentiation and outgrowth of neurons during development and adulthood (Lu et al. 2014). Additionally, several lines of evidence suggest that BDNF plays a role in the pathogenesis of some behavior problems, such as ADHD (Luo et al. 2020; Chang et al. 2020). Lower blood levels of BDNF have been observed among children with ADHD and with autism spectrum disorders (Skogstrand et al. 2019). Previous studies, mainly on mercury, have reported that different *BDNF* genotypes are able to moderate the impact of early exposure to this metal on child neurodevelopment (Julvez et al. 2013, 2019; Woods et al. 2014). In a previous study on the INMA cohort, the association between postnatal mercury exposure and children's behavior was modified by *BDNF* rs1519480, rs7934165, and rs7103411 (Lozano et al. 2021). The literature about other *BDNF*-metal interactions and neurotoxicity is still limited; however, exposure to As, Pb or Cd, has been associated with alterations in serum concentrations of BDNF (Karim et al. 2019; Malavika et al. 2021; Rodríguez-Carrillo et al. 2022) and in some studies these alterations were associated with children's neuropsychological development. Epigenetic changes of *BDNF* has been also associated with metal exposure (Karim et al. 2019). In the present study we observed a significant effect modification for the *BDNF* rs11030119, rs11030104 and rs962369 polymorphisms. Specifically, the genotypes at risk of obtaining worst internalizing and total problems with increasing prenatal exposure to Co, Pb or MMA were AA in rs11030104, GG and GA in rs11030119 and TT and TC in rs962369.

For *APOE* rs429358: children carrying the C allele had worse internalizing problems scores with increasing Co. APOE is a crucial factor involved in cholesterol metabolism, neurite growth, and neuron repair in the central nervous system. The rs429358 corresponds to an arginine to cysteine amino acid exchange at amino acid position 130. This change determines the Ɛ4 genotype, a major genetic susceptibility factor for neurodegenerative diseases, including Alzheimer (Jayadev 2022). Ng et al. (2013) found that ε4 carriers prenatally exposed to methyl-Hg had poorer neurodevelopment and higher scores in the general internalizing, emotionally reactive, and anxiety/depression, as well as CBCL total scores, at 2 years of age (Ng et al. 2013). Contrarily, a study in mother-infant pairs living in and around Mexico City (Wright et al. 2003) observed a possible protection against Pb exposure among Ɛ4 carriers; the negative association between Pb and the mental development scale at 24 months was stronger among non-Ɛ4 carriers. Polymorphisms in *APOE* seem to play a role on metals neurotoxicity and further studies exploring this mechanism should be developed to settle the role of this gene.

In relation to pre-adolescent's sex we observed some differences. Girls had a higher risk of total problems with increasing Se compared with boys. Boys had a higher risk of internalizing and externalizing problems with increasing Ni concentrations than girls. Although other interactions were not significant, they are worth noting. For instance, Pb concentrations were positively associated with all three CBCL scales only in boys, and Zn concentrations were positively associated with all scales only in girls. Boys only showed associations of MMA and Ni with internalizing and total problems, and tiAs and DMA with externalizing and total problems. Girls only showed significant associations of Co with internalizing and total problems and Cu and Tl with externalizing and total problems. Previous studies have evaluated the sex-specific toxic effect of some of these metals during pre-adolescence. Thus, although both sexes are affected by Pb exposure, the incidence, manifestation, and severity of outcomes appears to be stronger in boys than in girls (Singh et al. 2018). These differences have been explained by the neuroprotective role of some sex steroid hormones (estradiol and progesterone), sex-dependent effects of Pb on gene expression, and sex specific epigenetics changes induced by Pb (Khalid and Abdollahi 2019). Other metals seem to show sex differential neurotoxic effects. For instance, in a birth cohort from Massachusetts (USA), a doubling of cord blood Mn was positively associated with internalizing symptoms for girls (Rokoff et al. 2022). In the PRISM pregnancy cohort in Boston and New York (USA), prenatal exposure to a mixture of seven metals was associated with significantly increased scores on the temperamental domain of fear in infants, with girls showing particular sensitivity Information regarding sex differences in susceptibility of most part of the metals is still too scarce to draw any definitive conclusion and more research is highly warranted about this matter.

Strengths and Limitations

This study is based on a large prospective study of Spanish birth cohorts, with extensive information about environmental, dietary and life-style factors. We measured multiple maternal metals during the pregnancy and studied metals interactions and their effect using a multi-element approach. Also, we have included speciated As in the analyses and this is the first study, as far as we know, evaluating the effects of prenatal concentrations of inorganic As and its methylated forms on behavioural outcomes. One advantage of this study is its prospective design, which made it possible to obtain detailed information concerning maternal and child characteristics that may affect neuropsychological development. However, this study was conceived to specifically assess the prenatal effect and we did not assess the confounding effect of postnatal covariates which could potentially affect neurodevelopment such as breastfeeding (Amaro et al. 2022), tobacco exposure (Anadón et al. 2022), as well as lower social class, multiparity and greater number of older siblings (Pham et al. 2022). Also, postnatal covariates collected in the INMA study will cause a loss of sample size and a consequent lack of robustness in the confounding assessing. Additionally, the prospective follow-up will also enable us to study the persistence of the metal neurotoxicity at further ages, although the lack of later CBCL scores or school information did not allow us to evaluate potential differences in children's behavior across time points and locations. We also evaluated the influence of certain factors, such a genetic polymorphism and children's sex, on the effect of metals on children's behavior. This could contribute to the identification of high-risk subpopulations and improve public health interventions aimed at preventing the neurobehavioral impacts of metals exposure. The study has other limitations. First, metals concentrations were measured at two laboratories. However, we carried out sensitivity analyses excluding the Sabadell cohort without finding significant discrepancies. In addition, only 45% of the study population included initially in the cohorts participated in this study, the main causes being the unavailability of metals concentrations at both trimesters in order to assess the exposure during all the pregnancy or the lack of children's behavioral assessments at 9 years. Finally, the PCA used to assess the multi-element analysis showed inconclusive results. However, the results may be useful to develop further research focused in multi-pollutant effects.

Conclusions

In this large prospective birth cohort study we found that increasing prenatal levels of Cd, Co, Cu, tiAs, DMA, MMA, Mo, Ni, Pb, Se, Tl and Zn were associated with increased emotional and behavioral problems during pre-adolescence. We also observed that this association might be modified by certain genetic, sex and sociodemographic factors. Identifying susceptibility factors and other modifiers is important for understanding potential biological mechanisms and for the protection of vulnerable subpopulations. Larger prospective studies designed to address susceptibility factors may help unravel the complexity in metals-neurodevelopment associations. As we progress toward investigating the effects of the exposome, it is imperative that modifying factors are more fully examined. The present study shows that some modifiable lifestyles, food intakes, environmental factors, as well as sex and genetics, could be associated with prenatal exposure to metals and their effect on these disorders later in life, which may be considered in further studies to assess public health interventions.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s12403-023-00585-6.

Author Contributions All authors contributed to the study conception and design. Manuel Lozano: Writing - Original Draft, Formal análisis, Methodology. Karin Broberg: Conceptualization, Visualization, Supervision. Raquel Soler-Blasco: Validation, Methodology. Gabriel

Riutort-Mayol: Writing - Review & Editing. Ferran Ballester: Funding acquisition, Writing - Review & Editing. Llucia Gonzalez: Conceptualization, Writing - Review & Editing. Mario Murcia: Methodology, Data Curation. Joan O. Grimalt: Validation, Writing - Review & Editing. Fernando Gil: Validation, Writing - Review & Editing. Pablo Olmedo: Validation, Writing - Review & Editing. Simone Braeuer: Resources, Writing - Review & Editing. Maribel Casas: Funding acquisition, Writing - Review & Editing. Monica Guxens: Investigation, Funding acquisition. Amaia Irizar: Investigation, Writing - Review & Editing. Nerea Lertxundi: Investigation, Writing - Review & Editing. Miren Begoña Zubero: Investigation, Writing - Review & Editing. Loreto Santa Marina: Funding acquisition, Writing - Review & Editing. Sabrina Llop: Conceptualization, Writing - Original Draft, Supervision, Funding acquisition.

Funding Open Access funding provided thanks to the CRUE-CSIC agreement with Springer Nature. We are grateful to all the participants and their families for taking part in this study. We also appreciate the generous contribution in the study of all INMA members. A full roster of the INMA Project researchers can be found at http://www.proyectoinma.org/presentacion-inma/listado-investigadores/en_listado-investigadores.html. This study was supported by grants from Spanish government through the Ministry of Universities under the grant CAS21/00008 and grant for the requalification of the Spanish University, financed by the European Union, NextGeneration EU, Instituto de Salud Carlos III [FIS-FEDER: 13/1944, 16/1288, 17/00663 and 19/1338; FIS-FSE: 17/00260; Miguel Servet-FSE: MSII20/0006, FIS-PI06/0867, FIS-PI09/00090, CPII18/00018], CIBERESP, Department of Health of the Basque Government (2005111093, 2009111069, 2013111089, 2015111065), Generalitat Valenciana [BEST/2020/059, AICO/2020/285 and CIAICO/2021/132] and the Provincial Government of Gipuzkoa (DFG06/002, DFG08/001 and DFG15/221 and DFG 89/17). We also acknowledge support from the Spanish Ministry of Science and Innovation and the State Research Agency through the "Centro de Excelencia Severo Ochoa 2019–2023" Program (CEX2018-000806-S), and support from the Generalitat de Catalunya through the CERCA Program.

Data Availability The datasets generated during and/or analysed during the current study are not publicly available due to the Ethics Committee policy but are available from the corresponding author on reasonable request.

Declarations

Competing Interests The authors have no relevant financial or non-financial interests to disclose.

Ethics Approval This study conforms to the principles embodied in the Declaration of Helsinki.

Consent to Participate Informed consent was obtained from all participants in each phase, and the study was approved by the hospital ethics committees in each participating region.

References

Achenbach TM, Rescorla L (2001) Manual for the ASEBA school-age forms & profiles. an integrated system of multi-informant assessment

Achenbach TM, Ruffle TM (2000) The child behavior checklist and related forms for assessing behavioral/emotional problems and competencies. Pediatr Rev 21:265–271. https://doi.org/10.1542/pir.21-8-265

Amaro A, Baptista FI, Matafome P (2022) Programming of future generations during breastfeeding: the intricate relation between metabolic and neurodevelopment disorders. Life Sci 298:120526. https://doi.org/10.1016/j.lfs.2022.120526

Anadón A, Ares I, Martínez-Larrañaga MR, Martínez MA (2022) Chap. 21 - Cigarette and E-cigarettes smoking and reproductive and developmental toxicity. In: Gupta RC (ed) Reproductive and Developmental Toxicology (Third Edition). Academic Press, pp 395–420

Barr DB, Wilder LC, Caudill SP et al (2005) Urinary creatinine concentrations in the U.S. population: implications for urinary biologic monitoring measurements. Environ Health Perspect 113:192–200. https://doi.org/10.1289/ehp.7337

Benke KS, Nivard MG, Velders FP et al (2014) A genome-wide Association Meta-analysis of Preschool internalizing problems. J Am Acad Child Adolesc Psychiatry 53:667–676e7. https://doi.org/10.1016/j.jaac.2013.12.028

Bommarito PA, Kim SS, Meeker JD et al (2019) Urinary trace metals, maternal circulating angiogenic biomarkers, and preeclampsia: a single-contaminant and mixture-based approach. Environ Health 18:63. https://doi.org/10.1186/s12940-019-0503-5

Boucher O, Jacobson SW, Plusquellec P et al (2012) Prenatal methylmercury, postnatal lead exposure, and evidence of attention deficit/hyperactivity disorder among Inuit children in Arctic Québec. Environ Health Perspect 120:1456–1461. https://doi.org/10.1289/ehp.1204976

Broberg K, Taj T, Guazzetti S et al (2019) Manganese transporter genetics and sex modify the association between environmental manganese exposure and neurobehavioral outcomes in children. Environ Int 130:104908. https://doi.org/10.1016/j.envint.2019.104908

Chang JP-C, Mondelli V, Satyanarayanan SK et al (2020) Cortisol, inflammatory biomarkers and neurotrophins in children and adolescents with attention deficit hyperactivity disorder (ADHD) in Taiwan. Brain Behav Immun 88:105–113. https://doi.org/10.1016/j.bbi.2020.05.017

Cimino S, Carola V, Cerniglia L et al (2020) The μ-opioid receptor gene A118G polymorphism is associated with insecure attachment in children with disruptive mood regulation disorder and their mothers. Brain and Behavior 10:e01659. https://doi.org/10.1002/brb3.1659

Della Torre OH, Paes LA, Henriques TB et al (2018) Dopamine D2 receptor gene polymorphisms and externalizing behaviors in children and adolescents. BMC Med Genet 19:65. https://doi.org/10.1186/s12881-018-0586-9

Domingo-Salvany A, Bacigalupe A, Carrasco JM et al (2013) Proposals for social class classification based on the Spanish National classification of occupations 2011 using neo-weberian and neo-marxist approaches. Gac Sanit 27:263–272. https://doi.org/10.1016/j.gaceta.2012.12.009

Fonseca-Pedrero E, Ortuño-Sierra J, Pérez-Albéniz A (2020) Emotional and behavioural difficulties and prosocial behaviour in adolescents: a latent profile analysis. Rev Psiquiatr Salud Ment (Engl Ed) 13:202–212. https://doi.org/10.1016/j.rpsm.2020.01.002

Fruh V, Rifas-Shiman SL, Amarasiriwardena C et al (2019) Prenatal lead exposure and childhood executive function and behavioral difficulties in project viva. Neurotoxicology 75:105–115. https://doi.org/10.1016/j.neuro.2019.09.006

Garí M, Grzesiak M, Krekora M et al (2022) Prenatal exposure to neurotoxic metals and micronutrients and neurodevelopmental outcomes in early school age children from Poland. Environ Res 204:112049. https://doi.org/10.1016/j.envres.2021.112049

Gaynor JW, Nord AS, Wernovsky G et al (2009) Apolipoprotein E genotype modifies the risk of behavior problems after infant cardiac surgery. Pediatrics 124:241–250. https://doi.org/10.1542/peds.2008-2281

González L, Estarlich M, Murcia M et al (2021) Poverty, social exclusion, and mental health: the role of the family context in children aged 7–11 years INMA mother-and-child cohort study. Eur Child Adolesc Psychiatry. https://doi.org/10.1007/s00787-021-01848-w

Grandjean P, Landrigan PJ (2006) Developmental neurotoxicity of industrial chemicals. Lancet 368:2167–2178. https://doi.org/10.1016/S0140-6736(06)69665-7

Guxens M, Ballester F, Espada M et al (2012) Cohort Profile: the INMA—INfancia y Medio Ambiente—(Environment and childhood) project. Int J Epidemiol 41:930–940. https://doi.org/10.1093/ije/dyr054

Hamadani JD, Tofail F, Nermell B et al (2011) Critical windows of exposure for arsenic-associated impairment of cognitive function in pre-school girls and boys: a population-based cohort study. Int J Epidemiol 40:1593–1604. https://doi.org/10.1093/ije/dyr176

Heindel JJ, Balbus J, Birnbaum L et al (2015) Developmental Origins of Health and Disease: integrating environmental influences. Endocrinology 156:3416–3421. https://doi.org/10.1210/EN.2015-1394

Horton MK, Hsu L, Henn BC et al (2018) Dentine biomarkers of prenatal and early childhood exposure to manganese, zinc and lead and childhood behavior. Environ Int 121:148–158. https://doi.org/10.1016/j.envint.2018.08.045

Iñiguez C, Ballester F, Estarlich M et al (2009) Estimation of personal NO2 exposure in a cohort of pregnant women. Sci Total Environ 407:6093–6099. https://doi.org/10.1016/j.scitotenv.2009.08.006

Iyengar GV, Rapp A (2001) Human placenta as a 'dual' biomarker for monitoring fetal and maternal environment with special reference to potentially toxic trace elements. Part 3: toxic trace elements in placenta and placenta as a biomarker for these elements. Sci Total Environ 280:221–238. https://doi.org/10.1016/S0048-9697(01)00827-0

Jaspers M, de Winter AF, Huisman M et al (2012) Trajectories of psychosocial problems in adolescents predicted by Findings from Early Well-Child assessments. J Adolesc Health 51:475–483. https://doi.org/10.1016/j.jadohealth.2012.02.007

Jayadev S (2022) Genetics of Alzheimer Disease. Continuum (Minneap Minn) 28:852–871. https://doi.org/10.1212/CON.0000000000001125

Jones MR, Tellez-Plaza M, Vaidya D et al (2016) Estimation of Inorganic Arsenic exposure in populations with frequent Seafood Intake: evidence from MESA and NHANES. Am J Epidemiol 184:590–602. https://doi.org/10.1093/aje/kww097

Joo H, Choi JH, Burm E et al (2018) Gender difference in the effects of lead exposure at different time windows on neurobehavioral development in 5-year-old children. Sci Total Environ 615:1086–1092. https://doi.org/10.1016/j.scitotenv.2017.10.007

Julvez J, Smith GD, Golding J et al (2013) Prenatal methylmercury exposure and genetic predisposition to cognitive deficit at age 8 years. Epidemiology 24:643–650. https://doi.org/10.1097/EDE.0b013e31829d5c93

Julvez J, Davey Smith G, Ring S, Grandjean P (2019) A birth cohort study on the genetic modification of the association of prenatal Methylmercury with Child Cognitive Development. Am J Epidemiol 188:1784–1793. https://doi.org/10.1093/aje/kwz156

Kaiser HF (1958) The varimax criterion for analytic rotation in factor analysis. Psychometrika 23:187–200. https://doi.org/10.1007/BF02289233

Karakis I, Landau D, Gat R et al (2021) Maternal metal concentration during gestation and pediatric morbidity in children: an exploratory analysis. Environ Health Prev Med 26:40. https://doi.org/10.1186/s12199-021-00963-z

Karim Y, Siddique AE, Hossen F et al (2019) Dose-dependent relationships between chronic arsenic exposure and cognitive impairment and serum brain-derived neurotrophic factor. Environ Int 131:105029. https://doi.org/10.1016/j.envint.2019.105029

Khalid M, Abdollahi M (2019) Epigenetic modifications associated with pathophysiological effects of lead exposure. J Environ Sci Health C Environ Carcinog Ecotoxicol Rev 37:235–287. https://doi.org/10.1080/10590501.2019.1640581

Klipker K, Baumgarten F, Göbel K et al (2018) Mental health problems in children and adolescents in Germany. Results of the cross-sectional KiGGS Wave 2 study and trends. https://doi.org/10.17886/RKI-GBE-2018-084

Kornvig S, Wielsøe M, Long M, Bonefeld-Jørgensen EC (2021) Prenatal exposure to persistent organic pollutants and metals and problematic child behavior at 3–5 years of age: a greenlandic cohort study. Sci Rep 11:22182. https://doi.org/10.1038/s41598-021-01580-0

Lertxundi A, Andiarena A, Martínez MD et al (2019) Prenatal exposure to PM2.5 and NO2 and sex-dependent infant cognitive and motor development. Environ Res 174:114–121. https://doi.org/10.1016/j.envres.2019.04.001

Liu J, Chen X, Lewis G (2011) Childhood internalizing behaviour: analysis and implications. J Psychiatr Ment Health Nurs 18:884–894. https://doi.org/10.1111/j.1365-2850.2011.01743.x

Liu J, Chen Y, Gao D et al (2014) Prenatal and postnatal lead exposure and cognitive development of infants followed over the first three years of life: a prospective birth study in the Pearl River Delta region, China. Neurotoxicology 44:326–334. https://doi.org/10.1016/j.neuro.2014.07.001

Lozano M, Murcia M, Soler-Blasco R et al (2021) Exposure to mercury among 9-year-old children and neurobehavioural function. Environ Int 146. https://doi.org/10.1016/j.envint.2020.106173

Lozano M, Murcia M, Soler-Blasco R et al (2022) Exposure to metals and metalloids among pregnant women from Spain: levels and associated factors. Chemosphere 286. https://doi.org/10.1016/j.chemosphere.2021.131809

Lu B, Nagappan G, Lu Y (2014) BDNF and synaptic plasticity, cognitive function, and dysfunction. Handb Exp Pharmacol 220:223–250. https://doi.org/10.1007/978-3-642-45106-5_9

Luo L, Jiang X, Cao G et al (2020) Association between BDNF gene polymorphisms and attention deficit hyperactivity disorder in school-aged children in Wuhan, China. J Affect Disord 264:304–309. https://doi.org/10.1016/j.jad.2020.01.017

Maitre L, de Bont J, Casas M et al (2018) Human early life exposome (HELIX) study: a european population-based exposome cohort. BMJ Open 8:e021311. https://doi.org/10.1136/bmjopen-2017-021311

Maitre L, Julvez J, López-Vicente M et al (2021) Early-life environmental exposure determinants of child behavior in Europe: a longitudinal, population-based study. Environ Int 153. https://doi.org/10.1016/j.envint.2021.106523

Malavika L, Mitra P, Goyal T et al (2021) Association of blood lead levels with neurobehavior and BDNF expression in school

going children. J Trace Elem Med Biol 66:126749. https://doi.org/10.1016/j.jtemb.2021.126749
Merced-Nieves FM, Chelonis J, Pantic I et al (2022a) Sexually dimorphic associations between prenatal blood lead exposure and performance on a behavioral testing battery in children. Neurotoxicol Teratol 90:107075. https://doi.org/10.1016/j.ntt.2022.107075
Merced-Nieves FM, Chelonis J, Pantic I et al (2022b) Prenatal trace elements mixture is associated with learning deficits on a behavioral acquisition task among young children. New Dir Child Adolesc Dev. https://doi.org/10.1002/cad.20458
Muggeo VMR (2003) Estimating regression models with unknown break-points. Stat Med 22:3055–3071. https://doi.org/10.1002/sim.1545
Napier MD, Poole C, Satten GA et al (2016) Heavy metals, organic solvents, and multiple sclerosis: an exploratory look at gene-environment interactions. Arch Environ Occup Health 71:26–34. https://doi.org/10.1080/19338244.2014.937381
Narusyte J, Ropponen A, Alexanderson K, Svedberg P (2017) Internalizing and externalizing problems in childhood and adolescence as predictors of work incapacity in young adulthood. Soc Psychiatry Psychiatr Epidemiol 52:1159–1168. https://doi.org/10.1007/s00127-017-1409-6
Ng S, Lin C-C, Hwang Y-H et al (2013) Mercury, APOE, and children's neurodevelopment. Neurotoxicology 37:85–92. https://doi.org/10.1016/j.neuro.2013.03.012
Ng S, Lin C-C, Jeng S-F et al (2015) Mercury, APOE, and child behavior. Chemosphere 120:123–130. https://doi.org/10.1016/j.chemosphere.2014.06.003
Nordberg GF, Fowler BA, Nordberg M (2014) Handbook on the Toxicology of Metals. Fourth Edition
Ortuño-Sierra J, Fonseca-Pedrero E, Paíno M, Aritio-Solana R (2014) Prevalencia de síntomas emocionales y comportamentales en adolescentes españoles. Revista de Psiquiatría y Salud Mental 7:121–130. https://doi.org/10.1016/j.rpsm.2013.12.003
Paes LA, Torre OHD, Henriques TB et al (2018) Association between serotonin 2 C receptor gene (*HTR2C*) polymorphisms and psychopathological symptoms in children and adolescents. Braz J Med Biol Res 51. https://doi.org/10.1590/1414-431X20187252
Pham C, Bekkering S, O'Hely M et al (2022) Infant inflammation predicts childhood emotional and behavioral problems and partially mediates socioeconomic disadvantage. Brain Behav Immun 104:83–94. https://doi.org/10.1016/j.bbi.2022.05.011
R Core Team (2017) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Viena
Raîche G, Walls TA, Magis D et al (2013) Non-graphical solutions for Cattell's scree test. Methodology 9:23–29. https://doi.org/10.1027/1614-2241/a000051
Roberts Andrea L, Kristen L, Hart Jaime E et al (2013) Perinatal Air Pollutant Exposures and Autism Spectrum Disorder in the children of nurses' Health Study II participants. Environ Health Perspect 121:978–984. https://doi.org/10.1289/ehp.1206187
Rodríguez-Carrillo A, Mustieles V, D'Cruz SC et al (2022) Exploring the relationship between metal exposure, BDNF, and behavior in adolescent males. Int J Hyg Environ Health 239. https://doi.org/10.1016/j.ijheh.2021.113877
Rokoff LB, Shoaff JR, Coull BA et al (2022) Prenatal exposure to a mixture of organochlorines and metals and internalizing symptoms in childhood and adolescence. Environ Res 208:112701. https://doi.org/10.1016/j.envres.2022.112701
Rubio-Stipec M, Bird H, Canino G, Gould M (1990) The internal consistency and concurrent validity of a spanish translation of the child Behavior Checklist. J Abnorm Child Psychol 18:393–406. https://doi.org/10.1007/BF00917642
Singh G, Singh V, Sobolewski M et al (2018) Sex-dependent Effects of developmental lead exposure on the brain. Front Genet 9
Sioen I, Den Hond E, Nelen V et al (2013) Prenatal exposure to environmental contaminants and behavioural problems at age 7–8years. Environ Int 59:225–231. https://doi.org/10.1016/j.envint.2013.06.014
Skogstrand K, Hagen CM, Borbye-Lorenzen N et al (2019) Reduced neonatal brain-derived neurotrophic factor is associated with autism spectrum disorders. Transl Psychiatry 9:252. https://doi.org/10.1038/s41398-019-0587-2
Soler-Blasco R, Murcia M, Lozano M et al (2020) Prenatal manganese exposure and neuropsychological development in early childhood in the INMA cohort. Int J Hyg Environ Health 224. https://doi.org/10.1016/j.ijheh.2019.113443
Soler-Blasco R, Murcia M, Lozano M et al (2021) Urinary arsenic species and methylation efficiency during pregnancy: concentrations and associated factors in spanish pregnant women. Environ Res 110889. https://doi.org/10.1016/j.envres.2021.110889
Soler-Blasco R, Murcia M, Lozano M et al (2022) Prenatal arsenic exposure, arsenic methylation efficiency, and neuropsychological development among preschool children in a spanish birth cohort. https://doi.org/10.1016/j.envres.2021.112208. Environmental Research 207:
Supke M, Ferling C, Hahlweg K, Schulz W (2021) Persistence and course of mental health problems from childhood into adolescence: results of a 10-year longitudinal study. BMC Psychol 9:38. https://doi.org/10.1186/s40359-021-00535-4
Treble-Barna A, Wade SL, Pilipenko V et al (2022) Brain-derived neurotrophic factor Val66Met and behavioral Adjustment after Early Childhood Traumatic Brain Injury. J Neurotrauma 39:114–121. https://doi.org/10.1089/neu.2020.7466
Trucco EM, Villafuerte S, Hussong A et al (2018) Biological underpinnings of an internalizing pathway to alcohol, cigarette, and marijuana use. J Abnorm Psychol 127:79–91. https://doi.org/10.1037/abn0000310
Tung PW, Burt A, Karagas M et al (2022) Prenatal exposure to metal mixtures and newborn neurobehavior in the Rhode Island Child Health Study. Environ Epidemiol 6:e194. https://doi.org/10.1097/EE9.0000000000000194
Vahter M, Skröder H, Rahman SM et al (2020) Prenatal and childhood arsenic exposure through drinking water and food and cognitive abilities at 10 years of age: a prospective cohort study. Environ Int 139:105723. https://doi.org/10.1016/j.envint.2020.105723
Vasileva M, Graf RK, Reinelt T et al (2021) Research review: a meta-analysis of the international prevalence and comorbidity of mental disorders in children between 1 and 7 years. J Child Psychol Psychiatry 62:372–381. https://doi.org/10.1111/jcpp.13261
Vigeh M, Yokoyama K, Matsukawa T et al (2014) Low level prenatal blood lead adversely affects early childhood mental development. J Child Neurol 29:1305–1311. https://doi.org/10.1177/0883073813516999
Vioque J, Navarrete-Muñoz E-M, Gimenez-Monzó D et al (2013) Reproducibility and validity of a food frequency questionnaire among pregnant women in a Mediterranean area. Nutr J 12:26. https://doi.org/10.1186/1475-2891-12-26
Wahlberg K, Love TM, Pineda D et al (2018) Maternal polymorphisms in glutathione-related genes are associated with maternal mercury concentrations and early child neurodevelopment in a population with a fish-rich diet. Environ Int 115:142–149. https://doi.org/10.1016/j.envint.2018.03.015
Wang Y, Chen L, Gao Y et al (2016) Effects of prenatal exposure to cadmium on neurodevelopment of infants in Shandong, China. Environ Pollut 211:67–73. https://doi.org/10.1016/j.envpol.2015.12.038
Woods JS, Heyer NJ, Russo JE et al (2014) Genetic polymorphisms affecting susceptibility to mercury neurotoxicity in children: summary findings from the Casa Pia Children's amalgam clinical

trial. Neurotoxicology 44:288–302. https://doi.org/10.1016/j.neuro.2014.07.010

Wright RO, Hu H, Silverman EK et al (2003) Apolipoprotein E genotype predicts 24-month bayley scales infant development score. Pediatr Res 54:819–825. https://doi.org/10.1203/01.PDR.0000090927.53818.DE

Xu J, Hu H, Wright R et al (2015) Prenatal lead exposure modifies the impact of maternal self-esteem on children's inattention behavior. J Pediatr 167:435–441. https://doi.org/10.1016/j.jpeds.2015.04.057

Yorifuji T, Debes F, Weihe P, Grandjean P (2011) Prenatal exposure to lead and cognitive deficit in 7- and 14-year-old children in the presence of concomitant exposure to similar molar concentration of methylmercury. Neurotoxicol Teratol 33:205–211. https://doi.org/10.1016/j.ntt.2010.09.004

Yousef S, Adem A, Zoubeidi T et al (2011) Attention deficit hyperactivity disorder and environmental toxic metal exposure in the United Arab Emirates. J Trop Pediatr 57:457–460. https://doi.org/10.1093/tropej/fmq121

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Authors and Affiliations

Manuel Lozano[1,2] · Karin Broberg[3,4] · Raquel Soler-Blasco[2,5] · Gabriel Riutort-Mayol[6] · Ferran Ballester[2,5,7] · Llúcia González[2,5,6] · Mario Murcia[2,8] · Joan O. Grimalt[9] · Fernando Gil[10] · Pablo Olmedo[10] · Simone Braeuer[11] · Maribel Casas[5,12,13] · Mònica Guxens[5,12,13,14] · Amaia Irizar[15,16] · Nerea Lertxundi[15,17] · Miren Begoña Zubero[15,16] · Loreto Santa Marina[5,15,18] · Sabrina Llop[2,5]

✉ Manuel Lozano
manuel.lozano@uv.es

1 Preventive Medicine and Public Health, Food Sciences, Toxicology and Forensic Medicine Department, Universitat de València, Valencia, Spain

2 Epidemiology and Environmental Health Joint Research Unit, FISABIO–Universitat Jaume I–Universitat de València, Valencia, Spain

3 Department of Laboratory Medicine, Division of Occupational and Environmental Medicine, Lund University, Lund, Sweden

4 Unit of Metals and Health, Institute of Environmental Medicine, Karolinska Institute, Stockholm, Sweden

5 Spanish Consortium for Research on Epidemiology and Public Health (CIBERESP), Madrid, Spain

6 Foundation for the Promotion of Health and Biomedical Research in the Valencian Region, FISABIO- Public Health, Valencia, Spain

7 Department of Nursing, Universitat de València, Valencia, Spain

8 Health Information Systems Analysis Service, Conselleria de Sanitat, Generalitat Valenciana, Valencia, Spain

9 Institute of Environmental Assessment and Water Research, IDAEA-CSIC), Barcelona, Catalonia, Spain

10 Department of Legal Medicine and Toxicology, School of Medicine, University of Granada, Granada, Spain

11 Institute of Chemistry, University of Graz, Graz, Austria

12 ISGlobal, Barcelona, Spain

13 Universitat Pompeu Fabra, Barcelona, Spain

14 Department of Child and Adolescent Psychiatry/Psychology, Erasmus MC, University Medical Centre, Rotterdam, Netherlands

15 Group of Environmental Epidemiology and Child Development, Biodonostia Health Research Institute, San Sebastian, Spain

16 Departament of Preventive Medicine and Public Health, University of the Basque Country, UPV/EHU, Leioa, Spain

17 Faculty of Psychology, University of the Basque Country, UPV/ EHU, San Sebastian, Spain

18 Ministry of Health of the Basque Government, SubDirectorate for Public Health and Addictions of Gipuzkoa, San Sebastian, Spain