Multiple metals prenatal exposure and blood pressure throughout childhood: Multi-level modelling with complexity penalty and below detection limit metal imputation

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Abstract

Background: Studies assessing the effects of multiple chemical metals on blood pressure (BP), especially in childhood and adolescense, are scarce. Multi-pollutant studies are largely based on linear regression with independent prior distributions for the metal effects, which can lead to identifiability problems. On the other hand, discarding or imputing below detection limit metal observations (BDL) using point estimates or fixed imputations may have excessive influence on the model.

Objectives: We aimed to assess the association between prenatal exposure to multiple metals and BP in a longitudinal study during childhood, proposing an innovative modular probabilistic model that mitigates collinearity and makes practical imputations of BDL.

Methods: Prenatal urinary concentrations of metals (Mg, Al, Ca, Cr, Mn, Co, Ni, Cu, Zn, As, Se, Mo, Cd, Sn, Hg, Tl and Pb) and child BP assessed longitudinally at four, seven, and nine years of age in 740 mother-child pairs from the INMA Spanish birth cohort. The inference model was based on a Bayesian multi-level linear regression for longitudinal data, with Bayesian regularization for model complexity and collinearity, and the specification of hierarchical priors for practical imputation of BDL.

Results: Improved posterior inference of near-collinear metal regression coefficients compared to standard linear regression, avoiding estimates that are too large and with too large an uncertainty. The most consistent metal effects on child BP, relative to a 10% increase in exposure to the metal, were: Mn with a 5 mmHg increase in systolic BP, Zn with a 2.2 mmHg increase in diastolic BP, and Cu and Cd with a decrease of 4.6 and 3.6 mmHg, respectively, in diastolic BP.

Discussion: Regularization for model complexity and collinearity was shown to be essential for estimating associations in multi-pollutant studies using linear regression. Furthermore, obtaining full predictive distributions for BDL does not influence posterior inference, unlike point estimates or fixed imputations. The disagreement observed in the results between this study and other existing studies and, in general, between the related literature, highlights the need for robust analysis methodologies, such as the one proposed in this work, to draw reliable conclusions.

***Keywords*—** Blood pressure, Metals, Longitudinal model, Bayesian inference, Regularisation, Collinearity, Bayesian imputation, Detection limit, Quantification limit

1 Introduction

High blood pressure (BP) in children and adolescents is a growing health problem. A recent systematic review (Song et al., 2019) described a trend of increasing prevalence of childhood hypertension over the past two decades, increasing from 4.32% among children aged 6 years to 3.28% among those aged 19 in 2015. Moreover, many studies have recognised a significant degree of underdiagnosis of elevated paediatric BP and hypertension (Fuly et al., 2014; Hansen et al., 2007; Moin et al., 2021). Elevated paediatric BP could be a predictor of cardiovascular disease during adulthood (Chen and Wang, 2008; Theodore et al., 2015), high BP or hypertension being considered a major cause of death and morbidity in many populations (World Health Organization, 2023). Children and adolescents also experience cardiovascular target organ damage, including left ventricular hypertrophy and pathological vascular changes (i.e., carotid intima-media thickness) (de Simone et al., 2022; Lurbe and Ingelfinger, 2021; Stergiou et al., 2021; Falkner and Lurbe, 2021; Urbina et al., 2011).

Risk factors for the development of primary paediatric hypertension have not been clearly defined. However, an increasing body mass index, obesity, and abdominal circumference have been observed to be correlated with increased hypertension rates in children and adolescents (Falkner and Lurbe, 2021). Other paediatric subpopulations at risk of hypertension include children with chronic kidney disease (Barletta et al., 2018), premature birth, elevated intake of sodium, family history, disordered sleep, and high adiposity (Hardy and Urbina, 2021; Rosner et al., 2013). Identifying modifiable risk factors for high BP in childhood could be a key element in the prevention of cardiovascular diseases.

There is evidence that exposure to environmental pollutants could have a role in the worldwide increase in hypertension and other cardiometabolic diseases (Habeeb et al., 2022). In fact, environmental chemical exposure to heavy metals, phthalates, and arsenic has been established as a risk factor for the development of hypertension in adult populations; the established attributable risk factor for high BP of each chemical group being between 3 and 18% (Shiue and Hristova, 2014). However, there are a limited number of studies evaluating the association between early environmental exposure and BP during childhood. Pregnancy and childhood are life stages particularly sensitive to environmental exposure because of their immature systems and their rapid growth and development. In addition, the developmental origins of health and disease (DOHaD) hypothesis posits that an adverse intrauterine environment programs chronic disease including elevated BP and kidney disease later in life (Luyckx et al., 2013; Falkner and Lurbe, 2021).

Some studies have evaluated the association between early exposure to toxic metals, especially arsenic (As), cadmium (Cd), mercury (Hg), and lead (Pb), and hypertension during childhood (Ahn et al., 2018; Chen et al., 2019; Desai et al., 2021; Hawkesworth et al., 2013; Osorio-Yáñez et al., 2015; Yao et al., 2020); however most evaluated hypertension against a single metal exposure. A recent prospective study conducted on the Rhea cohort, in Greece, evaluated the impact of prenatal metal mixture exposure on longitudinal changes in BP during childhood and elevated BP at 11 years of age (Howe et al., 2021). They observed that molybdenum (Mo) and Pb were the mixture components most consistently associated with BP during childhood, with a statistically significant interaction between both metals. Another prospective study conducted in Mexico (Kupsco et al., 2019) evaluated the association between prenatal exposure to a metal mixture and cardiometabolic risk factors, including BP. They observed that prenatal metal mixture exposure was inversely associated with systolic BP (SBP), where copper (Cu) was the main contributor. In another study conducted on the Boston birth cohort, maternal concentrations of selenium (Se) and manganese (Mn) measured in red blood cells after birth were associated with decreasing child SBP between 3 to 15 years of age, especially among children born to mothers who smoked during pregnancy (Zhang et al., 2021).

Studies assessing the effects of multi-pollutants are largely based on multivariate linear regression analysis allowing for the assessment of the joint effects of several metals additively, with the specification of some specific low-order interactions. Many metal exposure variables and/or the existence of correlations between them increase the model complexity and collinear effects when using independent non-informative prior distributions on metal coefficients (Gelman et al., 2020). Ridge regression is a common procedure in traditional analysis to regularise/penalise the estimated parameters under overestimation and collinearity. From a Bayesian perspective, regularization for model complexity and collinearity in linear models is implicitly performed by using informative hierarchical prior distributions for the regression coefficients.

Furthermore, mixed-effects in linear regression models have been widely used for the analysis of longitudinal data due to their simplicity and interpretability (Stroup, 2012; Gibbons et al., 2010). However, they rely heavily on the parametric form of the linear functional predictor and lack modeling flexibility to estimate non-linear effects, multiple and high-order covariate interactions, and to account for correlated observations (Zhang et al., 2019; Kim et al., 2020), where flexible non-parametric Gaussian processes (Williams and Rasmussen, 2006;

Bobb et al., 2015; Riutort-Mayol et al., 2020) are more suitable. On the other hand, linear models can detect subtle linear associations with low signal-to-noise ratio more easily than the more complex Gaussian process models. In this regard, Kupsco et al. (2019) used Bayesian Gaussian processes to examine the association between prenatal exposure to metal mixtures with several cardiometabolic outcomes in a cross-sectional analysis of children aged four to six years. They obtained no clear relationships with large uncertainty intervals, maybe due to the signal-to-noise ratio being too low for regular Gaussian process models.

Another methodological issue in chemical exposure assessment is that some measurements of metal concentrations in some individuals might be below the limit of detection (LOD) of the analytical technique for a specific metal, forcing those observations to be discarded or estimated in advance, often using point estimates or fixed imputations, which may have an excessive influence on the model. In a Bayesian framework, those observations below the LOD can naturally be considered parameters to be estimated in a fully probabilistic model,where estimates can be limited by the LOD using upper-bounded prior distributions.

In this study we aimed to evaluate the relationship between maternal metal and metalloid urinary concentrations (Mg, Al, Ca, Cr, Mn, Co, Ni, Cu, Zn, As, Se, Mo, Cd, Sn, Hg, Tl, and Pb) and child BP longitudinally assessed at four, seven, and nine years of age in two cohorts of the INMA—INfancia y Medio Ambiente (Environment and Childhood)—project. We proposed an innovative modular probabilistic model based on multilevel linear regression for longitudinal data, with Bayesian regularization for model complexity and collinearity, and specification of hierarchical prior distributions for practical imputation of metal observations below the LOD.

2 Methodology

2.1 Study population

The participants in the present study were a subsample of the general population of mother-child pairs from the regions of Valencia (eastern area of Spain) and Gipuzkoa (northern of Spain) of the INMA Project. The INMA project (`http://www.proyectoinma.org/`) consists of a network of seven birth cohorts in Spain that aims to study the role of exposure to environmental pollutants in air, water and diet during pregnancy and childhood for child and adolescent growth and development. The study protocol can be consulted elsewhere (Guxens et al., 2012). Figure A.1 in Appendix A shows the flow diagram of the participants in the Valencia and Gipuzkoa cohorts of the INMA project and those selected for the present study. Briefly, 1493 pregnant women were recruited during their first antenatal visit (2003–2008, Gipuzkoa: 638, Valencia: 855). At birth, a total of 1399 mother-child pairs were followed. Of these, 1087 were followed at the four-year visit, 985 at the seven-year visit, and 472 at the none-year visit. The sample population included in the present study were those participants with available prenatal urinary metal concentrations and SBP and diastolic BP (DBP) measurements at the four-, seven-, and nine-year visits. Thus, from Valencia, 284, 414, and 363 participants from the four-, seven-, and nine-year visits, respectively, were included in the study; from Gipuzkoa, 333 participants belonging to the seven-year visit were included.

2.2 Data sources and variables

Metal and metalloid exposures

Metal and metalloid concentrations were analysed in maternal spot urine samples, collected in the first and third trimester of pregnancy (mean [SD] of weeks of gestation: 12.8 [1.3] and 32.4 [1.8], respectively) from the study participants. Specifically, magnesium (Mg), aluminium (Al), calcium (Ca), chromium (Cr), manganese (Mn), cobalt (Co), nickel (Ni), copper (Cu), zinc (Zn), arsenic (As), selenium (Se), molybdenum (Mo), cadmium (Cd), tin (Sn), mercury (Hg) thallium (Tl) and lead (Pb) were measured. The urine samples were kept frozen at $-20°$C and were analysed at the University of Granada (Spain). Urine samples were diluted 1:10 in ultrapure water (Milli-Q) with 2% HNO3 (Merck) and 1% HCl (Merck). Multi-element analyses were performed on an Agilent 8900 triple quadrupole ICP-MS-MS (Agilent Technologies, Santa Clara, CA, USA). To ensure the quality of the results, a multi-element 400 μg/L internal standard solution with Sc, Ge, Ir and Rh was added to the samples online. Additionally, one in every 12 samples was reanalysed at the end of each session. Further information can be found elsewhere (Lozano et al., 2022).

Blood pressure

BP was measured during clinical examination in the Valencia cohort at the four-, seven-, and nine-year follow-up visits, and in the Gipuzkoa cohort at seve-year follow-up visit. In both cohorts, a standardised protocol for measuring children's BP was used guaranteeing useful and quality measures (International Protocol of the European Society of Hypertension and the criteria suggested by the British Hypertension Society for its use in children and adolescents (Stergiou et al., 2012)). Three consecutive measurements were taken with an oscillometric device (OMRON M4-I) with at least one-minute time intervals between measurements. The device has an accuracy of ± 3 mmHg and is clinically validated according to the International Protocol of the European Society of Hypertension and the criteria suggested by the British Hypertension Society for its use in children and adolescents (Stergiou et al., 2012). SBP and DBP from the three measurements were recorded and the mean of the second and third measurements was calculated and used in further analyses.

Covariates and participant characteristics

Mothers filled in two questionnaires during pregnancy, in the first and third trimesters of gestation, and later, in the follow-up visits at four, seven, and nine years of age. Sociodemographic, environmental, anthropometric, and lifestyle information (covariates) was collected. The covariates considered in this study were: paternal employment status during pregnancy and at four, seven, and nine years (employed/ unemployed), maternal active smoking during pregnancy (yes/no), children's passive exposure to tobacco smoke at four, seven, and nine years, assessed as passive exposure either at home, or during leisure time (yes/no), children's physical activity (sedentary/slightly/moderately/quite/very active), children's living area, children's weeks of lactation. Also, we defined parental social class from parental occupation during pregnancy according to a widely used Spanish adaptation of the Inter- national Standard Classification of Occupations coding system (ISCO88). Class I + II included managerial jobs, technical staff, and commercial managers; Class III included skilled non-manual workers; and Class IV + V included manual and unskilled workers (Domingo-salvany et al., 2013). Sodium intake adjusted for energy during pregnancy and at four, seven, and nine years of age was estimated using a validated semi-quantitative food frequency questionnaire (FFQ) (Vioque et al., 2013). The FFQ contains items with nine possible responses, ranging from 'never or less than once per month' to 'six or more per day'. This was converted to average daily intake in grams for each participant. Sodium intake was also estimated using the food composition tables of the US Department of Agriculture (U.S. Department of Agriculture: Agricultural Research Service USDA, 2007) and with Spanish sources (Palma et al., 2008). The energy-adjusted intakes were computed using the residual method (Willet, 2013). The child's height and weight information were collected during clinical examination in the follow-up visits using standard protocols. We calculated z-scores for height, weight, and body mass index according to the WHO child growth standards (World Health Organization, 2006). The parents body mass index was also calculated. Overweight and obesity were derived from the BMI (z-scores) of participants.

2.3 Model framework

The statistical model to estimate the association effects between multiple metals, measured during gestation, and SBP and DBP, repeatedly measured over time (at the ages of four, seven, and nine years), was formulated under the framework of Bayesian probabilistic modelling. The model comprised a multi-output (a bivariate outcome with the SBP and DBP variables) observational model with a predictor function which was a function of age, metals, and adjustment covariates. The predictor function was based on mixed-effects linear models adapting the longitudinal structure of the outcome and the effects of metal exposure variables and adjustment covariates (Gibbons et al., 2010; Gelman and Hill, 2006).

To deal with instability in parameter estimation and overfitting in linear models due to nearly correlated metals or a large number of metals, Bayesian regularisation through using hierarchical prior distributions on metal coefficients was performed. Finally, a multivariate Gaussian prior distribution was defined for the group of metals and those metal observations below the LOD were considered parameters with LOD-bounded priors to be estimated by the model.

2.3.1 Multi-output (SBP and DBP) observational model

Jointly modelling SBP and DBP variables allows them to share information to better estimate model parameters. So, the observations of systolic ($\boldsymbol{y}^s$) and diastolic ($\boldsymbol{y}^d$) BP are jointly modelled through a multivariate student-t observational model:

$$\begin{pmatrix} y_{it}^s \\ y_{it}^d \end{pmatrix} \sim \text{t-student}\left(\begin{pmatrix} \mu_{it}^s \\ \mu_{it}^d \end{pmatrix}, \Sigma, \nu\right), \text{ with } \Sigma = \begin{pmatrix} \sigma_s^2 & \sigma_{sd} \\ & \sigma_d^2 \end{pmatrix} \text{ and } \nu \in \mathbb{R}^+,$$

where y_{it}^s and y_{it}^d represents the observed systolic (^{s}) and diastolic (^{d}) BP random variables, respectively, in individual i and time point t; μ_{it}^s and μ_{it}^d are the means of those random variables in individual i and time point t; Σ is the variance-covariance matrix shared by every pair (y_{it}^s, y_{it}^d). The parameter ν is the degrees of freedom of the student-t distribution. In this work we choose ν to be equal to 4. The subscripts i and t are the indices pointing to single individuals from the whole sample of N individuals ($i = 1, \ldots, N$) and the three single time points ($t = 1, 2, 3$) measured for each individual. The selection of a student-t distribution as the observational model is motivated to conduct robust analyses because of the observation of some extreme values. A prior distribution of the variance-covariance matrix Σ is defined in Appendix C.

2.3.2 Longitudinal mixed-effects linear mean predictor

A mixed-effects linear model adapts random-effects (varying effects), which can model heterogeneity among populations, and fixed-effects (non-varying effects), which model overall mean effects from the population, in the predictor function. The varying effects can collect differences among individuals (or groups of observations) by allowing individual effects (or group effects) to deviate from the overall mean effects. These differences can be due to, for example, specific behavioural and/or biological characteristics of the individuals (or groups).

In longitudinal data settings, where individuals are repeatedly measured over time (at different ages), responses tend to be correlated over time and predictor functions for individuals deviate systematically from the overall mean predictor function from the population. The basic modelling feature in longitudinal data and linear predictor functions is to allow an individual's intercept and time trend (i.e., tendency with respect to age) to deviate from the overall mean intercept and time trend for all individuals as a whole. This can be generalised to other covariates in the model, however, in the present work, varying effects were only considered for the intercept and time trend, so metal variables and adjustment covariates in our model were considered as non-varying effects, such that overall mean effects from the population were estimated for them.

Therefore, the longitudinal nature of the data as well as the overall mean effects of covariates and metals were defined in a mixed-effects linear predictor function and linked to the observations, y_{it}^s and y_{it}^d, through their means, μ_{it}^s and μ_{it}^d, respectively, as follows:

$$\begin{aligned} \mu_{it}^s &= m_i^s + \beta_i^s\, \tau_{t(i)} + \nu_i^s\, \tau_{t(i)}^2 + \boldsymbol{x}_{it}\, \boldsymbol{\gamma}^s + \boldsymbol{z}_i\, \boldsymbol{\delta}^s + \boldsymbol{z}_i\, \tau_{t(i)}\, \boldsymbol{\theta}^s + \sum_{j=1}^{J} z_{\Psi j 1_i}\, z_{\Psi j 2_i}\, \rho_j^s, \\ \mu_{it}^d &= m_i^d + \beta_i^d\, \tau_{t(i)} + \nu_i^d\, \tau_{t(i)}^2 + \boldsymbol{x}_{it}\, \boldsymbol{\gamma}^d + \boldsymbol{z}_i\, \boldsymbol{\delta}^d + \boldsymbol{z}_i\, \tau_{t(i)}\, \boldsymbol{\theta}^d + \sum_{j=1}^{J} z_{\Psi j 1_i}\, z_{\Psi j 2_i}\, \rho_j^d. \end{aligned} \tag{1}$$

For the sake of simplicity from herein we do not differentiate between parameters that correspond to the systolic (^{s}) and diastolic (^{d}) BP components. In the above eq. (1), $\tau_{t(i)}$, $\boldsymbol{x}_{it}$, and $\boldsymbol{z}_i = (z_{1_i}, \ldots, z_{K_i})$ are age, the vector of single and co-interaction adjustment covariates, and the K-vector of metals, respectively, of individual i at time point t. The parameters m_i, β_i, and ν_i are the varying intercept, linear, and quadratic time trend coefficients, respectively, per individual i. The parameter $\boldsymbol{\gamma}$ represents the vector of overall mean effects of single and co-interaction adjustment covariates. The parameter $\boldsymbol{\delta}$ is the K-vector of coefficients that represents the overall mean effects of single metals. The parameter $\boldsymbol{\theta}$ is the K-vector of coefficients that represents the overall mean effects of interactions between single metals and age. The parameter $\boldsymbol{\rho}$ is the J-vector of coefficients that represents the overall mean effects of the J-selected pairs of co-interaction metals. The matrix $\Psi \in \mathbb{R}^{J \times 2}$ is the matrix with the 2-tuples of metal indices for the J-selected pairs of co-interaction metals to evaluate.

Variation of the varying parameters across individuals, m_i, β_i, and ν_i, is clearly correlated, so they need to be

jointly modelled by a multivariate distribution to make inference properly:

$$\begin{pmatrix} m_i \\ \beta_i \\ \nu_i \end{pmatrix} \sim \text{Normal}\left(\begin{pmatrix} m_0 \\ \beta_0 \\ \nu_0 \end{pmatrix}, \Phi\right), \tag{2}$$

where m_0, β_0, and ν_0 represent the overall mean effects from the population around which the varying parameters deviate, Φ is the variance-covariance matrix of the varying parameters. Prior distributions for m_0, β_0, ν_0, and Ψ are defined in Appendix C. Also, prior distributions for $\boldsymbol{\gamma}$, $\boldsymbol{\delta}$, $\boldsymbol{\theta}$, and $\boldsymbol{\rho}$ are defined in Appendix C.

In this work, a quadratic linear function was used to capture time trends, however, more flexible semi-parametric functions, such as splines, can also be specified in this model framework.

2.3.3 Regularisation for model complexity and collinearity

To deal with model complexity and collinearity, which can cause instability in parameter estimation or overfitting in linear model frameworks, such as coefficient estimates with too large a magnitude and uncertainty, regularisation in inference through using hierarchical prior distributions on metal coefficients was performed. Thus, a hierarchical prior distribution was defined over the vectors of single metal coefficients $\boldsymbol{\delta} \in \mathbb{R}^K$, interaction between single metals and time coefficients $\boldsymbol{\theta} \in \mathbb{R}^K$, and co-interaction metal coefficients $\boldsymbol{\rho} \in \mathbb{R}^J$:

$$\begin{bmatrix} \boldsymbol{\delta} \\ \boldsymbol{\theta} \\ \boldsymbol{\rho} \end{bmatrix} \sim \text{Normal}(\mathbf{0}, \Omega), \quad \text{with} \quad \Omega = \begin{pmatrix} \boldsymbol{\sigma}_A^2 & \mathbf{0} & \mathbf{0} \\ & \boldsymbol{\sigma}_B^2 & \mathbf{0} \\ & & \boldsymbol{\sigma}_C^2 \end{pmatrix} \in \mathbb{R}^{(2K+J)\times(2K+J)}, \tag{3}$$

with zero-mean and diagonal variance-covariance matrix Ω. In the above eq. (3), $\boldsymbol{\sigma}_A^2 = (\sigma_A^2, \overset{\times K}{\ldots})$, $\boldsymbol{\sigma}_B^2 = (\sigma_B^2, \overset{\times K}{\ldots})$, and $\boldsymbol{\sigma}_C^2 = (\sigma_C^2, \overset{\times J}{\ldots})$ represent the variance parameters for $\boldsymbol{\delta}$, $\boldsymbol{\theta}$, and $\boldsymbol{\rho}$, respectively. Defining different variances for different groups of coefficients allows for different levels of regularisation for every group. Hierarchical prior distributions place low probability mass on large values and concentrate much of the probability towards zero, penalising estimates that are too large and with too large an uncertainty, reducing the impact of irrelevant predictors; unlike using a prior $p(\theta)$ that is uniform ($p(\theta) \sim 1$) or non-informative ($p(\theta) \sim \text{Normal}(0, 1/\epsilon)$ with $\epsilon \ll 1$), which does not perform regularisation. Prior distributions for σ_A^2, σ_B^2, and σ_C^2 are defined in Appendix C.

2.3.4 Modelling below detection limit metal observations

The methodology to estimate observations below the LOD consisted of defining a multivariate normal distribution for the vector of row metal concentrations $\boldsymbol{z}_i^* = (z_{1i}^*, \ldots, z_{Ki}^*)$ per individual i:

$$\begin{pmatrix} z_{1i}^* \\ \vdots \\ z_{Ki}^* \end{pmatrix} \sim \text{Normal}\left(\begin{pmatrix} \text{h}(\chi_{1i}) \\ \vdots \\ \text{h}(\chi_{Ki}) \end{pmatrix}, \Lambda\right), \quad \text{with} \quad \Lambda = \begin{pmatrix} \sigma_{11} & \ldots & \sigma_{1K} \\ & \ddots & \vdots \\ & & \sigma_{KK} \end{pmatrix}, \tag{4}$$

Then, those specific metal concentrations that are below the LOD are considered parameters and posterior distributions are estimated for them. In the above eq. 4, $\text{h}(\chi)$ represents a function of a set of covariates χ that defines the mean predictor function of the distribution, and Λ the variance-covariance matrix between the K metals which is common for all individuals. Furthermore, the LOD of a metal variable k (LOD_k) is established as the upper limit of prediction for those observations below the LOD of that metal k by defining a bounded prior distribution for each of them. Let $\boldsymbol{z}_k^* = (z_{k1}^*, \ldots, z_{kN}^*)$ be the row of metal concentrations for a specific metal k, which may contain observations above ($\boldsymbol{z}_k^{*a}$) and below ($\boldsymbol{z}_k^{*b}$) the LOD_k, then it follows $\boldsymbol{z}_k^* = \{\boldsymbol{z}_k^{*a}, \boldsymbol{z}_k^{*b}\}$. Bounded prior distributions for those observations below the LOD ($\boldsymbol{z}_k^{*b}$) can be defined as follows:

$$\boldsymbol{z}_k^{*b} \sim p(0, LOD_k),$$

where p represents a probability distribution with the lower bound of zero and the upper bound of LOD_k. As it is slightly more likely that the predictions of the observations below the LOD will be closer to the upper-bound

LOD_k than to the lower bound of zero, the probability distribution p is defined as a *beta* distribution scaled by the LOD_k, as follows:

$$\mathrm{p}(0, LOD_k) = LOD_k \cdot \mathrm{beta}(2, 1). \tag{5}$$

Additional prior information about the values to be predicted can be included through these distributions in equations (4) and (5).

Apart from the potential predictive capacity of this methodology based on the covariance matrix of the multivariate distribution for the group of metals, what is more interesting about the method for us in this work is that a posterior distribution was obtained for each observation below the LOD; distributions that will be more concentrated or more dispersed depending on the correlation between metals and the shape and information provided by the mean function $\mathrm{h}(\chi)$ of the multivariate distribution, as well as the number of actual observations that a certain variable has (i.e. the sample size of observations above the LOD of a variable).

Regardless of the accuracy of the predictions, which ultimately depend on the data and the form and information provided by the mean predictor function, an interesting thing about the method is, that in a model that involves variables of metal exposures, the inference will be based on full posterior distributions of those observations below the LOD, as opposed to point estimates or fixed imputations that can have excessive influence on the model. Furthermore, in Bayesian inference uncertainties are fully and adequately propagated between parameters and model assumptions.

2.3.5 Metal correction by creatinine

To correct the influence of urine sample dilution, metal concentrations need to be corrected by urinary creatinine. This correction was achieved by removing the effect of creatinine on each metal k, by including it as a predictor in the mean predictor function $\mathrm{h}(\cdot)$ in eq. (4), such as follows:

$$\mathrm{h}_k(creatinine_i) = a_k + b_k \cdot creatinine_i,$$

where a_k and b_k are the model coefficients for each metal k. The metal concentrations z_{ki} corrected by creatinine that are used in eq. (1) are then computed by subtracting the creatinine effect from the row concentrations z^*_{ki} as follows:

$$z_{ki} = z^*_{ki} - \mathrm{h}(creatinine_i).$$

2.3.6 Model inference and validation

The different pieces of the model explained in Sections 2.3.1, 2.3.2, 2.3.3, 2.3.4, 2.3.5 and Appendix C are jointly specified in a unique and fully probabilistic Bayesian hierarchical model, which is implemented in a modular probabilistic modelling framework, such as Stan (Stan Development Team, 2022). From the model specified, the joint posterior distribution of parameters, given data and posterior marginal distributions of each parameter, are derived. Stan software uses the Hamiltonian Monte Carlo sampling method (Neal, 2011) to estimate the posterior distributions. Four simulation chains with different initial values were launched. The convergence of the simulation chains was evaluated by the split-Rhat convergence diagnosis and the effective sample size of the chains (Gelman et al., 2013; Vehtari et al., 2019). In our case and in both models, a split-Rhat value ≤ 1.05 was obtained for all parameter simulation chains indicating good mixing of simulated chains. The convergence of the simulation chains shows that the samples did arise from the posterior distribution and that the model is correctly specified, without confusion or identifiability problems between parameters.

The magnitude and uncertainty of the parameters of interest are given by their marginal posterior distributions. The significance of parameters can be determined by evaluating whether their accumulated probabilities of being less than or greater than zero are lower than a probability threshold. In this study we set the commonly used probability threshold of 0.05.

For model checking, leave-one-out probability integral transformation (LOO-PIT) can be used to assess whether the model predictive distributions are calibrated, that is, they are describing the model predictive uncertainty well. In the case of a good calibration of predictive distribution, LOO-PIT values are uniformly distributed (Gelfand et al., 1992; Gelman et al., 2013). To compute LOO-PIT values, the R-package *loo* (Vehtari et al., 2022) was used, which performs efficient approximate leave-one-out cross-validation.

	Mg	Al	Ca	Cr	Mn	Co	Ni	Cu	Zn	As	Se	Mo	Cd	Sn	Hg	Tl	Pb
median	62193	7.59	162267	0.44	0.31	0.46	1.96	9.47	379.9	36.8	26.2	39.0	0.19	1.29	0.90	0.26	1.06
p25	41606	5.07	116774	0.35	0.22	0.28	1.41	6.07	250.1	19.8	19.3	28.4	0.11	0.67	0.55	0.18	0.67
p75	87991	13.3	229348	0.74	0.42	0.72	2.77	13.4	558.9	67.1	36.2	57.0	0.29	2.46	1.44	0.38	1.59
BDL (%)	0%	62%	0%	63%	27%	0%	46%	3%	0%	0%	0%	0%	0%	1%	1%	1%	3%

Table 1: Summary statistics (median and 25 and 75 percentiles) of metal concentrations (μg/g) and percentages of measurements below the LOD (Below detection limit (BDL)) of each metal.

2.4 Adjustment covariates and co-interaction metals selection

An iterative procedure to select the relevant sociodemographic, environmental, anthropometric, and lifestyle covariates as model adjustment covariates for BP was performed. First, a linear correlation between pairs of covariates was computed to check potential colliders. Then, regression models with BP outcome variables were fitted for each pair of covariates to confirm possible collinearity issues by checking the correlation between posterior distributions and variance inflate factors. Finally, after discarding those collider covariates, a full regression model with retained covariates was formulated and diagnosed, and covariates with significant relevance were selected. On the other hand, co-interaction metals on BP were also iteratively selected following a manual backward elimination procedure.

3 Results

3.1 Descriptive summary

Participant characteristics

Figure 1 depicts the descriptive histograms and frequency plots of covariates for the study sample population throughout the study period. There were no significant differences comparing with the total INMA cohort population (see Table D.1 in Appendix D), thus the study sample population can be considered a representative sample of the total INMA cohort population. The main characteristics of the study sample population could be summarized as: there was an approximately equal proportion of boys and girls among the children; approximately 43% of families belonged to a low social class, 29% to a medium social class, and 28% to a high social class at the four and nine-year visits; whereas at the seven-year visit, 36% belonged to a low social class, 26% to a medium social class, and 38% to a high social class; most participants lived in urban areas; nearly 50% of children received more than 24 weeks of lactation as newborns; approximately 80% of mothers did not smoke actively during pregnancy (*smok. preg.* in Figure1); nearly 50% of children were passively exposed to tobacco (*pasiv. smok.* in Figure1); the proportion of overweight and obese children increased with age, from 22% and 11%, respectively, at the four-year visit, to 28% and 23% at the nine-year visit; most children reported moderate or high physical activity (*phys. activity* in Figure 1); approximately 60% of parents were employed (*labour* in Figure 1); and most parents, either father or mother, were not overweight at moment of gestation, only approximately 20% of parents were overweight; most children had a calorie-adjusted estimated sodium intake under 2500 mg at the four-year visit and approximately 50% of children had a calorie-adjusted estimated sodium intake above 2500 mg at the seven-year visit.

Metal and metalloid exposures

The mean of metal measurements made in the first and third trimesters of gestation were computed and used for further analyses. Table 1 shows summary statistics (median and 25th and 75th percentiles) of metal concentrations in μg/g of creatinine and the percentage of measurements below the limit of detection (LOD) for each metal for the population study sample. Al, Cr, and Ni were the metals with the highest percentage of non-detected concentrations, that is, 62%, 63%, and 46%, respectively, and Mn had a significant 27% of non-detected observations. The LOD in μg/g for each metal are shown in Table B.1 in Appendix B.

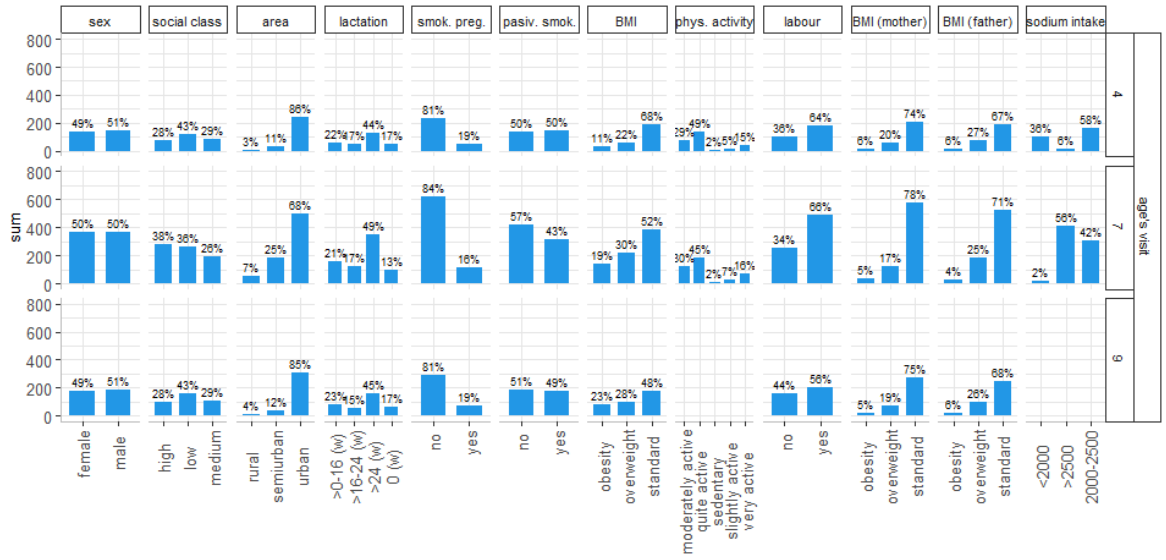

Figure 1: Histograms and frequency plots of sociodemographic, environmental, anthropometric, and lifestyle variables in the study sample population. (BMI: Body mass index).

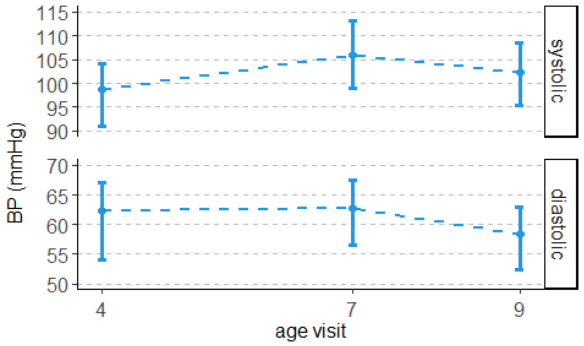

Figure 2: Mean and 2.5 and 97.5 percentiles of blood pressure (BP) measurements per age visit.

Systolic and diastolic blood pressure

Figure 2 shows the mean and 2.5 and 97.5 percentiles of BP in the sample population per age visit. In the population sample, the mean SBP observed throughout the period was 61.1 mmHg, and on BP average significantly increased between the four- and seven-year visits by approximately 7 mmHg, and decreased betweem the seven- and nine-year visits by approximately 3 mmHg. The mean DBP observed during the entire period was 102.3 mmHg, being mostly constant between the four- and seven-year visits, with the average decreasing significantly between the seven- and nine-year visits by approximately 4 mmHg.

3.2 Adjustment covariates

The sociodemographic, environmental, anthropometric, and lifestyle factors selected for inclusion in the model as adjustment covariates $\boldsymbol{x}$ were: child weight, social class at birth, smoking during pregnancy, calorie-adjusted estimated sodium intake, maternal and paternal working situation, and passive smoking throughout the follow-up. The model was also adjusted by the cohort that each individual belonged to. Some two-way interactions between covariates were also found to be relevant, such as child age and passive smoking, child weight and social class, child weight and smoking during pregnancy, child weight and passive smoking, and child weight and labour.

Single and co-interaction effects of adjustment covariates on BP are gathered by the vector of coefficients $\boldsymbol{\gamma}$ in eq. (1). In Appendix E and Figure E.1, estimates of single and co-interaction effects of adjustment covariates

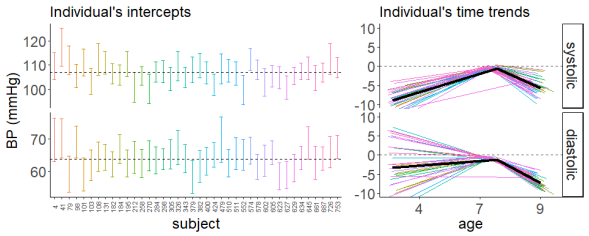

Figure 3: Mean and 90% credible intervals of the individual's base levels of BP (m_i) centred around the population's overall mean base level of BP (m_0) for SBP and DBP (Left). The mean of the individual's time trends of BP ($\beta_i \cdot \tau_{t(i)}$) centred around the population's overall mean time trend ($\beta_0 \cdot \tau_{t(i)}$) (bold-black line) plotted with respect to age, for SBP and DBP (Right). Notice that, for the sake of simplicity, only a small random sample of individuals ($n = 50$ in Figure 3-left and $n = 100$ in Figure 3-right) from the total sample are plotted.

are described.

3.3 Individual-specific BP base levels and time trends

The mean and 90% credible intervals of the individual's base levels of BP (m_i in (1)) are plotted centred around the overall mean base level of BP from the population (m_0 in (2)) in Figure 3-left, for SBP and DBP. The mean of the individual's age effects on BP ($\beta_i \cdot \tau_{t(i)}$ in (1)) are plotted centred around the overall mean age effect on BP ($\beta_0 \cdot \tau_{t(i)}$) in Figure 3-right, for SBP and DBP. Note that, for the sake of simplicity and interpretability, only a small random sample ($n = 50$ in Figure 3-left and $n = 100$ in Figure 3-right) of individuals are plotted.

The individual's BP base levels vary around the population's overall mean BP base level of 106.8 mmHg for SBP and 66.7 mmHg for DBP. The standard deviations of the individual's BP base levels were 5.5 mmHg for SBP and 3.1 mmHg for DBP (Figure 3-left). The overall mean age effect on BP increased between the four- and seven-year visits and decreased between the seven- and nine-year visits for both SBP and DBP (Figure 3-right).

3.4 Metal effects

Metal concentrations were previously log-transformed and standardizsed to have a zero-mean and unit variance to fit the model. After fitting the model, the coefficient estimates were transformed to refer to the original scale of concentrations, thus representing the change in BP (mmHg) with a 10% increase in corresponding metal concentrations.

3.4.1 Single metal effects

The overall mean effects from the population of every single metal k on BP are represented by coefficients δ_k in eq. (1). The mean and 90% credible intervals of those single metal regression coefficients δ_k are plotted in Figure 4-left, representing the change in BP given a 10% increase in metal concentrations. The largest and most significant single metal effects, with at least a 90% probability of being larger or smaller than zero, were for Al, Mn, Co, and Sb on SBP, and Al, Ca, Co, Cu, Zn, Se, Cd, and Sn on DBP. On SBP, Mn and Sb caused an increase in BP of approximately 5.5 and 1.2 mmHg, respectively; and Al and Co caused a decrease in BP of approximately 2 and 2.2 mmHg, respectively. On DBP, Al, Co, Zn, and Sn caused an increase in BP of approximately 2.8, 2.2, 4.5, and 1.3 mmHg, respectively, and Ca, Cu, Se, and Cd caused a decrease in BP of approximately 2.8, 4.6, 3.7, and 3.6 mmHg, respectively.

These effects were referred against the mean age of the period studied, which was 6.5 years. In the next section, we show the interaction effects between single metals and age, which represent the change in the effects of individual metals when increasing or decreasing age from the mean age.

3.4.2 Interaction effects of metals with age

The overall mean effects from the population of the interactions between every single metal k and age on BP are represented by coefficients θ_k in eq. (1). An interacting effect between a single metal and age represents

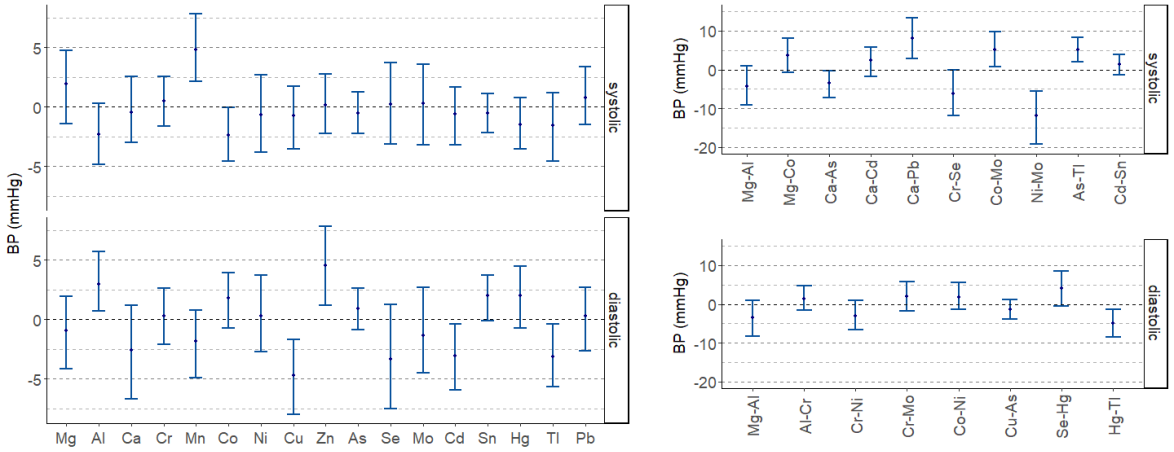

Figure 4: Mean and 90% credible intervals of single metal effects on SBP and DBP, representing the change in BP given a 10% increase in metal concentrations. These effects are referred to the mean age of the period studied which was 6.5 years (Left). Mean and 90% credible intervals of the largest and most significant metal co-interaction effects on SBP and DBP. These metal co-interaction coefficients represent the change in the single effect of one of the metals (single effects presented in previous Section 3.4.1) given a 10% increase in the concentration of the other metal (Right).

the change in a single metal effect (presented in previous Section 3.4.1) on BP given a one-year change in age. The mean and 90% credible intervals of the coefficients of these interaction effects between single metals and age are plotted in Figure G.1 in Appendix G. Most of the interaction effects were found to be very small and non-significant, no larger than ± 0.1 mmHg given a one-year change in age. Only interactions between Ni and age, for SBP, and Mg and age, for DBP, had a slightly larger and more significant effects, causing a decrease of 0.2 mmHg and an increase of 1.9 mmHg, respectively, given a one-year change in age.

3.4.3 Co-interaction metal effects

The overall mean effects from the population of selected co-interaction metals (selected interactions between two metals) on BP are represented by coefficients ρ_j in equation (1). These metal co-interaction coefficients represent the change in the effect of a single metal (presented in previous Section 3.4.1) with a 10% increase in the concentration of the interaction metal. The mean and 90% credible intervals of the most significant metal co-interaction effects on SBP and DBP are shown in Figure 4-right.

Most of these co-interaction metal effects were not less than 2 mmHg in the change of the effect of one of the metals given an increase of 10% in the other metal, indicating that the interactions had relatively large effects. The largest co-interaction effects positively associated with SBP were Mg-Co, Ca-Pb, Co-Mo and As-Tl, with a mean increasing change of 4.2, 7.8, 5.0, and 4.9 mmHg, respectively, in the effect of one of the metals given an increase of 10% in the other metal. The largest co-interaction effects negatively associated with SBP were Mg-Al, Cr-S, and Ni-Mo, with a mean decreasing change of 4.3, 5.4, and 12.2 mmHg, respectively, in the effect of one of the metals given an increase of 10% in the other metal. The largest co-interaction effects positively associated with DBP was Se-Hg with a mean increasing change of 5.0 mmHg in the effect of Se given an increase of 10% in Hg, and vice versa. The largest co-interaction effects negatively associated with DBP were Mg-Al, Cr-Ni, and Hg-Tl, with a mean decreasing change of 4.2, 3.1, and 4.9 mmHg, respectively, in the effect of one of the metals given an increase of 10% in the other metal.

3.4.4 Regularised versus non-regularised metal coefficients

As commented in Section 2.3.3, by using hierarchical priors, unlike uniform or independent non-informative priors, for metal coefficients, the coefficient estimates that are too large and with too large an uncertainty due to model complexity and collinearity are penalized. Figure 5 shows metal coefficient estimates using both hierarchical and independent non-informative priors. It can be seen how Bayesian regularisation through using hierarchical priors on metal coefficients overcomes the unstable coefficient estimates, with too wide an uncer-

Figure 5: Comparison between metal coefficient estimates using hierarchical priors and independent non-informative priors. These coefficients are referred to the log-transformed and standardized metals.

tainty and too large a magnitude, of using independent non-informative prior distributions. The coefficients shown in Figure 5 were referred to the log-transformed and standardised metals, as the aim was only to show how regularisation softens the effects of model complexity and collinearity on the coefficients.

3.5 Imputed below detection limit metal observations

Posterior distributions for metal concentrations below the LOD were estimated from the multivariate distribution as a function of the rest of the metals per individual i, as explained in Section 2.3.4. Furthermore, the estimates were upper bounded by the LOD of each specific metal k. Figure 6 shows the posterior for the observations below the LOD for some individuals and metals. It can be seen that the posterior distributions are mostly concentrated close to the upper-bound LOD as expected. Furthermore, the more observations below the LOD there are in a variable, the wider the posterior distribution of an imputed observation of that variable (see, for example, posteriors of imputed observations from the metals Al, Cr and Ni that have 62%, 63% and 46%, respectively, of observations below the LOD). The fewer observations below the LOD there are in a variable, the narrower the posteriors of an imputed observation of that variable (see, for example, posteriors of imputed observations from the metals Cd, Hg and Tl that have 0.8%, 0.5% and 1.3%, respectively, of observations below the LOD).

3.6 Model diagnosis and validation

The outliers, which are mainly present at the four-year visit, were well captured by the Student's t-distribution of 4 degrees of freedom, so they did not influence inference. Apart from the outliers, the remaining residuals were normal and homocedastic signifying that the model fits the linear model assumptions (Figure F.1).

The LOO-PIT values were nearly uniform (Figure F.2), which means that the posterior predictive distribution was well calibrated and the model captured the main variability patterns in the data well. There was a slight deviation from uniformity at the four-year visit caused by outliers present in that set of observations.

Due to the existence of outliers, calculating robust measures of predictive accuracy as well as the coefficient of determination (R^2) using the median of residuals instead of the mean is recommended. The R^2 of our model was around 0.75 for both SBP and DBP and the root mean square error (RMSE) was around 4 mmHg for both SBP and DBP (Figure F.3). Figure F.3 also shows the R^2 and the RMSE calculated using the mean of the residuals.

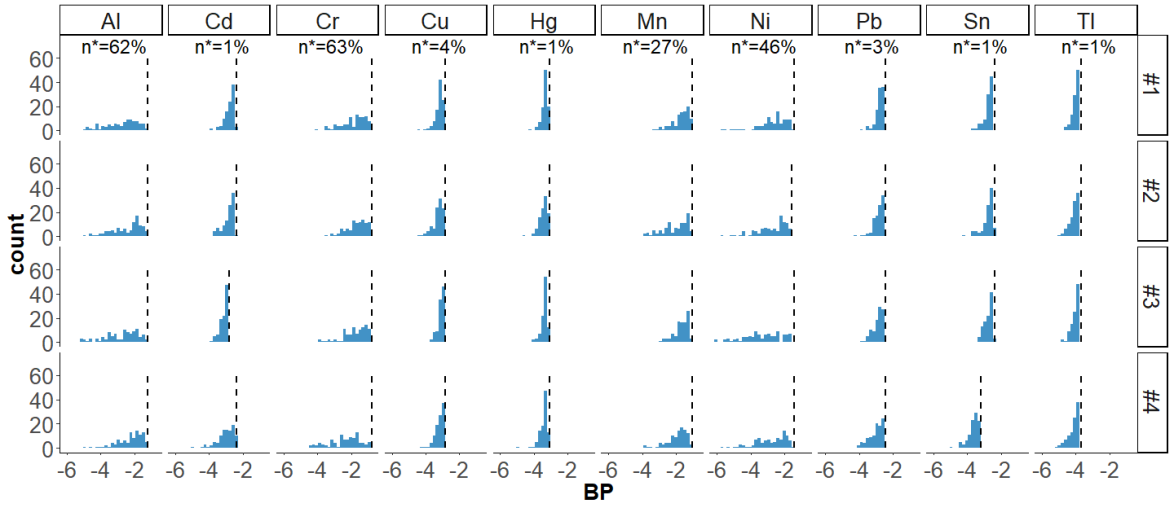

Figure 6: Posterior for observations below the LOD for some individuals and metals. The vertical dashed black line indicates the upper bound LOD of a metal. n^* indicates the percentage of observations below the LOD of a metal.

4 Discussion

This was a Spanish birth cohort study with a longitudinal assessment of BP during childhood and data on prenatal exposure to metals and metalloids. Specifically, we evaluated the association between maternal urinary concentrations of Mg, Al, Ca, Cr, Mn, Co, Ni, Cu, Zn, As, Se, Mo, Cd, Sn, Hg, Tl, and Pb and children's BP assessed at four, seven, and nine years of age using longitudinal Bayesian multilevel modelling with regularisation for model complexity and collinearity. We observed that prenatal concentrations of some metals and metalloids were associated with altered children's BP. Thus, positive relationships between maternal Mn and children's SBP and between maternal Al, Zn, and Sn and children's DBP were observed. Additionally, negative relationships between maternal Co and children's SBP and between maternal Cu, Se, and Cd and children's DBP were also found. We also observed significant interaction effects between some metals and metalloids on children's BP, such as, positive interaction effects between Ca & Pb, Co & Mo, As & Tl, and Se & Hg, and negative interaction effects between Ni & Mo. Al concentrations were significantly associated with children's BP, as well as the interaction between Cr and Se; however, the presence of 63% and 62% of observations below the LOD in Al and Cr metals, respectively, in our study requires that interpretation be carried out with caution.

Among the associations observed in the present study the most consistent ones are between maternal Mn, Cu, Zn, and Cd and children's BP. Prenatal Mn concentrations in our population were associated with an increase of around 5 mmHg in child SBP, prenatal Zn increased child DBP by 2.2 mmHg, and prenatal Cu was associated with a decrease of 4.6 mmHg in child DBP. These results differ from those reported by Zhang et al. (2021), where a negative relationship was observed between maternal Mn red blood cell concentrations and child SBP assessed at 3 to 15 years of age in the USA. However, two other prospective studies conducted in Mexican (Kupsco et al., 2019) and European (Warembourg et al., 2019) populations did not find any association between prenatal Mn and Cu concentrations and children's BP assessed at 4 to 6 and at 6 to 11 years of age, respectively. Both studies measured these compounds in maternal blood samples taken during pregnancy. Regarding Zn, only the Mexican study (Kupsco et al., 2019) evaluated the influence of maternal blood Zn on child BP, finding no association. Mn, Cu, and Zn are essential elements involved in numerous biological processes crucial for human health (Agency for Toxic Substances and Disease Registry, 2012a,b; Uriu-Adams et al., 2010). The bibliography regarding the influence of exposure to these elements on pregnancy or child health outcomes is still very limited. Prenatal Mn has been related to protective effects against hypertension (Borghese et al., 2023) and preeclampsia (Liu et al., 2020, 2019) in pregnant women and, contrarily, prenatal Cu has also been associated with altered maternal DBP and SBP (Liu et al., 2021) and preeclampsia (Lisa et al., 2021; Rafeeinia et al., 2014), both being prenatal situations related to altered child BP (Chen et al., 2023; Ouyang et al., 2023; Tenhola et al., 2006). In the same way, in a recent case-control study conducted in Palestine, low maternal dietary Zn was associated with hypertension (El Bilbeisi et al., 2023); additionally, serum Zn concentrations were lower in Bangladeshi pregnant women with preeclampsia (Uddin et al., 2023). It seems these

elements could be related to maternal cardiovascular health, however the action mechanism by which it could affect children's BP is still misunderstood and the bibliography is very limited and controversial. Furthermore, the complexity of evaluating exposure levels of essential metals must be taken into account, because homeostatic mechanisms maintain the levels of these compounds within physiological limits, and the levels evaluated in the urine may reflect the metabolic process, and not an excess of exposure (Martinez-Morata et al., 2023).

In our study, maternal urinary Cd concentrations were associated with a decrease of 3.6 mmHg in children's DBP. Among the reviewed studies H.1, most evaluated the relationship between Cd measured during pregnancy and BP assessed during childhood, however, none of them found any significant association. Only Zhang et al. (2021) observed an influence of the maternal Mn-Cd interaction on child BP, with an inverse association between Mn and child SBP stronger at higher levels of Cd. Maternal Cd concentrations have been measured in different biological samples during pregnancy in different studies, such as maternal red blood cells (Zhang et al., 2021), blood (Kupsco et al., 2019; Warembourg et al., 2019), serum (Ma et al., 2023), and urine (Akhtar et al., 2021; Hawkesworth et al., 2013; Howe et al., 2021), which hampers the comparability between them. Of note urinary Cd mainly reflects long-term exposure and blood Cd recent exposure, but there should be a noticeable overlap (Agency for Toxic Substances and Disease Registry, 2012b). Cadmium is a toxic metal of considerable concern, which has been identified as a human carcinogen by the International Agency for Cancer Research (International Agency for Research on Cancer, 1993) and has been related to several cardiovascular diseases (Guo et al., 2022). However maternal Cd is only partially transferred from the mother to the foetus suggesting that placenta acts as a partial barrier to this metal (Gundacker and Hengstschläger, 2012). The Cd accumulated in the placenta could affect the vascular system of the foetus, reducing placental blood flow and the gas and nutrient exchange between the mother and foetus, thus increasing the risk of adverse foetus development (Thompson and Bannigan, 2008). Additionally, prenatal Cd has been related to altered maternal BP (Osorio-Yañez et al., 2016) and preeclampsia (Borghese et al., 2023).

In our study, we observed a significant increase in children's DBP associated with the interaction between Se and Hg, with a mean change of 5 mmHg in the effect of Hg given an increase of 10% in Se. Evidence from epidemiological studies evaluating the interaction effect of Hg and Se on cardiovascular health is insufficient to draw any conclusions; nevertheless, according to our results, these metals seem to interact with each other. Evidence suggests that the cardiovascular protection of Se against Hg toxicity may be related to the antioxidant effects of selenoproteins (Mozaffarian, 2009). Xun et al. (2011) evaluated the relationship between long-chain omega-3 polyunsaturated fatty acids (LCω3PUFA), Se, and Hg on hypertension in a cohort of adults from the USA and observed that the cardiovascular protection of LCω3PUFA was more pronounced at higher Se and lower Hg levels measured in nails, although these conclusions seem to be inconsistent as interaction tests between both metals were not statistically significant in the same study. Furthermore, the heterogeneity in metal biomarkers and outcomes prevents any comparability with our study.

Another metal interaction that could be worth mentioning was that between maternal Pb and Ca concentrations. Specifically, we observed an increase of around 8 mmHg in children's SBP associated with Pb given an increase of 10% in Ca, and vice versa. Lead accumulates long-term in bones and the formation of the foetal skeleton during pregnancy could increase the mobilisation of this maternal bone Pb. Several studies have shown an increase in blood Pb levels during the third trimester, which was attributed to increased bone resorption to meet the Ca requirements of the developing foetus (Perng et al., 2019; Silbergeld, 1991). These results suggest an interaction between Ca and Pb during pregnancy; however, the possible effect of this interaction on child BP has hardly been studied. An experimental study conducted on rats has shown that dietary Ca prevents the Pb-associated increase in BP (Bogden et al., 1995); However, other similar studies reported contradictory results (Bogden et al., 1991). In a study conducted on pregnant women in Nigeria, higher blood levels of Pb and lower serum Ca were observed in preeclamptic and hypertensive women (Ikechukwu et al., 2012). In a cohort of older men, an increased likelihood of hypertension associated with high bone and blood Pb was demostrated, particularly in subjects with low dietary Ca intake (Elmarsafawy et al., 2006). Regarding the Pb single metal effect on hypertension, we did not find any significant effect in our study, while two studies observed a positive association between maternal Pb and BP among children from Mexico (Muciño-Sandoval et al., 2021) and the USA (Farzan et al., 2018), measuring Pb in maternal blood cells and toenails, respectively. However, neither of these two studies included the interaction between Pb and other metals in the analysis. Furthermore, Howe et al. (2021) observed a J-shape association between maternal urinary Pb and children's BP at ages 4 and 11, however, non-linear associations under these low signal-to-noise ratio relationships should be made with caution.

The interaction between Ni and Mo was also associated with child BP in our population. Specifically, a 10% increase in Ni concentrations triggered a decrease of 12.2 mmHg in child SPB associated with increasing Mo

concentrations. Maternal urinary Mo was positively associated with child SBP and DBP in the Greek cohort; however, the authors did not evaluate the effect of the interaction between Mo with other metals. Conversely, in other studies Mo concentrations were negatively associated with SBP Lan et al. (2021) and hypertension (Qu et al., 2022) in adults from China, similar to urinary Ni (Liu et al., 2022). However, as far as we know, no study has yet explored the possible interaction between both metals.

The prospective design of this study made it possible to obtain detailed information regarding maternal and child characteristics that may affect children's BP during childhood, as well as to study exposure to metals and metalloids and their possible adverse effects on cardiovascular health during childhood. Linear mixed-effects models for longitudinal data were formulated to assess the relationship between the multiple metal concentrations and BP in children during follow-up. Linear regression coefficients, due to their simplicity, were appropriate to detect subtle and low signal-to-noise ratio relationships, such as observations of metal exposure concentrations and BP in humans. Furthermore, to address the sensitivity of multivariate linear models to model complexity and collinear effects between correlated metal exposure variables, Bayesian regularisation was implemented through using hierarchical prior distributions on both individual metal and interacting metal coefficients. The improvement of the use of hierarchical prior distributions compared with the use of non-informative independent prior distributions on the coefficients or traditional linear regression was demonstrated.

One limitation of the measurement of metal concentrations in observational studies is that some might be below the LOD, forcing those observations to be discarded, thus reducing the study sample size, or estimated in advance often using point estimates or fixed imputations, which may have an excessive influence on the model. Within the Bayesian model framework developed in this study, those observations below the LOD were naturally considered parameters and estimated in a fully probabilistic fashion, where uncertainties were fully and adequately propagated between parameters and model assumptions, obtaining full predictive posterior distributions for those below detection limit observations. By defining upper-bounded prior distributions for these parameters, the parameter estimates were limited by the LOD. Furthermore, the accuracy of the estimates was improved by modelling the covariance structure between the group of metals.

A limitation of this study was that around 65% of the children who were included in the cohort at birth did not reach the nine-year-old assessment. The lost to follow-up population is an inherent characteristic in most longitudinal studies, which is usually related to socioeconomic status (lost to follow-up tends to be more pronounced among the less advantaged social classes and populations with lower levels of education). This situation could bias the observed associations; however, as the sample size of the cohort was quite large, the population characteristics of the sample population included in this study were not significantly changed compared to the initial cohort population. In this regard, Howe et al. (2013) concluded that even when more than half of the cohort was lost to follow-up, qualitative conclusions about the direction and approximate magnitude of inequalities did not change dramatically. Another possible limitation of the present study could be the use of urine to measure the metal concentration matrix. Urine is a reliable and established biomarker for assessing certain compounds, such as As, Cd, inorganic Hg, and Ni, among others; it is unclear whether it is appropriate to assess exposure to other metals, for example, Mn or Z (Martinez-Morata et al., 2023).

5 Conclusion

This study assessed the relationship between prenatal exposure to a group of metals and children's BP longitudinally measured from four- to nine-years of age in a prospective Spanish cohort, using a Bayesian linear mixed-effects model for longitudinal data with regularisation for model complexity and collinearity. We observed noteworthy associations between prenatal exposure to Mn, Cu, Zn, and Cd, as well as Se-Hg, Mo-Ni, and Pb-Ca interactions, and children's BP during the follow-up. As elevated paediatric hypertension could be a predictor of cardiovascular disease during adulthood, which is a major cause of death and morbidity in many populations, it is important to identify the possible risk factors. This study provides reliable insights about the potential effects of join exposure to multiple metals on the risk of altering children's BP, considering potential two-way interactions between metals. Regularisation for model complexity and collinearity using hierarchical prior distributions on metal coefficients has been proven essential to evaluate multiple exposures in linear models, compared with the use of independent priors or traditional linear regression. Furthermore, we proposed a powerful modelling approach to predict full posterior distributions for below detection limit metal observations based on modelling the group of metals with an upper bounded multivariate normal distribution. The evidence of the influence of prenatal metal and metalloid concentrations on children's BP and more longitudinal research on this topic would be advisable.

Acknowledgements

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A Flowgraph of participant selection

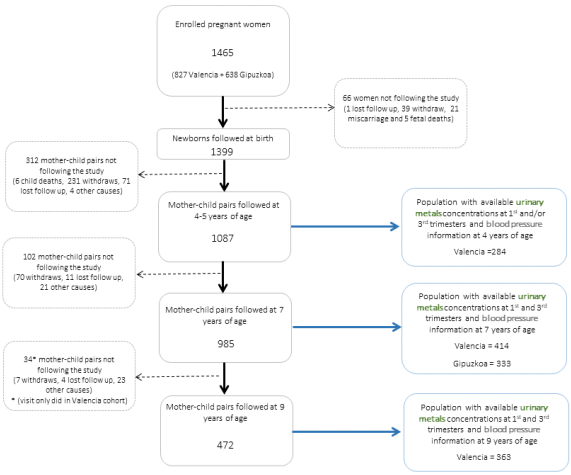

Figure A.1: Flowgraph of participant selection.

B Metal detection limits

The metal detection limits (LOD) in μg/g and the percentage of measurements below their corresponding LOD are shown for each metal and cohort in Table B.1. The measurements of the samples from the two cohorts, Valencia and Gipuzkoa, were carried out at different times, so they may have given slightly different LODs.

		Mg	Al	Ca	Cr	Mn	Co	Ni	Cu	Zn	As	Se	Mo	Cd	Sn	Hg	Tl	Pb
LOD	Valencia	9.22	5.20	47.91	0.30	0.13	0.01	0.91	1.18	3.27	0.33	1.75	0.06	0.03	0.11	0.08	0.04	0.16
	Gipuzkoa	5.82	3.07	40.88	0.25	0.10	0.03	0.85	0.29	4.05	0.33	1.27	0.07	0.03	0.06	0.05	0.03	0.08
%		0%	62%	0%	63%	27%	0%	46%	3%	0%	0%	0%	0%	1%	1%	1%	1%	3%

Table B.1: LODs (μg/g) and percentages of measurements below the LOD per metal.

C Prior distributions for hyperparameters

D Covariate descriptive frequency plots

As can be appreciated in Table D.1, there were no significant differences in participant characteristics between the study population and the total INMA cohorts population.

	4 years old (n=579)		9 years old (n=394)		11 years old (n=338)	
	Study population	**Total INMA population**	**Study population**	**Total INMA population**	**Study population**	**Total INMA population**
	[n (%)]	[n (%)]	[n (%)]	[n (%)]	[n (%)]	[n (%)]
Sex						
Female	140 (49%)	195 (49%)	371 (50%)	421 (50%)	178 (49%)	206 (49%)
Male	144 (51%)	205 (51%)	368 (50%)	422 (50%)	185 (51%)	211 (51%)
Living area						
Rural	9 (3%)	14 (4%)	53 (7%)	61 (7%)	13 (4%)	15 (4%)
Semiurban	31 (11%)	47 (12%)	184 (25%)	210 (25%)	42 (12%)	48 (12%)
Urban	244 (86%)	339 (85%)	502 (68%)	572 (68%)	308 (85%)	354 (85%)
Lactation						
>0-16 (weeks)	63 (22%)	91 (23%)	154 (21%)	176 (21%)	84 (23%)	93 (22%)
>16-24 (weeks)	47 (17%)	61 (15%)	120 (17%)	137 (17%)	55 (15%)	65 (16%)
>24 (weeks)	126 (44%)	185 (46%)	351 (49%)	407 (50%)	163 (45%)	192 (46%)
0 (weeks)	48 (17%)	63 (16%)	96 (13%)	102 (12%)	61 (17%)	67 (16%)
Social class						
CS I+II	121 (43%)	180 (45%)	265 (26%)	312 (25%)	156 (43%)	187 (45%)
CS III	83 (29%)	109 (27%)	194 (36%)	208 (37%)	105 (29%)	115 (28%)
CS IV+V	80 (28%)	111 (28%)	280 (38%)	323 (38%)	102 (28%)	115 (28%)
Smoking pregnan.						
No	231 (81%)	320 (80%)	621 (84%)	706 (84%)	293 (81%)	334 (80%)
Yes	53 (19%)	80 (20%)	118 (16%)	137 (16%)	70 (19%)	83 (20%)
Passive smoking						
No	141 (50%)	190 (48%)	423 (57%)	478 (57%)	186 (51%)	214 (51%)
Yes	143 (50%)	210 (52%)	316 (43%)	359 (43%)	177 (49%)	203 (49%)
Child BMI						
Obesity	30 (11%)	43 (11%)	137 (19%)	157 (19%)	84 (23%)	95 (23%)
Overweight	62 (22%)	89 (22%)	219 (30%)	247 (29%)	103 (28%)	108 (26%)
Standard	192 (68%)	268 (67%)	383 (52%)	439 (52%)	176 (48%)	214 (51%)
Physical activity						
Moderately active	81 (29%)	114 (28%)	122 (30%)	138 (30%)	-	-
Quite active	140 (49%)	186 (46%)	182 (45%)	201 (44%)	-	-
Sedentary	5 (2%)	6 (2%)	7 (2%)	7 (2%)	-	-
Slightly active	14 (5%)	23 (6%)	29 (7%)	34 (7%)	-	-
Very active	44 (15%)	71 (18%)	67 (16%)	79 (17%)	-	-
Labour						
No	101 (36%)	141 (35%)	250 (34%)	290 (35%)	158 (44%)	190 (46%)
Yes	183 (64%)	259 (65%)	489 (66%)	550 (65%)	205 (56%)	227 (54%)
Mother BMI						
Obesity	14 (5%)	17 (4%)	31 (4%)	33 (4%)	16 (4%)	18 (4%)
Overweight	56 (20%)	76 (19%)	119 (16%)	135 (16%)	69 (19%)	79 (19%)
Standard	214 (75%)	307 (77%)	589 (80%)	675 (80%)	278 (77%)	320 (77%)
Father BMI						
Obesity	15 (5%)	20 (5%)	28 (4%)	30 (4%)	18 (5%)	19 (5%)
Overweight	75 (26%)	106 (26%)	182 (25%)	204 (24%)	94 (26%)	109 (26%)
Standard	194 (68%)	274 (68%)	529 (72%)	609 (72%)	251 (69%)	289 (69%)
Sodium intake						
<2000	101 (36%)	147 (37%)	17 (2%)	18 (2%)	-	-
2000-2500	17 (6%)	24 (6%)	414 (56%)	465 (55%)	-	-
>2500	166 (58%)	229 (57%)	308 (42%)	357 (42%)	-	-

Table D.1: Frequency statistics of sociodemographic, environmental, anthropometric, and lifestyle variables in both the study sample population and total INMA cohort population. (BMI: Body mass index)

E Adjustment covariates effects

In Figure E.1, estimates of single and co-interaction effects of covariates are depicted. The most significant single and co-interaction covariate effects are: *child weight*, which is positively associated with BP, with mean effects from 2.2 to 2.7 mmHg for DBP, and 1.5 to 2.3 mmHg for SBP, given a standard deviation increase in weight; *sodium intake*, which increases SBP by 1.9 and 0.7 mmHg at the four and seven-year visits, respectively, and DBP by 1.0 mmHg at the seven-year visit, for a 500 mg increase in sodium intake. A significant interaction of *child weight* with *social class* was also found, especially at the seven-year visit, increasing SBP by 2 mmHg for medium social class and 1 mmHg for low social class, and increasing DBP by 1.5 mmHg for medium social class. *Child weight* was also found to interact with *passive smoking*, increasing BP at the seven-year visit and decreasing BP at four years. *Social class* was also found to be associated with BP, especially SBP, with a decreasing effect on BP for high social classes and an increasing effect for low social classes, except at four years, where low social class decreased BP.

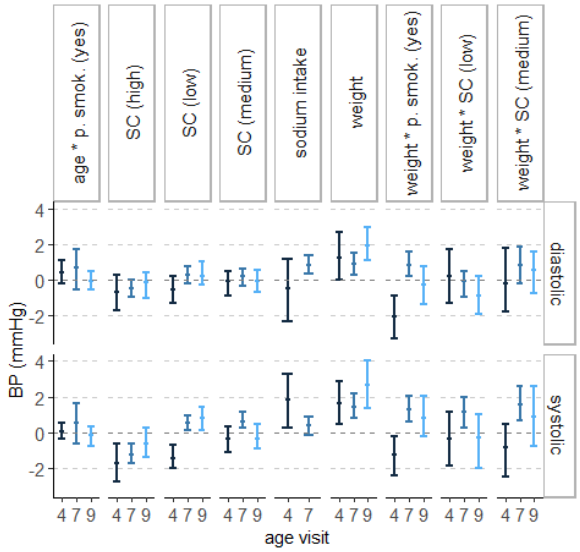

Figure E.1: Mean and 90% credible intervals of single and co-interaction effects of adjustment covariates.

F Diagnostic and validation plots

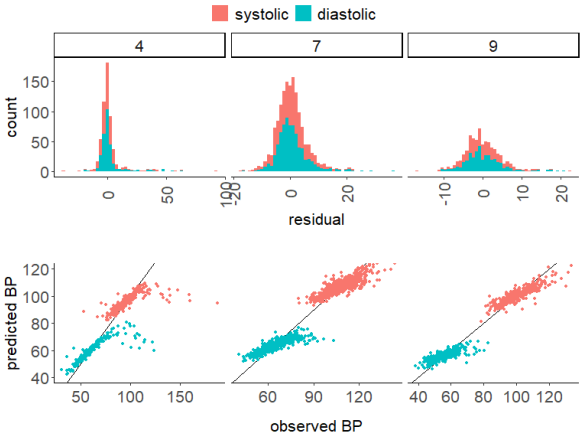

Figure F.1: Model residual histograms (top) and bivariate plots of observations and predictions (bottom) of systolic and diastolic BP per age.

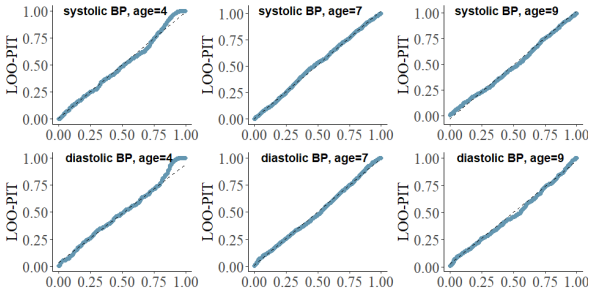

Figure F.2: Uniform Q-Q plot of LOO-PIT values per age and systolic and diastolic BP.

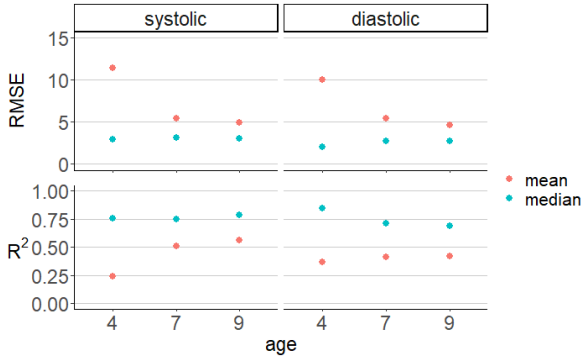

Figure F.3: Model root mean square error (RMSE) and coefficient of determination (R^2) per age and systolic and diastolic BP.

G Interacting effect estimates between metals and age

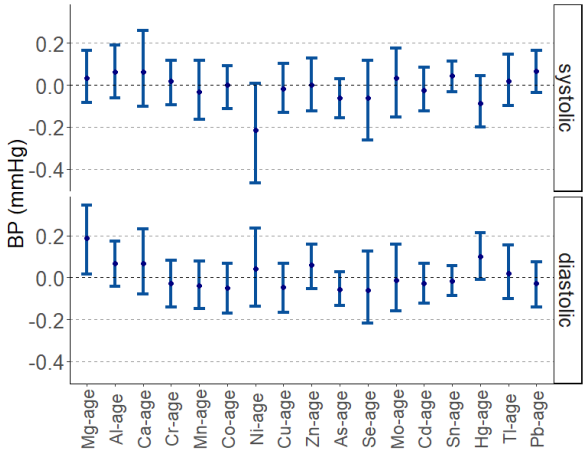

Figure G.1: Mean and 90% credible intervals of the coefficients of the interaction effects between single metals and age on systolic and diastolic BP. These interaction coefficients represent the change in the single metal coefficients (presented in previous Section 3.4.1) with a one-year change in age.

H Studies reviewed on prenatal exposure to metals and children's blood pressure

Study	Country	n	Matrix	Metals (median-$\mu g/L$)	Age exp	Age outcome	Results
Present study	Spain	284, 747 and 363	Maternal urine	Mg (62193), Al (7,59), Ca (162267), Cr (0,44) ,Mn (0,31), Co (0,46), Ni (1,96), Cu (9,47), Zn (379,9), As (36,8), Se (26,2), Mo (39,0), Cd (0,19), Sn (1,29), Hg (0,90), Tl (0,26), Pb (1,06)	1st and 3rd T	4, 7 and 9 yo	Single metals: Negative association Cu, Se and Cd and DBP Positive association Al, Zn and Sn and DBP Positive association Mn and SBP Negative association Al and Co and SBP Interactions metal-metal: Positive association Mg-Co, Ca-Pb, Co-Mo, As-Tl and SBP Negative association Cr-Se, Ni-Mo and SBP Positive association Se-Hg and DBP Negative association Cr-Ni and Hg-Tl and DBP
Ma, 2023	China	2535-2680	Maternal serum	As (1.77), Se (70.37), Cd (0.07), Hg (0.36)	1st, 2nd, and 3rd trimester of pregnancy	5-6 yo	Single metals: Negative association As (1stT) and MAP Positive association As (3rd T) and DBP, SBP, and MAP Positive association Hg (3rd T) and DBP and MAP Mixture: Positive association between metal mixture (3rd T) and risk of DBP, SBP and MAP. Potential As-Hg interaction
Howe, 2021	Greece	176	Maternal urine	As (12.2), Cd (0.47), Co (0.46), Pb (1.00), Mg (71.3 mg/L), Mo (64.9), Se (21.8), Sb (0.05)	12 wg	4, 6 and 11 yo	Single metals: Positive association Co and SBP and DBP at 4y (non-linear) Positive association Mo and SBP and DBP at 4y (J-shaped) Negative association Co and SBP and DBP per-year change in blood pressure from age 4 to age 11. Interaction: Mo and Pb synergistic interaction and BP
Zhang, 2021	US	1194	Maternal red blood cells	Pb (24.2), Hg (2.15), Cd (0.69), Se (278.0), Mn (37.30)	24-72h after delivery	3 to 15 yo	Single metals: Negative association Se and SBP. Negative association Mn and SBP Mn–Cd interaction: the inverse association between Mb and SBP was stronger at higher levels of Cd
Muciño-Sandoval, 2021	Mexico	601	Maternal blood, cord blood, and child blood	Pb (2.9, in maternal blood at 2nd trimester)	Prenatal exposure (2nd and 3rd T, delivery) Postnatal exposure (1, 2, 4 y)	6 and 8 yo	Pb prenatal exposure associated with less risk to have elevated DBP and SBP (>90th percentile)
Akhtar, 2021	Bangladesh	540	Maternal urine	Cd (0.58), As (76.07)	8 wg	4, 5 and 9 yo	No associations between As concentrations and BP at 4, 5 ans 9 yo. Positive association between maternal Cd concentrations and SBP at 9 yo.
Kupsco, 2019	Mexico	548	Maternal blood	As (0.85), Cd (0.29), Co (0.22), Cr (0.80), Cs (3.10), Cu (1580), Mn (15), Pb (37), Sb (3.8), Se (250), Zn (6130)	2nd T	4-6 yo	Single metals: no association Mixture: Overall mixture negative associated with SBP
Warembourg, 2019	HELIX cohort (Europeans cohorts)	1277	Maternal blood	As (1.4), Cd (0.1), Co (0.2), Cs (1.4), Cu (903), Hg (0.9), Mn (8.6), Mo (0.8), Pb (8.5), Tl (2.0)	na	6 to 11 yo	No association between prenatal metal concentrations and SBP or DBP.
Farzan, 2018	US	323	Toenails	Pb (0.30)*, As (0.10)*	28 wg	5 yo	Positive association prenatal Pb and SBP.
Hawkesworth, 2013	Bangladesh	1488	Urine	As (80.5)*, Cd (0.63)*	Early gestation (8 wg) and late gestation (30 wg)	4,5 yo	Positive association between late As and SBP.

Table H.1: Longitudinal studies on prenatal exposure to metals and children's blood pressure. As: arsenic; Ba: barium ; Cd: cadmium; Co: cobalt; Cu: cupper; Cr: chromium; Cs: cesium; DBP: diastolic arterial pressure ; Hg: mercury; MAP: mean arterial pressure; Mg: magnesium; Mo: molybdenum; Na: not available; Pb: lead; Sb: antimony; SBP: sistolic arterial pressure; Se: selenium; T: trimestre of pregnancy; Wg: weeks of gestation; Yo: years old; Zn: zinc. *Mean.

References

Agency for Toxic Substances and Disease Registry (2012a) Toxicological profile for cadmium. Tech. rep.

Agency for Toxic Substances and Disease Registry (2012b) Toxicological profile for manganese. Tech. rep., Agency for Toxic Substances and Disease Registry:Georgia

Ahn J, Kim NS, Lee BK, Park J, Kim Y (2018) Association of blood pressure with blood lead and cadmium levels in korean adolescents: analysis of data from the 2010–2016 korean national health and nutrition examination survey. Journal of Korean Medical Science 33(44)

Akhtar E, Roy AK, Haq MA, Von Ehrenstein OS, Ahmed S, Vahter M, Ekstrom EC, Kippler M, Wagatsuma Y, Raqib R (2021) A longitudinal study of rural bangladeshi children with long-term arsenic and cadmium exposures and biomarkers of cardiometabolic diseases. Environmental Pollution 271:116333

Barletta GM, Pierce C, Mitsnefes M, Samuels J, Warady BA, Furth S, Flynn J (2018) Is blood pressure improving in children with chronic kidney disease? a period analysis. Hypertension 71(3):444–450

Bobb JF, Valeri L, Claus Henn B, Christiani DC, Wright RO, Mazumdar M, Godleski JJ, Coull BA (2015) Bayesian kernel machine regression for estimating the health effects of multi-pollutant mixtures. Biostatistics 16(3):493–508

Bogden JD, Gertner SB, Kemp FW, McLeod R, Bruening KS, Chung HR (1991) Dietary lead and calcium: effects on blood pressure and renal neoplasia in wistar rats. The Journal of nutrition 121(5):718–728

Bogden JD, Kemp FW, Han S, Murphy M, Fraiman M, Czerniach D, Flynn CJ, Banua ML, Scimone A, Castrovilly L, et al. (1995) Dietary calcium and lead interact to modify maternal blood pressure, erythropoiesis, and fetal and neonatal growth in rats during pregnancy and lactation. The Journal of nutrition 125(4):990–1002

Borghese MM, Fisher M, Ashley-Martin J, Fraser WD, Trottier H, Lanphear B, Johnson M, Helewa M, Foster W, Walker M, et al. (2023) Individual, independent, and joint associations of toxic metals and manganese on hypertensive disorders of pregnancy: results from the mirec canadian pregnancy cohort. Environmental Health Perspectives 131(4):047014

Chen X, Wang Y (2008) Tracking of blood pressure from childhood to adulthood: a systematic review and meta–regression analysis. Circulation 117(25):3171–3180

Chen Y, Wu F, Liu X, Parvez F, LoIacono NJ, Gibson EA, Kioumourtzoglou MA, Levy D, Shahriar H, Uddin MN, et al. (2019) Early life and adolescent arsenic exposure from drinking water and blood pressure in adolescence. Environmental research 178:108681

Chen Y, Wang Y, Li Y, Ding G, Zhang Y (2023) Association of the severity of hypertensive disorders in pregnancy with birthweight, childhood obesity, and blood pressure at age 7. Nutrients 15(14):3104

Desai G, Niu Z, Luo W, Frndak S, Shaver AL, Kordas K (2021) Low-level exposure to lead, mercury, arsenic, and cadmium, and blood pressure among 8-17-year-old participants of the 2009–2016 national health and nutrition examination survey. Environmental research 197:111086

Domingo-salvany A, Bacigalupe A, Miguel J, Sociedad D (2013) Propuestas de clase social neoweberiana y neomarxista a partir de la Clasificación Nacional de Ocupaciones 2011. Gaceta Sanitaria 27(3):263–272, DOI 10.1016/j.gaceta.2012.12.009

El Bilbeisi AH, Abo Khosa SM, Taleb MH, El Afifi AM (2023) Assessment of serum, dietary zinc levels, and other risk factors during the third trimester among pregnant women with and without pregnancy-induced hypertension: a case-control study. Frontiers in Nutrition 10:1155529

Elmarsafawy SF, Jain NB, Schwartz J, Sparrow D, Nie H, Hu H (2006) Dietary calcium as a potential modifier of the relationship of lead burden to blood pressure. Epidemiology 17(5):531–537

Falkner B, Lurbe E (2021) Primary hypertension beginning in childhood and risk for future cardiovascular disease. The Journal of Pediatrics 238:16–25

Farzan SF, Howe CG, Chen Y, Gilbert-Diamond D, Cottingham KL, Jackson BP, Weinstein AR, Karagas MR (2018) Prenatal lead exposure and elevated blood pressure in children. Environment international 121:1289–1296

Fuly JTB, Giovaninni NPB, Marcato DG, Alves ERB, Sampaio JD, Moraes LId, Nicola TC, Jesus JSAd, Costalonga EF (2014) Evidence of underdiagnosis and markers of high blood pressure risk in children aged 6 to 13 years. Jornal de pediatria 90:65–70

Gelfand AE, Dey DK, Chang H (1992) Model determination using predictive distributions with implementation via sampling-based methods. Tech. rep., Stanford Univ CA Dept of Statistics

Gelman A, Hill J (2006) Data analysis using regression and multilevel/hierarchical models. Cambridge University Press

Gelman A, Carlin JB, Stern HS, Dunson DB, Vehtari A, Rubin DB (2013) Bayesian data analysis, 3rd edn. FL: CRC press

Gelman A, Hill J, Vehtari A (2020) Regression and other stories. Cambridge University Press

Gibbons RD, Hedeker D, DuToit S (2010) Advances in analysis of longitudinal data. Annual review of clinical psychology 6:79–107

Gundacker C, Hengstschläger M (2012) The role of the placenta in fetal exposure to heavy metals. Wiener Medizinische Wochenschrift (1946) 162(9-10):201–206

Guo X, Su W, Li N, Song Q, Wang H, Liang Q, Li Y, Lowe S, Bentley R, Zhou Z, et al. (2022) Association of urinary or blood heavy metals and mortality from all causes, cardiovascular disease, and cancer in the general population: a systematic review and meta-analysis of cohort studies. Environmental Science and Pollution Research 29(45):67483–67503

Guxens M, Ballester F, Espada M, Ferna MF, Grimalt JO, Ibarluzea J, Olea N, Rebagliato M, Tardón A, Torrent M, Vioque J, Vrijheis M, Sunyer J, on behalf of the INMA project (2012) Cohort Profile : The INMA — INfancia y Medio Ambiente —(Environment and Childhood) Project. International Journal of Epidemiology 41:930–940, DOI 10.1093/ije/dyr054

Habeeb E, Aldosari S, Saghir SA, Cheema M, Momenah T, Husain K, Omidi Y, Rizvi SA, Akram M, Ansari RA (2022) Role of environmental toxicants in the development of hypertensive and cardiovascular diseases. Toxicology Reports 9:521–533

Hansen ML, Gunn PW, Kaelber DC (2007) Underdiagnosis of hypertension in children and adolescents. Jama 298(8):874–879

Hardy ST, Urbina EM (2021) Blood pressure in childhood and adolescence. American Journal of Hypertension 34(3):242–249

Hawkesworth S, Wagatsuma Y, Kippler M, Fulford AJ, Arifeen SE, Persson LA, Moore SE, Vahter M (2013) Early exposure to toxic metals has a limited effect on blood pressure or kidney function in later childhood, rural bangladesh. International journal of epidemiology 42(1):176–185

Howe CG, Margetaki K, Vafeiadi M, Roumeliotaki T, Karachaliou M, Kogevinas M, McConnell R, Eckel SP, Conti DV, Kippler M, et al. (2021) Prenatal metal mixtures and child blood pressure in the rhea mother-child cohort in greece. Environmental Health 20(1):1–16

Howe LD, Tilling K, Galobardes B, Lawlor DA (2013) Loss to follow-up in cohort studies: bias in estimates of socioeconomic inequalities. Epidemiology 24(1):1–9

Ikechukwu IC, Ojareva OIA, Ibhagbemien AJ, Okhoaretor OF, Oluwatomi OB, Akhalufo OS, Oluwagbenga AT, Chigaekwu MN (2012) Blood lead, calcium, and phosphorus in women with preeclampsia in edo state, nigeria. Archives of Environmental & Occupational Health 67(3):163–169

International Agency for Research on Cancer (1993) Iarc monographs on the evaluation of carcinogenic risks to humans. beryllium, cadmium, mercury, and exposures in the glass manufacturing industry. Tech. rep., In: IARC MONOGRAPHS ON THE EVALUATION OF CARCINOGENIC RISKS TO HUMANS. Lyon

Kim SS, Meeker JD, Aung MT, Yu Y, Mukherjee B, Cantonwine DE, McElrath TF, Ferguson KK (2020) Urinary trace metals in association with fetal ultrasound measures during pregnancy. Environmental Epidemiology 4(2)

Kupsco A, Kioumourtzoglou MA, Just AC, Amarasiriwardena C, Estrada-Gutierrez G, Cantoral A, Sanders AP, Braun JM, Svensson K, Brennan KJ, et al. (2019) Prenatal metal concentrations and childhood cardiometabolic risk using bayesian kernel machine regression to assess mixture and interaction effects. Epidemiology (Cambridge, Mass) 30(2):263

Lan C, Liu Y, Li Q, Wang B, Xue T, Chen J, Jiangtulu B, Ge S, Wang X, Gao M, et al. (2021) Internal metal (loid) s are potentially involved in the association between ambient fine particulate matter and blood pressure: A repeated-measurement study in north china. Chemosphere 267:129146

Lisa S, Akhter Q, Nessa A, Munna M, Eza L (2021) Serum copper level and its relation with blood pressure and urinary protein level in preeclampsia. Mymensingh Medical Journal: MMJ 30(2):473–477

Liu T, Zhang M, Guallar E, Wang G, Hong X, Wang X, Mueller NT (2019) Trace minerals, heavy metals, and preeclampsia: findings from the boston birth cohort. Journal of the American Heart Association 8(16):e012436

Liu T, Hivert MF, Rifas-Shiman SL, Rahman ML, Oken E, Cardenas A, Mueller NT (2020) Prospective association between manganese in early pregnancy and the risk of preeclampsia. Epidemiology 31(5):677–680

Liu T, Zhang M, Rahman ML, Wang X, Hinkle SN, Zhang C, Mueller NT (2021) Exposure to heavy metals and trace minerals in first trimester and maternal blood pressure change over gestation. Environment international 153:106508

Liu Y, Wu M, Xu B, Kang L (2022) Association between the urinary nickel and the diastolic blood pressure in general population. Chemosphere 286:131900

Lozano M, Murcia M, Soler-Blasco R, Casas M, Zubero B, Riutort-Mayol G, Gil F, Olmedo P, Grimalt JO, Amorós R, et al. (2022) Exposure to metals and metalloids among pregnant women from spain: Levels and associated factors. Chemosphere 286:131809

Lurbe E, Ingelfinger J (2021) Developmental and early life origins of cardiometabolic risk factors: novel findings and implications. Hypertension 77(2):308–318

Luyckx VA, Bertram JF, Brenner BM, Fall C, Hoy WE, Ozanne SE, Vikse BE (2013) Effect of fetal and child health on kidney development and long-term risk of hypertension and kidney disease. The Lancet 382(9888):273–283

Ma Y, Liang C, Wang Z, Wang X, Xie L, Tao S, Yan S, Wu X, Wei Z, Tong J, et al. (2023) Association between prenatal metals exposure and blood pressure in 5–6 years children: A birth cohort study. Environmental Research 219:114974

Martinez-Morata I, Sobel M, Tellez-Plaza M, Navas-Acien A, Howe CG, Sanchez TR (2023) A state-of-the-science review on metal biomarkers. Current environmental health reports 10(3):215–249

Moin A, Mohanty N, Tedla YG, Carroll AJ, Padilla R, Langman CB, Smith JD (2021) Under-recognition of pediatric hypertension diagnosis: Examination of 1 year of visits to community health centers. The Journal of Clinical Hypertension 23(2):257–264

Mozaffarian D (2009) Fish, mercury, selenium and cardiovascular risk: current evidence and unanswered questions. International journal of environmental research and public health 6(6):1894–1916

Muciño-Sandoval K, Ariza AC, Ortiz-Panozo E, Pizano-Zárate ML, Mercado-García A, Wright R, Maria Téllez-Rojo M, Sanders AP, Tamayo-Ortiz M (2021) Prenatal and early childhood exposure to lead and repeated measures of metabolic syndrome risk indicators from childhood to preadolescence. Frontiers in Pediatrics 9:750316

Neal RM (2011) Handbook of Markov Chain Monte Carlo, CRC press, chap MCMC using Hamiltonian dynamics

Osorio-Yáñez C, Ayllon-Vergara JC, Arreola-Mendoza L, Aguilar-Madrid G, Hernández-Castellanos E, Sánchez-Peña LC, Del Razo LM (2015) Blood pressure, left ventricular geometry, and systolic function in children exposed to inorganic arsenic. Environmental Health Perspectives 123(6):629–635

Osorio-Yañez C, Gelaye B, Miller R, Enquobahrie D, Baccarelli A, Qiu C, Williams M (2016) Associations of maternal urinary cadmium with trimester-specific blood pressure in pregnancy: role of dietary intake of micronutrients. Biological trace element research 174:71–81

Ouyang F, Wells JC, Zhang GH, Du K, Wang X, Shen L, Luo ZC, Zhang J (2023) Maternal prenatal factors and child adiposity in associations with cardiometabolic risk factors in term-born chinese children at the age of 2 years. Nutrients 15(15):3342

Palma I, Farran A, Cervera P (2008) Tables of Food Composition for household measures of habitual consumption in Spain: CESNID, 4th edn. McGraw Hill, Madrid

Perng W, Tamayo-Ortiz M, Tang L, Sánchez BN, Cantoral A, Meeker JD, Dolinoy DC, Roberts EF, Martinez-Mier EA, Lamadrid-Figueroa H, et al. (2019) Early life exposure in mexico to environmental toxicants (element) project. BMJ open 9(8):e030427

Qu Y, Lv Y, Ji S, Ding L, Zhao F, Zhu Y, Zhang W, Hu X, Lu Y, Li Y, et al. (2022) Effect of exposures to mixtures of lead and various metals on hypertension, pre-hypertension, and blood pressure: A cross-sectional study from the china national human biomonitoring. Environmental Pollution 299:118864

Rafeeinia A, Tabandeh A, Khajeniazi S, Marjani AJ (2014) Serum copper, zinc and lipid peroxidation in pregnant women with preeclampsia in gorgan. The open biochemistry journal 8:83

Riutort-Mayol G, Gómez-Rubio V, Lerma JL, del Hoyo-Meléndez JM (2020) Correlated functional models with derivative information for modeling microfading spectrometry data on rock art paintings. Mathematics 8(12):2141

Rosner B, Cook NR, Daniels S, Falkner B (2013) Childhood blood pressure trends and risk factors for high blood pressure: the nhanes experience 1988–2008. Hypertension 62(2):247–254

Shiue I, Hristova K (2014) Higher urinary heavy metal, phthalate and arsenic concentrations accounted for 3–19% of the population attributable risk for high blood pressure: Us nhanes, 2009–2012. Hypertension Research 37(12):1075–1081

Silbergeld EK (1991) Lead in bone: implications for toxicology during pregnancy and lactation. Environmental health perspectives 91:63–70

de Simone G, Mancusi C, Hanssen H, Genovesi S, Lurbe E, Parati G, Sendzikaite S, Valerio G, Di Bonito P, Di Salvo G, et al. (2022) Hypertension in children and adolescents: A consensus document from e. council on hypertension, european association of preventive cardiology, european association of cardiovascular imaging, association of cardiovascular nursing & allied professions, esc council for cardiology practice and association for european paediatric and congenital cardiology. European heart journal 43(35):3290–3301

Song P, Zhang Y, Yu J, Zha M, Zhu Y, Rahimi K, Rudan I (2019) Global prevalence of hypertension in children: a systematic review and meta-analysis. JAMA pediatrics 173(12):1154–1163

Stan Development Team (2022) Stan modeling language users guide and reference manual, 2.30. URL `https://mc-stan.org`

Stergiou GS, Parati G, Asmar R, O'Brien E (2012) Requirements for professional office blood pressure monitors. Journal of hypertension 30(3):537–542, DOI 10.1097/HJH.0b013e32834fcfa5., URL https://pubmed.ncbi.nlm.nih.gov/22241143/

Stergiou GS, Palatini P, Parati G, O'Brien E, Januszewicz A, Lurbe E, Persu A, Mancia G, Kreutz R, et al. (2021) 2021 european society of hypertension practice guidelines for office and out-of-office blood pressure measurement. Journal of hypertension 39(7):1293–1302

Stroup WW (2012) Generalized linear mixed models: modern concepts, methods and applications. CRC press

Tenhola S, Rahiala E, Halonen P, Vanninen E, Voutilainen R (2006) Maternal preeclampsia predicts elevated blood pressure in 12-year-old children: evaluation by ambulatory blood pressure monitoring. Pediatric research 59(2):320–324

Theodore RF, Broadbent J, Nagin D, Ambler A, Hogan S, Ramrakha S, Cutfield W, Williams MJ, Harrington H, Moffitt TE, et al. (2015) Childhood to early-midlife systolic blood pressure trajectories: early-life predictors, effect modifiers, and adult cardiovascular outcomes. Hypertension 66(6):1108–1115

Thompson J, Bannigan J (2008) Cadmium: toxic effects on the reproductive system and the embryo. Reproductive toxicology 25(3):304–315

Uddin SN, Haque M, Barek MA, Chowdhury MNU, Das A, Uddin MG, Islam MS (2023) Analysis of serum calcium, sodium, potassium, zinc, and iron in patients with pre-eclampsia in bangladesh: A case–control study. Health Science Reports 6(2):e1097

Urbina EM, Khoury PR, McCoy C, Daniels SR, Kimball TR, Dolan LM (2011) Cardiac and vascular consequences of pre-hypertension in youth. The Journal of Clinical Hypertension 13(5):332–342

Uriu-Adams JY, Scherr RE, Lanoue L, Keen CL (2010) Influence of copper on early development: prenatal and postnatal considerations. Biofactors 36(2):136–152

Vehtari A, Gelman A, Simpson D, Carpenter B, Bürkner PC (2019) Rank-normalization, folding, and localization: An improved $\widehat{R}$ for assessing convergence of MCMC. ArXiv pre-print arXiv: 1903.08008

Vehtari A, Gabry J, Magnusson M, Yao Y, Bürkner PC, Paananen T, Gelman A (2022) loo: Efficient leave-one-out cross-validation and waic for bayesian models. URL https://mc-stan.org/loo/, r package version 2.5.1

Vioque J, Navarrete-Muñoz EM, Gimenez-Monzó D, García-de-la Hera M, Granado F, Young IS, Ramón R, Ballester F, Murcia M, Rebagliato M, Iñiguez C (2013) Reproducibility and validity of a food frequency questionnaire among pregnant women in a Mediterranean area. Nutrition Journal 12:26, DOI 10.1186/1475-2891-12-26, URL http://nutritionj.biomedcentral.com/articles/10.1186/1475-2891-12-26

Warembourg C, Maitre L, Tamayo-Uria I, Fossati S, Roumeliotaki T, Aasvang GM, Andrusaityte S, Casas M, Cequier E, Chatzi L, et al. (2019) Early-life environmental exposures and blood pressure in children. Journal of the American College of Cardiology 74(10):1317–1328

Willet W (2013) Nutritional epidemiology. Oxford University Press, Oxford

Williams CK, Rasmussen CE (2006) Gaussian processes for machine learning, vol 2. MIT press Cambridge, MA

World Health Organization (2006) WHO child growth standards: length/height-for-age, weight-for-age, weight-for-length, weight-for-height and body mass index-for-age: methods and development., vol 52. World Health Organization, Geneva, URL https://www.who.int/childgrowth/standards/technical_report/en/, arXiv:1011.1669v3

World Health Organization (2023) Hypertension. URL https://www.who.int/news-room/fact-sheets/detail/hypertension

Xun P, Hou N, Daviglus M, Liu K, Morris JS, Shikany JM, Sidney S, Jacobs D, He K (2011) Fish oil, selenium and mercury in relation to incidence of hypertension: a 20-year follow-up study. Journal of internal medicine 270(2):175–186

Yao B, Lu X, Xu L, Wang Y, Qu H, Zhou H (2020) Relationship between low-level lead, cadmium and mercury exposures and blood pressure in children and adolescents aged 8–17 years: an exposure-response analysis of nhanes 2007–2016. Science of the Total Environment 726:138446

Zhang M, Liu T, Wang G, Buckley JP, Guallar E, Hong X, Wang MC, Wills-Karp M, Wang X, Mueller NT (2021) In utero exposure to heavy metals and trace elements and childhood blood pressure in a us urban, low-income, minority birth cohort. Environmental health perspectives 129(6):067005

Zhang W, Chen Z, Liu A, Buck Louis GM (2019) A weighted kernel machine regression approach to environmental pollutants and infertility. Statistics in Medicine 38(5):809–827