Nonparametric longitudinal modeling of the relationship between mercury exposure and behaviour from 9 to 15 years

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Abstract

Mercury (Hg), a toxic compound primarily accumulated through industrial processes and consumption of contaminated fish, poses risks to the central nervous system and development, particularly its methylmercury (MeHg) form. While prenatal exposure to MeHg has been linked to behavioral problems in childhood, its effects in adolescents remain controversial. We investigated this association among 870 children and adolescents aged 9, 11, and 15 from the INfancia y Medio Ambiente (INMA) cohort in Valencia. Hair samples were analyzed for total Hg at the Public Health Laboratory of Alava in Spain, ensuring accurate results through rigorous quality control measures. Behavioral assessments were conducted using the validated Spanish version of the Child Behavior Checklist (CBCL), providing valuable insights into children's well-being…

1. Introduction

Mercury (Hg), recognized as a environmental pollutant, poses a notable risk to human health owing to its capacity for bioaccumulation within the human body over prolonged periods [@Bernhoft2012]. Its primary dissemination into the environment stems from industrial activities, coal combustion, and natural occurrences, wherein Hg undergoes transformation into methylmercury (MeHg) within aquatic ecosystems [@Almeida2019]. Consequently, the most notable source of human exposure arises from the consumption of contaminated fish, particularly those occupying upper trophic levels within aquatic food webs [@Sheehan2014; @Castano2015].

MeHg is highly toxic due to its ability to easily cross biological membranes, including the blood-brain barrier and the placenta [@Hong2012]. The central nervous system is particularly vulnerable to MeHg toxicity as it accumulates in the brain, leading to neurotoxic effects [@Branco2021] . Early exposure to Hg can lead to developmental delays, cognitive impairments, and motor dysfunction [@AlSaleh2020]. Historical incidents, such as Minamata in the 1950s and Iraq in the early 1970s, highlight the serious consequences of exposure to elevated levels of Hg. In Minamata, fetuses exposed to Hg showed central nervous alterations and physical deformities, with a hair Hg concentration 280 to 760 times higher than normal [@Ekino2007]. In Iraq, acute exposure caused dysesthesia, paralysis, and even death [@Skerfving1976]. More recent studies in New Zealand, the Faroe Islands, and Korea reveal cognitive and motor skill disorders in children even at lower concentrations of Hg. This underscores the need for continued comprehensive research on different exposure scenarios and Hg concentrations at this vulnerable stages of development.

Hair samples have been widely used for evaluating the total Hg (THg) exposure because is a non-invasive method. Hair also accumulates Hg over longer periods than other biormarkers, such as blood, so it is a better biomarker of chronic exposure. In addition, it allows to determine better the MeHg exposure, because hair sample are greatly correlated with MeHg exposure. In our study, we hypothesized that higher hair levels of THg, as a proxy of MeHg total exposure, would worse the child behaviour at 9, 11 and 15 years-old. Thus, our aim was to assess associations between Hg exposure using THg on hair and behaviour problems, measured by the Child Behavior Checklist (CBCL), in a longitudinal study during childhood and adolescence, including 9-, 11- and 15-years-old participants from the Infancia y Medio AMbiente (INMA) birth cohort of Valencia.

2. Material and methods

2.1. Study population and design

We used data on 855 pregnant women participants in the INMA project from Valencia sub-cohort, in Spain. The main objective of the INMA project was to assess the impact of various environmental pollutants and exposures during pregnancy and early life, as well as their consequences on child growth and development [@Guxens2012]. Participants were recruited during their initial antenatal visit from 2003 to 2005. Inclusion criteria encompassed: a) being at least 16-years old, b) 10–13 weeks into gestation, c) having a singleton pregnancy, d) intending to undergo follow-up and delivery at the designated center (La Fe Hospital), e) no communication impediment, and f) no assisted conception. After excluding those who withdrew, were lost to follow-up, or experienced induced or spontaneous abortions or fetal deaths, the study followed 787 women until delivery (2004–2006) in Valencia. Their newborns were enrolled at birth and tracked until 9-years ($n = 472$), 11-years ($n = 385$) and 15-years ($n = 279$). The final study population included in our analysis consisted of 368 9-years-old children (*46.7%* of total births), 297 11-years-old children (*37.7%* of total births) and 205 15-years-old children (*26.0%* of total births). All the participants have available data for behavioral assessment at each age, as well as hair THg concentrations and information available for all variables of interest. Supplemental **Figure S1** shows a flowchart that provides an overview.

Informed consent for the prenatal period was obtained from the mother, and additional consent was secured from one of the parents or a legal representative at each phase of the postnatal period. The study protocol received approval from the Public Health Research Centre in Valencia (CSISP) and La Fe Hospital ethics committees.

2.2. Mercury exposure

Hair samples were collected from the occipital scalp at the ages of 9-, 11-, and 15-years. Each sample, obtained as close to the root as possible, met the criteria of a minimum length of 2 cm or a weight of at least 100 mg. Subsequently, these samples were carefully stored in plastic zip bags at room temperature until analysis. The assessment of THg was conducted at the Public Health Laboratory of Alava in the Basque Country, Spain, utilizing a direct Hg analyzer.

To prepare the samples for analysis, they were rinsed with 10 mL of 1% Triton X-100 (Panreac, Barcelona), then weighed in a weigh boat. The Hg analyses were performed directly using AMA 254 and DMA-80 equipment through catalytic combustion, gold amalgamation, thermal desorption, and atomic absorption spectrometry. Replicate analyses were carried out for each sample to ensure accuracy. The method's limit of quantification (LOQ) was determined to be 0.01 μg/g, and no measurements of Hg in hair fell below this threshold at any of the three time points.

The quality control of the hair sample batches involved using IAEA-086 (International Atomic Energy Agency, Austria) and NCS ZC 81002b (NCS Institute, Beijing, China) reference materials. Furthermore, the method's accuracy was externally validated by participating in various inter-laboratory exercises organized by the Centre de toxicologie du Québec (Quebec Multielement External Quality Assessment Scheme, QMEQAS program). In all instances, satisfactory results were achieved, as evidenced by z-scores for Rounds 2013-3 and 2015-3, which were +0.42 and +0.13, respectively.

2.3. Behavioural assessment

The CBCL was employed to evaluate emotional and behavioral issues in children aged 9, 11, and 15 years. Designed by Achenbach and Ruffle (2000), the CBCL is a well-established and validated instrument for the Spanish population [ref]. It relies on reports from parents or caregivers regarding children's behavior and their social, emotional, and adaptive functioning.

This widely recognized questionnaire comprises 112 items. Parents or caregivers assess the frequency or intensity of specific behaviors exhibited by the child, using a three-point response scale (0 = not at all true, 1 = somewhat or sometimes true, and 2 = very or often true) to evaluate issues that have occurred in the preceding two months. The CBCL includes various subscales that aid in identifying potential behavioral problems across three domains: (1) emotional issues (internalizing scales: anxiety, depression, somatic complaints); (2) conduct problems (externalizing scales: rule-breaking and aggressive behavior); and (3) total problems (the sum of internalizing, externalizing, and other scales: social, cognitive, and attention problems). Elevated scores on the CBCL indicate an escalation in conduct issues. This comprehensive evaluation furnishes valuable insights into the behavioral well-being of children within the specified age groups.

2.4. Covariates

In order to identify additional variables of interest to include in the multivariable models, we performed a Directed Acyclic Graph (DAG) based on our prior knowledge from the scientific literature (**Figure S2**) [@Textor2016]. The minimum set to establish associations between Hg exposure and behaviour was: breastfeeding (yes/no), social class (Class I + II: managerial jobs, senior technical staff and commercial managers; class III: skilled non-manual workers; and class IV + V:manual and unskilled workers), children's sex (male/female), smoking during pregnancy (yes/no) and total fish consumption (grams/day). We took into account also other relevant covariates about children and adolescents lifestyles and exposures in our analysis: child's body mass index (BMI), passive smoking (Does anyone in the household smoke? yes/no). Other variables about the mother or the pregnancy period were also included: weeks of gestation (weeks), parity (0, 1, >2). The child's BMI was calculated using z-scores according to the WHO child growth standard (World Health Organization, 2006; World Health Organization, 2008). Total fish consumption was determined by a a semi-quantitative food frequency questionnaire previously validated in the same study population at the age of 7 years [@Vioque2019].

2.5. Statistical analysis

A descriptive analysis was performed to assess the distribution and variability within the data. Measures of spread including the range, mean, standard deviation, median and interquartile range were used to assess continuous variables. Frequency and proportion distributions were used to assess categorical variables. Histograms and box-plots were used to visualise the data and identify potential outliers.

The model formulated to assess the relation between exposure to Hg and CBCL scores consisted in a regression model for longitudinal data. The CBCL scales are discrete score variables, which were longitudinally measured at 9, 11 and 15 year's visit. Hg exposure concentrations are continuous variables, also longitudinally measured at 9, 11 and 15 year's visit. Some of the adjustment variables are time-varying covariables followed up at 9, 11 and 15 year's visit. Other adjustment variables were static covariables. The negative binomial model is a probability distribution for counting/discrete score variables that accounts for overdispersion of the observations. Therefore, the observational model for the outcome variable of CBCL scores (let us denote y as the CBCL score variables) was a negative binomial probability distribution of the mean μ and a parameter ϕ that accounts for the overdispersion of the observations in relation to their mean (μ),

$$y \sim NegBinomial(\mu, \phi),$$

were $E(y) = \mu$ is the expectation of y and $Var(y) = \mu + \mu^2 / \phi$ is the variance of y.

A longitudinal predictive function is defined for the mean μ of the y scores, which is based on an additive function composed of a Gaussian process as a function of time and the adjustment variables, and the time-varying linear random effects of Hg exposure. Gaussian processes (Rasmussen and Williams, 2006; Neal, 1997) are non-parametric probabilistic models for multidimensional functions that have become a popular approach for longitudinal data, time series data, spatial and spatio-temporal data, and multidimensional functions in general (Riutort-Mayol et al., 2020; Carlin et al., 2014; Diggle, 2013). Gaussian processes are reliable and powerful models as they take into account high-order interactions between covariables and infer and exploit the covariance structure of data to estimate the underlying functions. However, single covariable effects are difficult to interpret using a multivariate Gaussian process function as it is a multidimensional function. Furthermore, they are more sensitive to low signal to noise ratio than linear models.

In this study, we were not interested in interpreting time and adjustment covariable effects, but Hg exposure effects on CBCL scores. Furthermore, Hg exposure effects were expected to be subtle effects on CBCL scores.

Therefore, additive Hg exposure effects were defined as time-varying linear random effects and, on the other hand, time and adjustment variables effects were modelled using a Gaussian process function. Thus, the predictor function was defined over the logarithm of the mean μ of the scores y as follows:

$$\log(\mu) = f(t, x) + b_t \, Hg,$$

where b_t are time-varying linear random effects of Hg exposure,

$$b_t \sim \mathrm{Normal}(\mu_{b_t}, \sigma^2_{b_t}),$$

and f(t, x) is a GP function of time t and adjustment variables x,

$$f(t, x) \sim GP(0, k(\{t, x\}, \{t', x'\})$$

with k being a squared exponential covariance function (Rasmussen and Williams, 2006),

$$k(\{t,t'\},\{x,x'\}) = \sigma^2 \exp(-1 / (2\ell^2) * (t-t')^2 + (x'-x')^2),$$

where ℓ is the lengthscale parameter that controls the smoothness of the underlying GP function, or grade of decay in the correlation between pairs of observations as a function of the square distance ($(t-t')^2 + (x'-x')^2$) between them in terms of the covariates time (t) and x. The parameter $\sigma>0$ is the marginal variance of the GP function. The defining property of a GP is that the collection of function values $\{f(t_i,x_i)\}_1^n$ follows a multivariate normal with an arbitrary mean function (Let us considerer a null mean function for simplicity) and a variance-covariance matrix K,

$$\{f(t_1,x_1),\ldots,f(t_n,x_n)\} \sim \mathrm{Normal}(0, K),$$

where each element (i,j) of matrix K is given by the covariance function,

$$K_{ij} = k(\{t_i,x_j\},\{t_j,x_j\}).$$

In negative binomial regression, similar to Poisson regression, the value of a linear coefficient or random effect associated with a covariate in the predictor function (i.e., a continuous or categorical independent variable) represents a multiplicative increase in the outcome by 1 unit increase in the covariate (Gelman et al. 2021), such that $100*b_t$ represents the approximate % increase in CBCL score with 1 ug/g Hg.

1.1 Model Inference

The model was formulated from a Bayesian perspective and adjusted using sampling methods in the probabilistic programming software Stan (Carpenter et al., 2017; Team, S. D, 2017). Stan software uses the Hamiltonian Monte Carlo sampling method (Neal 2011) to estimate the marginal posterior probability distributions of every parameter of interest. Four simulation chains with different initial values have been 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 for both models, a split-Rhat value lower than 1.05 has been obtained for all parameter simulation chains indicating good mixing of simulated chains. The convergence of the simulation chains indicates that the samples do come from the posterior distribution, and that the model is correctly specified without confusion or identifiability problems between parameters.

Magnitude and uncertainty of the parameters of interest, the random effects of Hg exposure, are given by their marginal posterior distributions. Significance of random effects 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.

The preprocessing of the data before sending it to Stan to perform model inference, as well as its postprocessing, was carried out using R software for data analysis and processing (R Core Team, 2020).

3. Results

The median (IQR) of hair THg levels was 1.0 (1.2) µg/g for 9-years, 0.9 (0.9) µg/g for 11-years and 0.6 (0.7) µg/g for 15-years. Sociodemographic characteristics and lifestyle among mothers and their 9-, 11- and 15-years children are describe in **Table 1**. In regard to mothers, most of them were employed (9-years= 69.6%; 11-years= 68.4%; 15-years=79.0%), belonged to the highest social class (9-years= 44.3%; 11-years= 37.7%; 15-years=36.6%), had only 1 child (9-years= 58.2%; 11-years= 59.3%; 15-years=57.6%) and did not smoke during pregnancy (9-years= 79.3%; 11-years= 78.1%; 15-years=81.0%). The median age was about the same in the three periods (30-31-30 years) and the WAIIS score was also similar (9.8-10.5-10.5). With regard to children, they were mainly female (50.5%-55.9%-52.7%)…

4. Discussion

In this Spanish study we observed that hair THg levels at 9-years were associated with behaviour scales, specifically in regard to ¿an increase? in internalizing and total problems dimensions. However, this association was not maintained for 11- and 15-years visit.

We have to acknowledge that our study presents certain limitations and strengths. The first pertains to the design. Although follow-up studies are more useful for determining causal relationships and avoiding certain biases, such as reverse causality, they also have a limitation concerning attrition . In our study, we analyzed information from 3 different periods with progressively smaller samples. Thus, we went from 368 participants in the first period (9-years) to 205 in the last period analyzed (15-years), representing an attrition of ~ 45% from the initial sample. This reduction in the study sample could have led to a loss of statistical power, which is one of the reasons we only obtained statistically significant results for the 9-year-old visit. Regarding the tool used to evaluate changes in behavior associated with Hg exposure, it also presents some limitations worth mentioning. The CBCL scale heavily relies on reports from parents and caregivers, thus allowing for the possibility of underestimation, overestimation, or misinterpretation of children's behavior. Additionally, other characteristics of the parents and/or caregivers could also influence, such as their ability to observe and recall the child's behavior or their honesty in responding faithfully. This tool may also exhibit cultural differences, often determined by socioeconomic status, as the interpretation of certain behaviors can vary, being considered normal in some contexts but problematic in others. This context-dependent interpretation could thus affect the validity of the tool. However, we decided to use this scale due to its versatility, wide usage in the literature for behavior assessment, and ease of administration. In addition, it can be repeatedly used to monitor the child's progress over time, allowing for the evaluation of any changes that occur.

5. Conclusions

Ackwoledgments

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Authors contribution

GR-M contributed to methodology and formal statistical analysis; AO-C contibuted to methodology and writing; AJS-P contributed to conceptualization, methodology, visualization, support in statistical analysis, reviewing of manuscript and obtaining funding;

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Conflicts of Interest

The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

References

Table 1. Sociodemographic characteristics and lifestyle among mothers and their 9-, 11- and 15-years children of the INMA cohort study (*n* = 870).

Variables	**9 years (*n* = 368)**	**11 years (*n* = 297)**	**15 years (*n* = 205)**
Mother			
Age (years)	30 (5)[1]	31 (6)	30 (5)
Work, *n* (%)			
Employed	256 (69.6)	203 (68.4)	162 (79.0)
No-employed	112 (30.4)	94 (31.6)	43 (21.0)
Social class[2], *n* (%)			
I+II (highest)	102 (27.7)	94 (31.6)	66 (32.2)
III	103 (28.0)	91 (30.6)	64 (31.2)
IV+V (lowest)	163 (44.3)	112 (37.7)	75 (36.6)
Parity, *n* (%)			
1	214 (58.2)	176 (59.3)	118 (57.6)
2	136 (37.0)	103 (34.7)	74 (36.1)
>2	18 (4.9)	18 (6.1)	13 (6.3)
Smoke during pregnancy, *n* (%)			
Yes	76 (20.7)	65 (21.9)	39 (19.0)
No	292 (79.3)	232 (78.1)	166 (81.0)
WAIIS (IC measure)	9.8 (3.8)	10.5 (4.4)	10.5 (4.4)
Children			
Age (years)	9.1 (0.3)	10.9 (0.2)	15.6 (0.6)
Gestational age (weeks)	39.8 (1.7)	39.7 (1.8)	39.8 (1.8)
Sex, *n* (%)			
Male	182 (49.5)	131 (44.1)	97 (47.3)
Female	186 (50.5)	166 (55.9)	108 (52.7)
z-BMI, (Kg/m^2)	0.8 (2.0)	0.8 (2.0)	0.4 (1.4)
Hair THg levels (µg/g)	1.0 (1.2)	0.9 (0.9)	0.6 (0.7)
Fish consumption (g/day)	4.8 (3.1)	5.5 (4.0)	5.1 (4.1)
Breastfeeding (weeks)	13.9 (19.6)	14.9 (18.6)	17.1 (17.1)
Passive exposure to tobacco smoke, *n* (%)			
Yes	183 (49.7)	131 (44.1)	83 (40.5)
No	185 (50.3)	166 (55.9)	122 (59.5)

BMI, body mass index. [1]Median (IQR) (all such values). [2]Social Class = I-II (managers, professionals), III (technicians and associate professionals, clerical support workers, skilled agricultural, forestry and fishery workers), IV-V (craft and related trades workers, plant and machine operators and assemblers).

Figure 1. Association between Hg exposure among 9-, 11- and 15-years participants and behaviour problems scales.

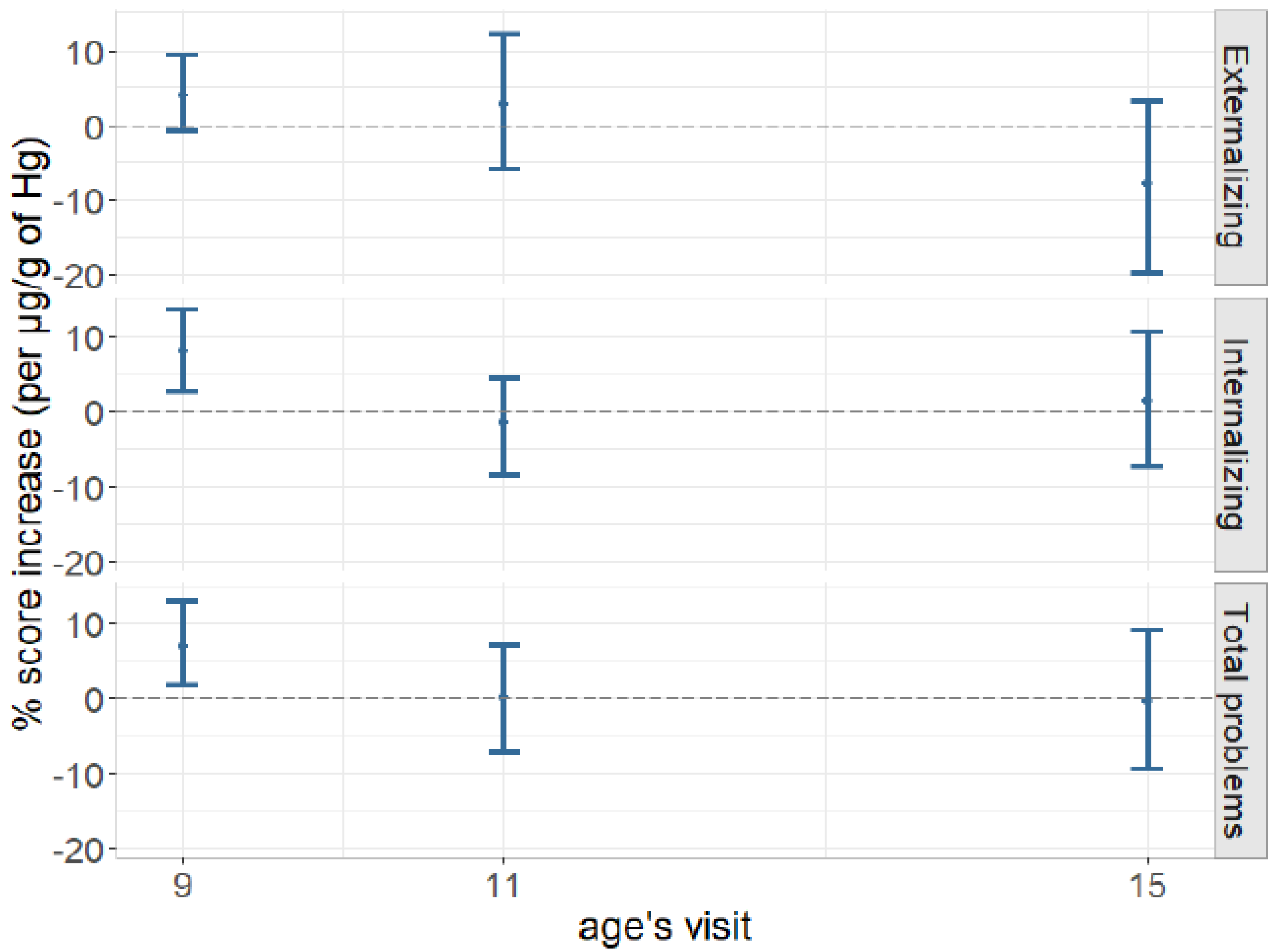

Figure S1. Flowchart of participants included in the present analysis from the INfancia y Medio Ambiente (INMA) Cohort Study.

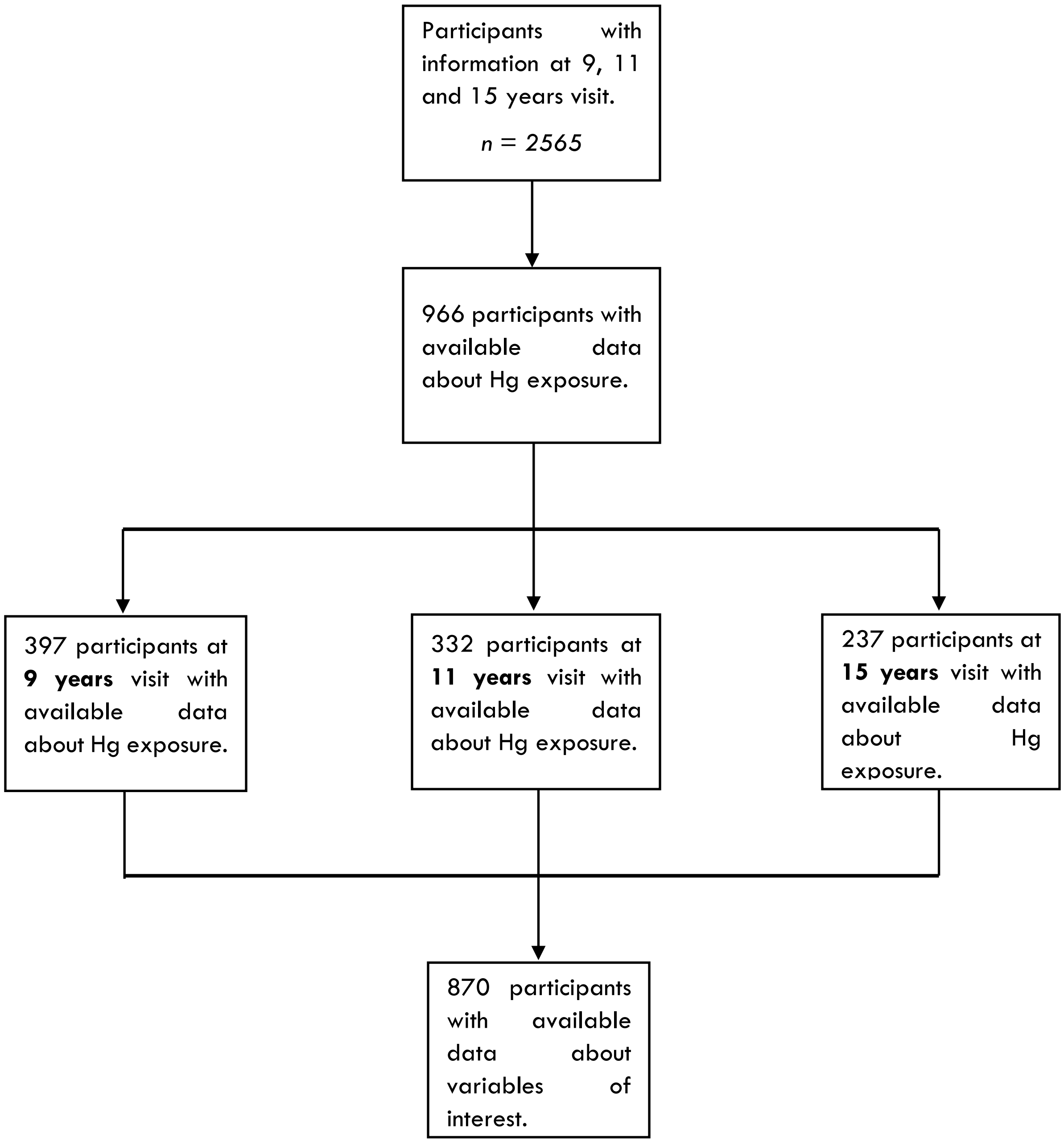

Table S1. Differences in sociodemographic characteristics among mothers and their 9-, 11- and 15- years old children included and excluded from the study.

	9 years		11 years		15 years	
Variables	**Included (*n* = 368)**	**Excluded***	**Included (*n* = 297)**	**Excluded***	**Included (*n* = 205)**	**Excluded***
Mother						
Age (years)	30 (5)[1]	29.5 (7)	31 (6)	29 (7)	30 (5)	30 (6)
Work, *n* (%)						
Employed	256 (69.6)	36 (59.0)	203 (68.4)	53 (67.9)	162 (79.0)	65 (80.2)
Unemployed	112 (30.4)	25 (41.0)	94 (31.6)	25 (32.1)	43 (21.0)	16 (19.8)
Social class[2], *n* (%)						
I+II (highest)	102 (27.7)	61 (17.2)	94 (31.6)	69 (16.2)	66 (32.2)	97 (18.8)
III	103 (28.0)	94 (26.6)	91 (30.6)	106 (24.9)	64 (31.2)	133 (25.7)
IV+V (lowest)	163 (44.3)	199 (56.2)	112 (37.7)	205 (58.8)	75 (36.6)	287 (55.5)
Children						
Age (years)	9.1 (0.3)	9.2 (0.4)	10.9 (0.2)	11.0 (0.4)	15.6 (0.6)	15.6 (0.6)
Sex, *n* (%)						
Male	182 (49.5)	200 (56.5)	131 (44.1)	251 (59.1)	97 (47.3)	285 (55.1)
Female	186 (50.5)	154 (43.5)	166 (55.9)	174 (40.9)	108 (52.7)	232 (44.9)
z-BMI, (kg/m^2)	0.8 (2.0)	0.9 (2.3)	0.8 (2.0)	0.9 (2.1)	0.4 (1.4)	0.3 (1.7)

BMI, body mass index. [1]Median (IQR) (all such values). [2]Social Class = I-II (managers, professionals), III (technicians and associate professionals, clerical support workers, skilled agricultural, forestry and fishery workers), IV-V (craft and related trades workers, plant and machine operators and assemblers). *The sample size of the excluded population depends on the variable described.

Figure S2. Directed acyclic graph (DAG) between Hg exposure and behaviour problems scales (CBCL).