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# Exposure to mercury among Spanish preschool children: Trend from birth to age four

Sabrina Llop[a,b,*], Mario Murcia[b,a], Xabier Aguinagalde[c], Jesus Vioque[b,d], Marisa Rebagliato[b,e], Amparo Cases[a], Carmen Iñiguez[a,b], Maria-Jose Lopez-Espinosa[a,b], Ascensión Amurrio[c], Eva María Navarrete-Muñoz[b,d], Ferran Ballester[a,b,f]

[a] Foundation for the Promotion of Health and Biomedical Research in the Valencian Region, FISABIO-Public Health, Av. Catalunya 21, 46020 Valencia, Spain
[b] Spanish Consortium for Research on Epidemiology and Public Health (CIBERESP), Madrid, Spain
[c] Laboratorio de Salud Pública de Alava, Santiago 11, 01002 Vitoria Gasteiz, Spain
[d] Universidad Miguel Hernandez, Avenida de Alicante KM 87, 03550 Sant Joan d´Alacant, Spain
[e] Medicine Department, Jaume I University, Av. Vicent Sos Baynat, s/n, 12071 Castelló de la Plana, Spain
[f] Faculty of Nursing, University of Valencia, Jaume Roig s/n, 46010 Valencia, Spain

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ABSTRACT

The purpose of this study is to describe the total hair mercury concentrations and their determinants in preschool Spanish children, as well as to explore the trend in mercury exposure from birth to the age four. This evolution has been scarcely studied in other birth cohort studies.

The study population was 580 four year old children participating in the INMA (i.e. Childhood and Environment) birth cohort study in Valencia (2008–2009). Total mercury concentration at age four was measured in hair samples by atomic absorption spectrometry. Fish consumption and other covariates were obtained by questionnaire. Multivariate linear regression models were conducted in order to explore the association between mercury exposure and fish consumption, socio-demographic characteristics and prenatal exposure to mercury.

The geometric mean was 1.10 μg/g (95%CI: 1.02, 1.19). Nineteen percent of children had mercury concentrations above the equivalent to the Provisional Tolerable Weekly Intake proposed by WHO. Mercury concentration was associated with increasing maternal age, fish consumption and cord blood mercury levels, as well as decreasing parity. Children whose mothers worked had higher mercury levels than those with non working mothers. Swordfish, lean fish and canned fish were the fish categories most associated with hair mercury concentrations. We observed a decreasing trend in mercury concentrations between birth and age four. In conclusion, the children participating in this study had high hair mercury concentrations compared to reported studies on children from other European countries and similar to other countries with high fish consumption. The INMA study design allows the evaluation of the exposure to mercury longitudinally and enables this information to be used for biomonitoring purposes and dietary recommendations.



## 1. Introduction

Mercury is a ubiquitous pollutant that appears naturally, however it has been the anthropological sources which have brought about a significant increase of its concentrations in the environment. Mercury can exist in several forms, but it is organic methylmercury (MeHg) that is one of the most relevant from the public health point of view, due to its neurotoxic effects (Driscoll et al., 2013; World Health Organization (WHO), 2010). Most MeHg originates in aquatic systems where it is formed from the inorganic form through the action of bacteria present in water and sediments (Parks et al., 2013).

The dominant pathway of human mercury exposure to MeHg is through eating seafood. Predatory fish such as swordfish, shark, and

*Abbreviations:* AESAN, Asociación Española de Sanidad Ambiental y nutrición; CI, confidence intervals; Democophes, demostration of a study to coordinate and perform human biomonitoring on a European scale; EFSA, European Food Safety Authority; EPIC, European Investigation into Cancer and Nutrition; GM, geometric mean; Hg, mercury; INMA, Infancia y Medio Ambiente; MeHg, methylmercury; PTWI, provisional tolerable weekly intake; RfD, reference dose; sd, standard deviation; T-Hg, total mercury; US EPA, Environmental Protection Agency in United States; WHO, World Health Organization

* Corresponding author at: Foundation for the Promotion of Health and Biomedical Research in the Valencian Region, FISABIO-Public Health, Av. Catalunya 21, 46020 Valencia, Spain.
*E-mail address:* llop_sab@gva.es (S. Llop).

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fresh tuna have the highest concentrations of MeHg (Martorell et al., 2011). Moreover, MeHg bioaccessibility in these fish, for example, swordfish, may reach 94% (Torres-Escribano et al., 2010). In fact, a direct relationship between fish intake and mercury concentrations has been observed in different populations (Bjornberg et al., 2003; Freire et al., 2010; Miklavcic et al., 2013; Ramon et al., 2011; Schoeman et al., 2010; Steuerwald et al., 2000). MeHg is effectively absorbed from the gastrointestinal tract and readily crosses the placenta and blood–brain barrier (World Health Organization (WHO), 2007).

Mercury is known to be neurotoxic to humans and children are especially vulnerable to this exposure, since their immune system and detoxification mechanisms are not yet fully developed. Moreover, the human brain continues to develop postnatally, since most neurons have been formed by the time of birth, and growth of glial cells and myelinisation of axons continues for several years (Grandjean and Landrigan, 2006). Other effects have been associated with early exposure to mercury, such as cardiovascular and immunologic disorders (Karagas et al., 2012).

Given that fish has important components of healthy diets, such as omega-3 polyunsaturated fatty acids, iodine, selenium and vitamin D, there is concern that exposure to MeHg at concentrations reached by pregnant women and children with regular fish consumption may impair child development despite these beneficial nutrients (Institute of Medicine (IOM), 2006).

Results from the birth cohort study conducted in Faroe (Grandjean et al., 1997) provided the basis for the National Research Council's and US Environmental Protection Agency (EPA)'s 2001 validation of a reference dose (RfD) for MeHg intake of 0.1 μg/kg of body weight per day, as an estimate of a daily exposure where no appreciable risk of deleterious effects during a lifetime would occur (US Environmental Protection Agency, 2007). This level would correspond to a maternal hair MeHg concentration of 1.0 μg/g and a MeHg concentration of 5.8 μg/L in whole cord blood (Budtz-Jorgensen et al., 2000). Other agencies have recommended regulatory levels that are significantly less stringent than EPA's RfD. Thus, the Food and Agriculture Organization (FAO) and the World Health Organization (WHO) revised in 2003 and confirmed in 2006 the Provisional Tolerable Weekly Intake (PTWI) of MeHg to a level of 1.6 μg/kg of body weight per week (0.23 μg/kg per day) (United Nations Environment Programme, 2007).

The two biomarkers most frequently used to determine individual exposures to this contaminant are the mercury concentrations in scalp hair and in whole blood (Grandjean et al., 2005). The association between the two exposure biomarkers was determined in a number of studies (mean ratio range between 140 and 370). The WHO Expert Committee on food additives used a value of 250 to represent the overall average ratio (World Health Organization (WHO), 2004).

The INMA study (i.e. Childhood and Environment) is a Spanish birth cohort study that aims to investigate the role of the most relevant toxicants in the environment on child health and development. In previous studies, high levels of prenatal exposure to mercury were reported in the INMA cohort, with 24% of newborns having concentrations above the WHO PTWI and 64% above the US EPA RfD (Ramon et al., 2008, 2011). Despite this, prenatal exposure to mercury was not associated with a delay in cognitive and psychomotor development during the second year of age (Llop et al., 2012). The purpose of this study is to describe the hair mercury concentration at age four of those children and its relationship with socio-demographic, environmental and dietary factors, as well as to explore the trend in mercury exposure between birth and age four.

## 2. Materials and methods

### 2.1. Study population and study design

Subjects were participants in the INMA (Environment and Childhood) Project, a multicenter birth cohort study that aims to investigate the effect of environmental exposures and diet during pregnancy on fetal and child development in different geographic areas of Spain (http://www.proyectoinma.org). The study protocol has been reported elsewhere (Guxens et al., 2012). Briefly, the original population in this study consisted of 855 women recruited at the first trimester of pregnancy (2003–2005) in the INMA cohort of Valencia (Spain). Excluding the women who withdrew from the study, were lost to follow-up, and had induced or spontaneous abortions or fetal deaths, we followed up a total sample of 787 (92%) women until delivery (2004–2006). Their children were enrolled at birth and were followed up until the age of four ($n=593$; 69%).

The final study population was made up of 580 children with available mercury concentrations and covariates data. Informed consent was obtained from all participants in each phase, and the study was approved by the hospital ethics committee.

### 2.2. Mercury analysis

Whole blood samples were collected from cord vessels using venipuncture before the placenta was delivered and then frozen to −80 °C until analysis. Hair samples were collected from the occipital scalp when children were 4 years old, placed in a plastic bag and stored at room temperature until analysis.

The analyses of total mercury (T-Hg) were carried out in the Public Health Laboratory of Alava (Basque Country, Spain) using, for both types of samples, a single-purpose mercury analyzer (AMA 254. Leco Corporation. St. Joseph, Michigan). The samples were weighed in a nickel boat and analyzed in the quoted equipment by catalytic combustion, gold amalgamation, thermal desorption, and atomic absorption spectrometry. Replicate analyses were made for each sample. The limits of determination of the method (LOD) were 2.0 μg/L for blood samples and 0.01 μg/g for hair samples. For measurements in cord blood below the LOD (<5%) we used the approach $LOD/\sqrt{2}$ (Centers for Disease Control and Prevention, 2009).

The laboratory analyzed daily reference materials with each batch of samples, as internal quality control to check the accuracy of the results. The reference materials used were: Seronorm Trace Elements in Whole Blood (L-2, L-3 Billingstad, Norway) for blood samples and NCS ZC 81002b Human hair (Beijing, China) for hair.

Additionally, from 2009 onwards, the Public Health Laboratory of Alava participates regularly (3 times per year) in inter-laboratory comparisons organized by the New York State Department of Health in the Wadsworth Center with satisfactory results.

We assumed that a high proportion of the T-Hg measured in our study population was MeHg and used the T-Hg concentrations to calculate the percentage of samples above the EPA's RfDs or the WHO's PTWI.

### 2.3. Covariates

Women filled in 2 questionnaires during their pregnancy, at the first and the third trimesters of gestation. The questionnaires were administered by trained interviewers and focused on socio-demographic, environmental, and lifestyle information during pregnancy. The covariates used in this study were maternal and paternal age at conception, maternal and paternal educational level (up to primary, secondary, university), country of birth (Spanish, other), and parity (0, 1, ≥2 deliveries).

We defined parental social class according to the most privileged occupation during pregnancy of the mother or the father using a widely used Spanish adaptation of the international ISCO88 coding system (Domingo-Salvany et al., 2000). Class I included managerial jobs, senior technical staff, and commercial managers; class II included skilled non-manual workers; and class III, manual workers.

In a subsequent interview, when the children were between 11 and 21 months of age we obtained information on duration of breastfeeding (0, >0–16, >16–24, >24 weeks). Breastfeeding was defined as ingesting breast milk regardless of whether or not infants were receiving any other supplementation with food or liquid including nonhuman milk.

When children were age four, we obtained information about maternal and paternal employment status (working, not working), children had lunch in the school canteen (yes, no), parental cohabitation (yes, no), and type of zone of residence (urban, sub-urban, metropolitan, rural). In addition, a semi-quantitative food frequency questionnaire (FFQ) of 105 food items was administered in order to assess the usual daily intake for main foods and nutrients among children (available upon request at: http://bibliodieta.umh.es/cfa-105-inma-infancia/). The FFQ was a modified version from a previous FFQ based on the Harvard questionnaire (Willett et al., 1985) which we developed and validated among mothers of the INMA-Valencia study when they were pregnant (Vioque et al., 2013). To adapt it for children, additional foods items and suitable portion sizes for children 4–5 years old were used in the questionnaire.