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MERCURY LEVELS IN BLOOD, URINE AND HAIR IN A NATION-WIDE SAMPLE OF SPANISH ADULTS.

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I.- INTRODUCTION

Mercury is a global environmental contaminant with high toxicity both for human beings and animal life. This metal is naturally occurring in earth crust but human activities during the last decades have mobilized it and made it available to the biosphere (Lamborg et al., 2014; Mason et al., 2012). Currently the main sources of mercury emissions are from coal burning in coal-fired power-plants and from artisanal gold mining when mercury is vaporized over open fire to recover the gold. Mercury can be present in both inorganic and organic forms, and in addition, the metallic form of mercury, Hg(0), is volatile and vaporised upon heating. Of importance with respect to human exposure is that mercury in the environment is biologically transformed to organic forms, mostly methylmercury, which has a higher bioavailability and toxicity than inorganic forms. Atmospheric mercury is deposited in the oceans, methylated by microorganisms, taken up and accumulated by organisms and is concentrated up in the food chain to reach the highest levels in top predators. Therefore, consumption of food items from the marine environment is probably the main source of mercury exposure in the general population today (Castaño et al., 2015). Consequently, the capacity of long range atmospheric transport, the biotransformation to organic forms, the bioaccumulation and biomagnification along the food chain in combination with its high toxicity has resulted in mercury being a priority environmental pollutant to be biomonitored as reflected by its third position in the ATSDR (Agency for Toxic Substances and Disease Registry, US Department of Health and Human Services) priority list of hazardous substances.

The toxicity and the adverse health effects of mercury exposure are well described (Nordberg et al., 2014, Kurland et al., 1960, Yorifuji et al., 2013). Of particular importance are the adverse effects associated with life-long exposure to low levels as well as impacts on the developing fetus during pregnancy (Bjørklund et al., 2017, Grandjean et al., 2010). Therefore, from a public health perspective, mercury is clearly an environmental contaminant with capacity to affect health, well-being and life quality of the general population at a broad scale. Consequently, information on the actual mercury exposure in the population is important when advising and deciding on mitigation strategies. Many countries have national programs aimed at reducing mercury emissions. The European Union launched the Mercury Strategy 2005 and most recently the Minamata Convention, under the auspices of the United Nations, has been ratified with commitments to reduce global mercury emissions, given that around 50% of the anthropogenic mercury deposited annually in Europe originates from outside Europe (EEA, 2018).

Human biomonitoring (HBM) programs, in which environmental contaminants are analysed in different tissues or body fluids, are the most straight-forward approaches to get information on the actual exposure levels in the population. The European Human Biomonitoring project DEMOCOPHES revealed significant differences in mercury, and particularly methylmercury, exposure between countries across Europe (Den Hond et al., 2015). In an in-depth analysis, the country variation was strongly related to dietary habits (Castaño et al., 2015). Individuals from countries with relatively high consumption of marine products had higher values than individuals from countries in which marine fish consumption was not prominent.

With respect to mercury absorption in humans, the chemical form strongly influences the amount absorbed and the tissues in which the metal accumulates. Organic mercury is readily absorbed in the gastrointestinal tract (approx. 95%), while the absorption of elemental mercury after ingestion is negligible (<0.01%). On the other hand, mercury vapor is efficiently absorbed in the lungs (approx. 80%) (ATSDR, 1999). Furthermore, different chemical forms of mercury are associated with specific exposure sources, for example food is the primary source of methylmercury, while dental amalgams or inhalation of mercury vapor are sources of inorganic mercury. This must be taken into account when mercury exposure is assessed because although several biological matrices can be analyzed, they provide information on different sources of exposure.

In Spain, levels of mercury have been studied in specific regional surveys, small-scale studies targeting industrial sources, occupational settings and other exposed populations groups. Until now, there has not been a representative study of mercury exposure nationwide. In 2007, the Spanish Ministry of Food, Agriculture and the Environment funded a Human Biomonitoring program to study the levels of priority environmental pollutants in Spain following commitments to the European Union Environment and Health Action Plan (EC, 2004) and the National implementation of the Stockholm Convention (BOE, 2004). The objectives are to establish reference levels for some heavy metals and persistent organic pollutants within the Spanish population. BIOAMBIENT.ES is a cross-sectional HBM study designed to this aim in Spanish adults (Esteban et al., 2013, Perez-Gomez et al., 2013) and was the first human biomonitoring study in Spain at nationwide scale. The current paper aims to provide reference levels for mercury in blood, urine and hair in Spanish adults from the BIOAMBIENT.ES survey, under the hypothesis that each matrix will reflect information from different sources of mercury exposure.

II.- MATERIALS AND METHODS

Study population

BIOAMBIENT.ES was a nation-wide cross-sectional epidemiological study recruiting 1,936 volunteers from March 2009 to July 2010. Participants were workers aged 18 years or older, living in Spain for five years or more that attended the health facilities of the Societies for Prevention of IBERMUTUAMUR, MUTUALIA, MC-PREVENCIÓN, MUGATRA, UNIMAT PREVENCIÓN and PREVIMAC for their annual medical check-up. After rejection of the subjects and biological samples not fulfilling the criteria (Esteban et al., 2013, Perez-Gomez et al., 2013), a total of 1,880 blood samples, 1,770 urine samples and 577 hair samples were included in the analysis of mercury levels.

To guarantee the nationwide representativeness, volunteers can be found following a stratified cluster sampling procedure covering all geographical areas, genders and occupational sectors. The detailed description of the design and fieldwork is described in Esteban et al., (2013) and Perez-Gomez et al., (2013).

Briefly, 12 geographical areas were defined by combining neighbouring regions to obtain the following strata: Northwest I (Galicia), Northwest II (Asturias, Cantabria), North (Basque Country), Northeast I (Navarre, La Rioja, Aragon), Northeast II (Catalonia), Central I (Castile-Leon), Central II (Madrid), Central III (Castile-La Mancha, Extremadura), East (Valencia, Balearic Islands), South I (Andalusia, Ceuta), South II (Murcia) and Canary Islands. A total of 38 Health Prevention Centers were selected from the above-mentioned geographical strata following a proportional distribution according to data from the Spanish Working population Survey 2007 (INE, 2009). The economic sector was stratified defining two groups according to the National Classification of Economic Activities for 2009 (INE, 2011). The first group included "service activities" and the second, named "others", included farming, industry and construction activities. Finally, to ensure a good seasonal dispersion of the sampling, samples were collected in four quarterly recruitment periods (January–March, April–June, July–September, and October–December). Samples of first-morning urine, blood, serum and hair (optional) were collected from those volunteers that signed the informed consent.

Epidemiological questionnaires and health data

All participants completed a self-administered epidemiological questionnaire to get basic demographic data and information about risk factors related to mercury exposure (and other pollutants studied in BIOAMBIENT.ES). The exposure to mercury through the diet was widely investigated by different questions focused on fish consumption including frequency and species consumed. The questionnaire included also questions about amalgam fillings (number and amalgams fillings in the last year).

Health related data were collected by the doctors using a specific clinical sheet form designed *ad hoc* to gather basic clinical information on all the participants in a uniform manner. In addition, participants were asked to grant access to their medical records in order to obtain both the complete results of the occupational health exam and the data needed to assess possible occupational exposures.

Ethical approval

The study presented was approved by both the Comité Ético Científico and the legal department of IBERMUTUAMUR. The participation was voluntary and altruistic and all participants gave their written informed consent. They received a small token of appreciation, a pen drive, for their collaboration in the study.

The study was performed in accordance with legal/ethical principles and regulations concerning research involving individual information and biological samples, including organic law 15/1999 on Personal Data protection and its Regulations, Law 41/2002, on Autonomy of Patients and rights and obligations relating to health information and documentation, as well as General Health Law 14/1986. Since the study involved the collection of blood samples, the principles of the Declaration of Helsinki and those contained in the UNESCO Universal Declaration on the Human Genome and Human Rights have been observed.

Results communication

A multidisciplinary committee was established to study the results considered as anomalous on a case-by-case in order to confirm the results and take any appropriate measures. The participants received feedback about the research by an individual letter.

Statistical analysis

The complex design of the study was taken into account in all analyses. The post-stratified weights were calculated taking into account occupational sector, gender and geographic region from the Spanish Active Population census of 2007 (INE, 2009). Weights were applied to all estimates presented herein. For the analyses, the following socio-demographic factors have been included: gender; age group (≤29 years; 30–39 years; 40–49 years; ≥50 years); geographic area and sampling period (January–March, April–June, July–September, and October–December).

Mercury concentrations in the three matrices were log-transformed and the geometric mean, confidence intervals (95%) and percentiles (10, 25, 50, 75, 90 and 95) were calculated by gender, age group, geographical region, economical sector, sampling period and fish consumption. After adjusting linear models, postestimation contrasts were used to assess the possible association between mercury levels and the previously mentioned variables. Statistical analyses were conducted using the SVY commands in STATA (version.11, Stata Corp, College Station, TX, USA), a software package that incorporates the sample weights and adjusts the analyses to take into account the complex sample design of the survey.

Sample collection and storage

Since this study was a multicenter study and samples were collected by different fieldwork teams, special care was taken in the harmonization of the pre-analytical phase (Esteban et al., 2013). All centers were visited by two technicians that gave precise instructions and training on how to collect and send the biological samples to the central laboratory located at the Environmental Toxicology Area of the National Centre for Environmental Health of the Instituto de Salud Carlos III. All samples collected were sent by courier to the central laboratory within 96h after the collection.

Blood samples were collected in 5-6 mL tubes treated with sodium heparin for trace metals analysis (VACUETTE® NH Trace Element Sodium Heparin). The tubes were tested prior to the sample collection in order to assess the background contamination (Cañas et al., 2010). The blood samples were kept in a portable isothermal bag with ice packs (at about 4–8◦C) and sent to the lab. Once in the laboratory, they were aliquoted and stored at −20◦C until analysis in polypropylene tubes previously washed with 10% HNO_3 (Esteban et al., 2013).

First morning urine samples were collected in polypropylene vessels previously washed with 10% HNO_3 in the central laboratory and sent to the health prevention centers. The health-care workers provided the vessels to the volunteers together with the instructions on how to collect the first-morning urine sample that should be collected on the day of the occupational medical check-up. The samples were kept in a portable isothermal bag with ice packs (at about 4–8◦C) and sent to the lab. They were then aliquoted and stored at − 20 °C in polypropylene tubes washed with 10% HNO_3 until analysis (Esteban et al., 2013).

Fieldworkers were trained for collecting the hair samples. Hands-on training sessions were conducted during the visit of the technicians to the centers and a video was also provided. The hair sample was optional, not mandatory for participation. Since for the analysis the 3 cm of hair closest to the scalp were used, the procedure followed for hair sampling differed slightly with the length of the hair. Two strands were collected in the case of long hair, one on each side of the occipital region, and immobilised using tape adhesive avoiding to put it in the first 3 cm closest to the scalp. The locks were introduced in a zip-lock plastic bag after identifying the extreme closest to the scalp. In case of short hair (shorter than 3.5 cm), small strands were cut from different places but within the same area of the head. The strands were directly put into the zip-lock plastic bag. Once in the laboratory the samples were processed and stored at room temperature until analysis. In the case of long hair, the first 3 cm were cut, put into a PP vessel and chopped into the smallest possible pieces with scissors. Samples of short hair were directly put into the PP vessel and chopped.

More details about hair sampling and processing can be seen in the video available in the website of the National Centre for Environmental Health of the Instituto de Salud Carlos III (http://portal-videos.isciii.es/?p=249)

Chemical analysis

Mercury in urine

Urinary mercury determinations were performed in clean-room facilities with ISO 6 air quality, differential pressures and controlled temperatures suitable for the analysis of trace element concentrations. An Inductively Coupled Plasma Mass Spectrometer (PerkinElmer ELAN DRC-e) was used. The instrument was calibrated between 0.05 – 10.0 µg/L of mercury to obtain quantitative urinary mercury (m/z 202).

First morning urine samples were mixed for homogenization and diluted 1:10 in HCL Suprapure® 2% containing 100 µg/L of gold to reduce the memory effect of mercury and 10 µg/L bismuth as internal standard. Clincheck®-Control (Recipe, Germany) was used for internal quality control every 10 samples. Participation in the 3 rounds per year of Quebec Multielement External Quality Assessment Scheme (QMEQAS) was used as external quality control, with satisfactory results according to the established criteria. The limit of quantification of the method was 0.05 µg/L.

In order to control the effect of urine dilution the creatinine concentration was determined in 1:40 dilutions using Jaffé's alkaline picrate method (Spinreact Kit, Spain).

Mercury in blood

Mercury concentration in blood was also determined by Inductively Coupled Plasma Mass Spectrometry (PerkinElmer ELAN DRC-e) in clean-room facilities.

The instrument was calibrated between 0.05 – 100.0 µg/L of mercury using matrix-matched calibration standards, and a calibration curve was used to obtain quantitative blood mercury concentrations (m/z 202). Blood samples were mixed gently for homogenization and diluted 1:50 in an aqueous solution containing 10 ppb of rhodium as internal standard, triton X-100 (0.05%, v/v), EDTA (0.05%, v/v), propanol (10%, v/v), 10 µg/L H (AuCl)4 and tetramethylammonium hydroxide (TMAH) 1% (v/v). Seronorm® Trace Elements Whole Blood (Sero, Norway) was used for internal quality control every ten blood samples. Participation in the 3 rounds per year of Quebec Multielement External Quality Assessment Scheme (QMEQAS) was used as external quality control with satisfactory results according the established criteria. The limit of quantification of the method was 0.1 µg/L.

Mercury in hair

Total mercury was analysed by Thermal Decomposition Gold Amalgamation Atomic Absorption Spectroscopy at 254nm in a Direct Mercury Analyzer (Milestone DMA-80). Method was accredited under UNE/EN ISO 17025 by the Spanish National Accreditation Entity (ENAC). The calibration curve was elaborated with hair reference material NIES CRM No.13 (4.42 ng/mg) and IAEA-086 (0.573 ng/mg) between 1 to 100 ng Hg. The LOQ was 10 pg Hg/mg (0.01 µg/g) hair and 3 mg of hair sample were required for the determination.

Samples of NIES CRM No.13 and IAEA-086 were analysed between batches of samples as internal QCs. The recovery for both control materials was > 99 %. As external QC the laboratory performing the analysis participated in 3 round per year of the Quebec Multielement External Quality Assessment Scheme (QMEQAS) and in the Mercury in Hair Interlaboratory Comparison Program (MHICP) with satisfactory results according to the established criteria.

III.- RESULTS AND DISCUSSION

The purpose of the present paper is to present mercury exposure data obtained within the BIOAMBIENT.ES project from a cross section of the Spanish population. This is the first study covering the whole country following a standardised and representative protocol, with the objective to provide national baseline data on mercury exposure in Spain.

In tables 1-4 we report Hg values for the 3 different biomarkers. The tables include information on gender, age, occupational sector, Spanish regions, time of sampling and fish consumption. We report the values, but a detailed discussion of the differences or relationships observed are outside the scope and ambition of this paper. However, a general observation is that consumption of fish and seafood is a major determinant of mercury exposure in the Spanish population, which we have already discussed on basis of the DEMOCOPHES material (Castaño et al., 2015) and also recently reported in other Spanish studies (Pérez et al., 2019).

Naturally, fish and seafood consumption patterns show regional differences. Based on information from the Ministry of Agriculture, Food and Fisheries (MAPA, 2010), fish/sea food consumption, expressed as kilogram/per person and year, is higher in coastal regions than in-land regions. Coastal populations from Andalusia-Ceuta, Murcia, Valencia-Balearic Islands and the North West coast have an average consumption of more than 30 kg/person, exceeding the national average of 27 kg/person. Particularly the consumption of tuna fish is prominent in these regions, 1.5 kg/person compared to 0.6 kg as the national average. The national average for swordfish is 0.5 kg/year. Besides fresh fish, canned fish represent an important part of fish consumption in the Southern and Eastern regions with more than 4 kg per person per year. In Andalusia and Murcia consumption of canned tuna is 2.5 kg/person and year.

Regarding Hg levels in fish, recent studies report 0.47 mg/kg muscle in fresh tuna, 0.222 mg/kg in canned tuna and 0.54 mg/kg in swordfish (Olmedo et al., 2013).

1. Mercury in blood

All 1880 valid blood samples from the BIOAMBIENT.ES were tested. The limit of quantification (LOQ) in blood is 0.1 µg/L. Only 5 samples (0.27%) fell below this limit. For these samples, geometric means were imputed by using the LOQ divided by the square root of two.

The geometric mean (GM) for blood mercury concentrations was 6.35 µg/L (CI 6.00-6.72) (Table 1, Figure 1). This value is slightly higher than the 5 µg/L as a general average value reported by Basu et al., (2018) in non-exposed populations in their review of mercury biomarkers in human populations based on 312 publications.

Concerning the values obtained for the mercury on blood, there were no differences by sex. An age dependence ($p<0.001$), compatible with the cumulative effect of mercury exposure, was observed. Individuals from the service sector showed a significantly higher GM than those from other sectors ($p=0.006$). With respect to the geographical distribution (Figure 1), levels in the Valencia-Balearic Islands, Andalusia-Ceuta and Murcia areas were significantly higher than the national GM, while in Navarra-La Rioja-Aragon and in the Canary Islands were significantly lower ($p=0.005$). The distribution by geographic areas is, therefore, heterogeneous (Figure 1). In terms of sampling, the July-September period presented a value slightly lower than the mean, although this difference was not significant.

In terms of fish consumption, significant associations ($p<0.001$) were found with the level of mercury in blood, with a factor of 2 between daily consumers and those very occasional ones reporting less than one serving a week. This finding is in line with the conclusion by Sheehan et al., (2014) that blood mercury better reflects actual MeHg exposure in comparison to total hair mercury, which probably is a more relevant biomarker for long-term MeHg exposure.

2. Mercury in urine

The exposure source of mercury found in urine is complicated and it has been generally assumed that mercury in urine represents exposure to inorganic mercury. Dental amalgam is a source of exposure to inorganic Hg in the general population (Halbach et al., 2008). However, Sherman et al., (2013), based on the analysis of stable Hg isotopes, conclude that within populations that consume fish more than once per week, urine Hg concentrations may overestimate Hg exposure from inorganic sources (dental amalgams) and that the most of the

Hg found in urine originates from food-borne MeHg, which is de-methylated in the body. They also remark that for those occupationally exposed to inorganic Hg the situation may be different. From the initial 1770, 1704 urine samples were analysed, after excluding those that were too concentrated or too diluted with reference to the creatinine concentration (validity interval 0.3-3 g/L) (WHO, 1996). The LOQ of the technique is 0.05 µg/L. 36 samples (2.1%) did not reach the limit of quantification. As mentioned above, for these samples, geometric means were imputed by using the LOQ divided by the square root of two.

The GM of unadjusted and creatinine adjusted concentrations in urine were 1.11 µg/L (CI 1.03-1.19), and 0.80 µg/g (CI 0.74-0.86), respectively (Table 2 and Table 3). As a comparison, the unadjusted value is markedly lower than the 3 µg/L in non-exposed populations quoted by Basu et al., (2018). Figure 2A shows the distribution of urine mercury GM by sex, age and fish consumption.

Women presented higher levels in urine than men when measured in µg/g (creatinine adjusted). With respect to age, older groups showed higher geometric means than the younger ones. The mercury levels of workers in the service sector were higher than those working in other sectors. Catalonia was the geographical area showing the lowest levels of mercury whereas the highest levels were found in Murcia. Our study confirms the conclusion of Sherman et al., (2013) that mercury levels in urine partly reflect fish consumption. In general, there is a 50% difference between regular and very occasional (less than once a week) consumers.

3. Mercury in hair

There is a consensus in recent reviews (Sherman et al., 2013, Sheehan et al., 2014, Basu et al., 2018) that total mercury in hair primarily represents MeHg exposure from food. In this study, 577 hair samples were measured. All samples were above the limit of quantification (0.01 µg/g). The GM was 1.90 µg/g (CI 1.76-2.05) (Table 4) which is in the range of the 2 µg/g reported in non-exposed populations (Basu et al., 2018). Figure 3 shows the mercury levels (GM) in participants by sex, age, and fish consumption.

No differences were observed in terms of gender. Levels of Hg were found to increase with age, with the value for the older group (≥50 years; 2.46 µg/g, CI 2.24-2.70) being almost 1.5 higher than that for the youngest one (≤29 years; 1.65 µg/g, CI 1.24-2.20) ($p=0.0001$). This is consistent with the bioaccumulative character of mercury. In relation to the occupational sector, individuals working in the service sector show a tendency for higher GM than those from other sectors, although the difference is not significant. Mercury levels of people living in the coastal regions Asturias-Cantabria, Valencia-Balearic Islands, Andalusia-Ceuta and Murcia, were higher than the national mean. In contrast, lower levels were found in land regions Navarre-La Rioja-Aragon, Castile-Leon Castile-La Mancha-Extremadura, although also in coastal regions like the Canary Islands.

As a general comment, Basu et al., (2018) in their extensive review of mercury exposure data based on human biomarkers, remark that one factor limiting comparisons and conclusions between studies is the lack of quality which makes comparisons difficult. The Spanish National Centre of Epidemiology has designed the current study according to established methodology. Our laboratory together with a German laboratory were instrumental in harmonising and quality assuring human biomonitoring methodology within the European Union funded project COPHES (Schindler et al., 2014). The objectives of COPHES were to develop a harmonised protocol for human biomonitoring in Europe. The protocols were applied in the DEMOCOPHES project, an EU wide human biomonitoring project (Den Hond et al., 2015) in which our laboratory quality assured the mercury analysis for the 15 participating laboratories. Although BIOAMBIENT.ES was not part of the COPHES or DEMOCOPHES projects, the methodology and the quality criteria developed in the 2 EU projects were also followed in the BIOAMBIENT.ES.

IV.- CONCLUSION

Consumption of fish is the major determinant of exposure in the general Spanish population. This is reflected in all the 3 different biomarkers (blood, urine, hair) of Hg exposure analysed. The regional differences in Hg biomarker responses parallels the regional consumption patterns of fish and seafood, being higher in coastal than in inland regions. In a European context the Spanish values fall in the group of Mediterranean countries with traditionally high consumption of marine fish and seafood including top predators like tuna and swordfish, while populations with a lower fish consumption in Northern and Eastern Europe have lower values (Den Hond et al., 2015). The same applies in an international comparison. Aquatic food chains are the main source of mercury exposure globally, and the common denominator is a combination of food habits and occurrence of Hg contamination. Human consumers of marine food-chain apex mammals like whales and seals reach high body burdens (20-60 µg/L blood) (AMAP, 2015), as well as consumers feeding on local freshwater fish in areas contaminated by small-scale artisanal gold mining (Sheehan et al., 2014).

Most of the population in the current study (Figure 3) has blood mercury levels between the health-based reference values, HBM-I (5 µg/l) and HBM-II (15 µg/l) established by the German Human Biomonitoring Commission (UBA, 2018) and hair levels below by the health-based guidance value, 2.3 µg/g, established by WHO (WHO, 2008). However, there are a few individuals who have significantly higher values which should be followed up with targeted monitoring and recommendations.

There is an on-going discussion among public health and food safety authorities on the maximum permissible level of Hg in fish and seafood products (EU DG SANTE 2018). Currently, the European Commission recommends Member States to develop specific national consumption advice balancing health benefits from fish consumption with the health risks of elevated mercury exposure. In order to fine tune recommendations and risk assessments a continuous input of information is necessary, and Members States are urged by the

Commission to submit new information to the European Food Safety Authority. The strength of the present study is the regional separation which makes it possible to fine tune recommendations and consumption advice in relation to cultural habits and life style habits and pinpoint vulnerable groups which should be more closely followed.

Conflict of interest

The authors declare no conflicts of interest to disclose.

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Figure 1. Maps of Spain with the distribution of mercury levels (Geometric means) in blood (µg Hg/L), urine (µg Hg/g creatinine) and hair (µg Hg/g) of participants recruited according the regions designed in the study BIOAMBIENT.ES: 1. Galicia; 2. Asturias, Cantabria; 3. Basque country; 4. Navarre, La Rioja, Aragon; 5. Catalonia; 6. Castile- Leon; 7. Madrid; 8. Castile-La Mancha, Extremadura; 9. Valencia, Balearic Islands; 10. Andalusia, Ceuta; 11. Murcia; 12. Canary Islands

Figure 2. Mercury levels (Geometric means) in BIOAMBIENT.ES participants by sex, age, and fish consumption. A: mercury in urine creatinine adjusted (µg/g). B mercury in hair (µg/g)

Figure 3. Distribution of blood (A) and hair (B) mercury levels (Geometric means, GM) in BIOAMBIENT.ES participants. HBM-I-Value 5µg/L; HBM-II-Value 15µg/L; WHO Health based value 2.3 µg/g

Table 1. Geometric means (GM) with 95% confidence intervals, selected percentiles and number of samples below the limit of quantification (LOQ) of **blood total mercury concentrations (µg/L)** in BIOAMBIENT.ES participants, stratified by gender, age, occupational sector, geographic area sampling period and fish consumption.

	N	GM (CI95%)	P10	P25	P50	P75	P90	P95	<LOQ	pvalue
Total	1880	6.35 (6.00-6.72)	2.52	4.11	6.60	10.60	15.60	19.30	5	
Gender										0.602
Male	962	6.41 (5.95-6.91)	2.32	3.99	6.77	11.10	17.40	21.60	4	
Female	918	6.27 (5.88-6.69)	2.70	4.18	6.35	10.00	14.20	16.90	1	
Age										0.000
≤29	356	5.00 (4.46-5.62)	1.65	3.32	5.76	8.98	13.00	16.20	2	
30 – 39	774	6.56 (6.16-7.00)	2.79	4.40	6.62	10.50	15.50	19.50	1	
40 – 49	470	6.36 (5.76-7.03)	2.55	4.05	6.42	11.10	15.90	19.90	2	
≥50	268	8.01 (7.14-8.99)	3.04	5.09	8.83	12.80	18.20	24.80	0	
Occupational sector										0.006
Others	655	5.67 (5.19-6.20)	2.10	3.80	6.13	9.62	15.10	17.70	4	
Service	1225	6.64 (6.23-7.08)	2.71	4.26	6.73	11.00	15.80	20.00	1	
Geographical area										0.005
Galicia	147	7.00 (5.07-9.65)	2.65	4.52	7.45	11.30	16.20	21.60	0	
Asturias, Cantabria	99	6.36 (1.82-22.19)	2.89	4.65	6.81	8.59	13.10	14.60	0	
Basque country	149	6.02 (4.79-7.55)	2.68	4.51	6.50	10.60	14.20	16.30	3	
Navarre, La Rioja, Aragon	145	4.91 (3.85-6.27)	1.97	3.77	5.08	8.13	10.30	11.80	0	
Catalonia	247	5.40 (4.57-6.37)	2.09	3.65	6.01	8.95	12.10	15.20	1	
Castile- Leon	153	5.81 (4.62-7.29)	2.25	3.85	5.98	9.41	14.90	17.40	0	
Madrid	199	6.22 (4.36-8.88)	2.46	3.76	6.11	11.80	17.50	21.70	0	
Castile-La Mancha, Extremadura	150	6.02 (3.49-10.39)	2.31	3.55	5.76	9.97	15.70	19.50	0	
Valencia, Balearic Islands	205	7.66 (6.38-9.21)	2.97	4.85	8.53	13.70	18.90	23.60	1	
Andalusia, Ceuta	239	7.47 (5.95-9.38)	2.93	4.83	7.74	12.30	17.80	19.50	0	
Murcia	97	7.81 (3.16-19.32)	3.87	5.57	7.98	11.20	15.80	17.70	0	
Canary Islands	50	5.15 (2.89-9.17)	2.16	3.17	5.91	8.08	10.50	11.10	0	
Sampling period										0.512
January – March	349	6.65 (5.90-7.49)	2.83	4.14	6.81	10.60	15.90	19.30	1	
April – June	562	6.29 (5.64-7.01)	2.36	4.00	6.65	10.80	15.80	19.80	4	
July – September	373	6.12 (5.72-6.54)	2.73	4.44	6.35	9.36	12.80	14.90	0	
October - December	561	6.48 (5.78-7.28)	2.51	4.12	6.70	11.10	17.30	19.60	0	
Fish consumption										0.000
< once a week	213	3.71 (3.24-4.24)	1.23	2.34	4.11	6.70	8.92	12.30	3	
Once a week	575	5.38 (4.97-5.83)	2.09	3.48	5.91	9.03	12.90	16.50	2	
2-4 times a week	833	7.66 (7.28-8.06)	3.30	5.01	7.80	11.70	17.50	21.60	0	
> 5 times a week	221	8.38 (7.30-9.63)	3.49	5.15	8.87	14.00	18.20	27.20	0	

Table 2. Geometric means (GM) with 95% confidence intervals, selected percentiles and number of samples below the limit of quantification (LOQ) of **urinary total mercury concentrations (µg/L)** in BIOAMBIENT.ES participants, stratified by gender, age, occupational sector, geographic area, sampling period and fish consumption.

	N	GM (CI95%)	P10	P25	P50	P75	P90	P95	<LOQ	pvalue
Total	1704	1.11 (1.03-1.19)	0.39	0.72	1.19	2.12	3.11	4.14	36	
Gender										0.490
Male	872	1.09 (0.97-1.22)	0.34	0.68	1.17	2.11	3.15	4.23	21	
Female	832	1.14 (1.05-1.23)	0.42	0.73	1.21	2.15	3.01	3.99	15	
Age										0.015
≤29	313	0.98 (0.80-1.21)	0.29	0.66	1.22	2.11	2.89	3.97	12	
30 – 39	694	1.20 (1.11-1.30)	0.47	0.78	1.28	2.17	3.17	4.31	8	
40 – 49	441	1.08 (0.99-1.18)	0.39	0.66	1.13	2.06	3.00	3.82	9	
≥50	248	1.08 (0.94-1.25)	0.35	0.67	1.13	2.18	3.63	4.39	7	
Occupational sector										0.021
Others	600	0.96 (0.84-1.10)	0.30	0.63	1.08	1.89	3.03	4.12	18	
Service	1104	1.17 (1.08-1.28)	0.42	0.73	1.27	2.18	3.15	4.14	18	
Geographical area										0.000
Galicia	130	1.40 (0.51-3.84)	0.51	0.88	1.49	2.58	4.07	5.98	3	
Asturias, Cantabria	95	1.30 (0.36-4.66)	0.50	0.82	1.51	2.18	3.60	4.41	1	
Basque country	127	1.03 (0.40-2.62)	0.31	0.61	1.01	2.00	3.26	3.76	3	
Navarre, La Rioja, Aragon	133	1.12 (0.91-1.38)	0.53	0.85	1.22	1.64	2.44	2.88	2	
Catalonia	228	0.91 (0.62-1.32)	0.30	0.63	0.99	1.87	2.79	3.15	6	
Castile- Leon	136	1.01 (0.61-1.67)	0.32	0.64	1.10	1.91	2.79	3.28	3	
Madrid	184	0.92 (0.70-1.21)	0.29	0.58	0.93	1.88	3.00	3.95	7	
Castile-La Mancha, Extremadura	143	1.03 (0.53-2.00)	0.35	0.61	1.24	1.91	2.30	2.76	3	
Valencia, Balearic Islands	180	1.42 (1.23-1.63)	0.53	0.88	1.76	2.57	3.82	4.41	4	
Andalusia, Ceuta	211	1.30 (1.21-1.40)	0.46	0.82	1.44	2.46	3.67	4.25	2	
Murcia	92	1.69 (1.03-2.77)	0.50	1.11	1.85	3.04	4.24	4.41	1	
Canary Islands	45	0.82 (0.24-2.85)	0.36	0.52	0.90	1.38	1.99	2.19	1	
Sampling period										0.503
January – March	318	1.22 (1.05-1.40)	0.51	0.76	1.21	2.00	3.04	4.33	1	
April – June	497	1.04 (0.88-1.24)	0.34	0.68	1.13	2.00	3.02	3.90	16	
July – September	343	1.19 (1.04-1.37)	0.39	0.73	1.28	2.28	3.22	4.27	3	
October - December	514	1.08 (0.89-1.31)	0.35	0.68	1.24	2.26	3.18	4.16	15	
Fish consumption										0.000
< once a week	193	0.67 (0.57-0.79)	0.24	0.46	0.81	1.21	2.20	2.71	13	
Once a week	520	0.97 (0.83-1.14)	0.31	0.63	1.08	2.03	3.08	3.96	17	
2-4 times a week	754	1.33 (1.24-1.42)	0.51	0.84	1.43	2.36	3.29	4.36	3	
> 5 times a week	203	1.30 (1.09-1.55)	0.50	0.81	1.33	2.14	3.15	4.19	1	

Table 3. Geometric means (GM) with 95% confidence intervals, selected percentiles and number of samples below the limit of quantification (LOQ) of **urinary total mercury concentrations normalized to urinary creatinine concentrations (µg/g creat)** in BIOAMBIENT.ES participants, stratified by gender, age, occupational sector, geographic area, sampling period and fish consumption.

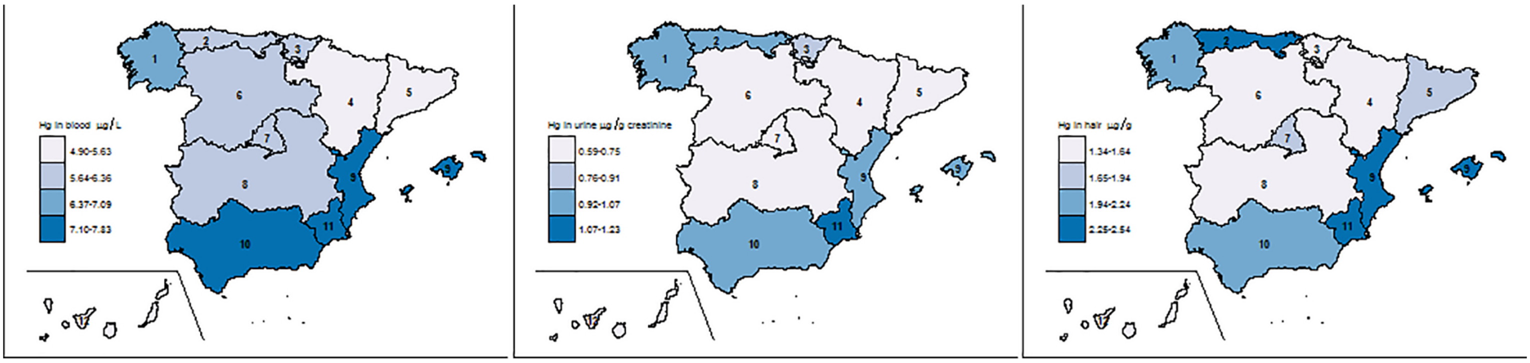
	N	GM (CI95%)	P10	P25	P50	P75	P90	P95	<LOQ	pvalue
Total	1704	0.80 (0.74-0.86)	0.29	0.49	0.87	1.47	2.30	2.82	36	
Gender										0.000
Male	872	0.69 (0.62-0.78)	0.25	0.42	0.78	1.32	2.00	2.41	21	
Female	832	0.95 (0.89-1.02)	0.35	0.57	1.03	1.67	2.67	3.38	15	
Age										0.035
≤29	313	0.64 (0.51-0.80)	0.19	0.43	0.76	1.26	1.90	2.48	12	
30 – 39	694	0.81 (0.74-0.89)	0.31	0.47	0.86	1.44	2.33	2.89	8	
40 – 49	441	0.83 (0.76-0.89)	0.30	0.53	0.92	1.49	2.13	2.72	9	
≥50	248	0.94 (0.83-1.06)	0.32	0.56	0.95	1.83	2.41	3.13	7	
Occupational sector										0.012
Others	600	0.68 (0.59-0.78)	0.25	0.42	0.75	1.33	2.08	2.41	18	
Service	1104	0.85 (0.78-0.93)	0.32	0.52	0.94	1.52	2.37	3.01	18	
Geographical area										0.000
Galicia	130	1.03 (0.50-2.13)	0.41	0.58	1.07	1.81	2.59	3.50	3	
Asturias, Cantabria	95	0.93 (0.22-3.83)	0.40	0.60	0.91	1.44	2.38	2.83	1	
Basque country	127	0.79 (0.35-1.78)	0.27	0.52	0.86	1.49	2.19	2.87	3	
Navarre, La Rioja, Aragon	133	0.73 (0.58-0.92)	0.30	0.49	0.82	1.18	1.86	2.10	2	
Catalonia	228	0.60 (0.39-0.94)	0.22	0.41	0.63	1.11	1.92	2.39	6	
Castile- Leon	136	0.68 (0.42-1.12)	0.25	0.44	0.76	1.22	1.78	2.33	3	
Madrid	184	0.65 (0.48-0.88)	0.24	0.38	0.70	1.23	2.20	2.80	7	
Castile-La Mancha, Extremadura	143	0.72 (0.39-1.33)	0.31	0.46	0.78	1.18	1.77	2.05	3	
Valencia, Balearic Islands	180	1.02 (0.93-1.12)	0.34	0.65	1.16	1.74	2.85	3.42	4	
Andalusia, Ceuta	211	1.02 (0.83-1.25)	0.35	0.65	1.20	1.86	2.57	3.10	2	
Murcia	92	1.23 (0.86-1.76)	0.49	0.88	1.38	1.90	2.57	2.83	1	
Canary Islands	45	0.74 (0.50-1.11)	0.25	0.47	0.86	1.20	1.81	2.15	1	
Sampling period										0.390
January – March	318	0.86 (0.74-0.99)	0.36	0.53	0.85	1.39	2.14	2.59	1	
April – June	497	0.74 (0.62-0.88)	0.26	0.41	0.75	1.44	2.23	2.80	16	
July – September	343	0.88 (0.77-1.01)	0.35	0.52	0.94	1.47	2.43	3.00	3	
October - December	514	0.79 (0.63-1.00)	0.27	0.49	0.93	1.54	2.28	2.86	15	
Fish consumption										0.000
< once a week	193	0.48 (0.42-0.56)	0.15	0.32	0.55	0.95	1.38	1.98	13	
Once a week	520	0.67 (0.56-0.79)	0.24	0.42	0.74	1.28	2.09	2.66	17	
2-4 times a week	754	0.96 (0.89-1.04)	0.37	0.58	1.05	1.63	2.43	3.06	3	
> 5 times a week	203	1.02 (0.86-1.19)	0.43	0.61	1.05	1.74	2.46	2.86	1	

Table 4. Geometric means (GM) with 95% confidence intervals, selected percentiles and number of samples below the limit of quantification (LOQ) of **hair total mercury concentrations (µg/g)** in BIOAMBIENT.ES participants, stratified by gender, age, occupational sector, geographic area, sampling period and fish consumption.

	N	GM (CI95%)	P10	P25	P50	P75	P90	P95	<LOQ	pvalue
Total	577	1.90 (1.76-2.05)	0.80	1.20	2.00	3.40	4.60	5.20	0	
Gender										0.853
Male	250	1.92 (1.63-2.27)	0.80	1.10	1.90	3.70	4.90	6.80	0	
Female	327	1.87 (1.58-2.22)	0.80	1.30	2.00	3.30	3.90	4.60	0	
Age										0.001
≤29	85	1.65 (1.24-2.20)	0.70	1.10	2.10	3.00	4.60	4.70	0	
30 – 39	240	1.81 (1.64-1.99)	0.80	1.10	1.70	3.40	4.10	4.70	0	
40 – 49	151	1.97 (1.70-2.29)	0.80	1.30	1.90	3.30	4.70	7.90	0	
≥50	101	2.46 (2.24-2.70)	1.00	1.60	2.70	3.90	5.30	5.50	0	
Occupational sector										0.090
Others	127	1.58 (1.28-1.96)	0.70	1.10	1.50	2.50	4.30	5.30	0	
Service	450	2.06 (1.81-2.34)	0.80	1.40	2.20	3.40	4.60	5.10	0	
Geographical area										0.006
Galicia	70	1.97 (1.56-2.50)	0.90	1.30	2.00	3.00	4.30	7.90	0	
Asturias, Cantabria	12	2.29 (1.24-4.26)	1.60	1.60	2.00	3.00	4.10	5.50	0	
Basque country	75	1.62 (0.74-3.51)	0.60	1.10	2.00	3.30	4.20	4.40	1	
Navarre, La Rioja, Aragon	37	1.35 (0.41-4.47)	0.50	0.90	1.20	2.10	3.30	3.70	0	
Catalonia	16	1.76 (1.18-2.64)	1.10	1.10	1.70	3.40	3.40	3.40	0	
Castile- Leon	6	1.60 (0.36-7.17)	0.90	1.50	1.50	2.00	2.20	2.20	0	
Madrid	63	1.92 (1.62-2.28)	0.80	1.10	1.80	3.70	4.60	8.00	0	
Castile-La Mancha, Extremadura	69	1.59 (0.67-3.73)	0.70	0.90	1.70	2.20	4.10	4.80	0	
Valencia, Balearic Islands	39	2.30 (1.16-4.56)	0.70	1.70	2.90	4.50	4.90	5.20	0	
Andalusia, Ceuta	134	2.15 (1.38-3.35)	0.90	1.50	2.30	3.90	5.30	5.60	3	
Murcia	10	2.53 (0.18-35.40)	1.60	2.10	3.00	3.10	3.70	3.70	0	
Canary Islands	46	1.51 (1.41-1.62)	0.70	0.90	1.60	2.50	2.90	3.90	0	
Sampling period										0.006
January – March	87	1.61 (1.32-1.96)	0.70	0.90	1.80	3.00	3.70	4.60	0	
April – June	155	2.05 (1.75-2.39)	0.70	1.40	2.30	3.70	4.70	4.80	0	
July – September	88	1.63 (1.37-1.92)	0.90	1.10	1.70	3.00	3.40	3.40	0	
October - December	242	2.08 (1.76-2.46)	0.90	1.40	2.00	3.90	4.90	7.00	0	
Fish consumption										0.001
< once a week	70	0.99 (0.63-1.54)	0.15	0.50	1.10	2.00	3.80	4.10	0	
Once a week	177	1.77 (1.63-1.93)	0.80	1.10	1.60	3.20	4.30	4.90	0	
2-4 times a week	255	2.33 (2.09-2.60)	1.10	1.70	2.40	3.60	4.60	5.30	0	
> 5 times a week	70	2.21 (1.80-2.72)	0.70	1.40	2.40	3.90	4.90	6.80	0	

Highlights

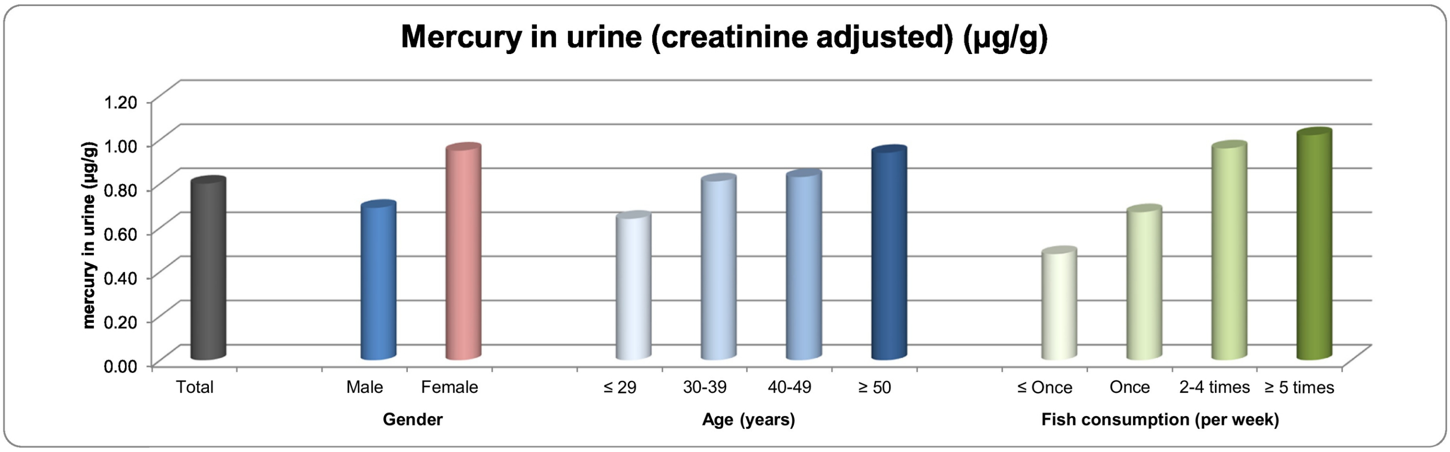
- Mercury levels of a representative sample of Spanish active population reported.
- Values of 1880 blood, 1704 urine and 577 hair samples from all Spanish regions reported.
- The major source of mercury exposure in Spanish adults is dietary fish intake.
- Coastal regions inhabitants have higher exposure levels than those from inland regions.



Hg in blood µg/L
4.90-5.63
5.64-6.36
6.37-7.09
7.10-7.83
Hg in urine µg/g creatinine
0.59-0.75
0.76-0.91
0.92-1.07
1.07-1.23
Hg in hair µg/g
1.34-1.64
1.65-1.94
1.94-2.24
2.25-2.54

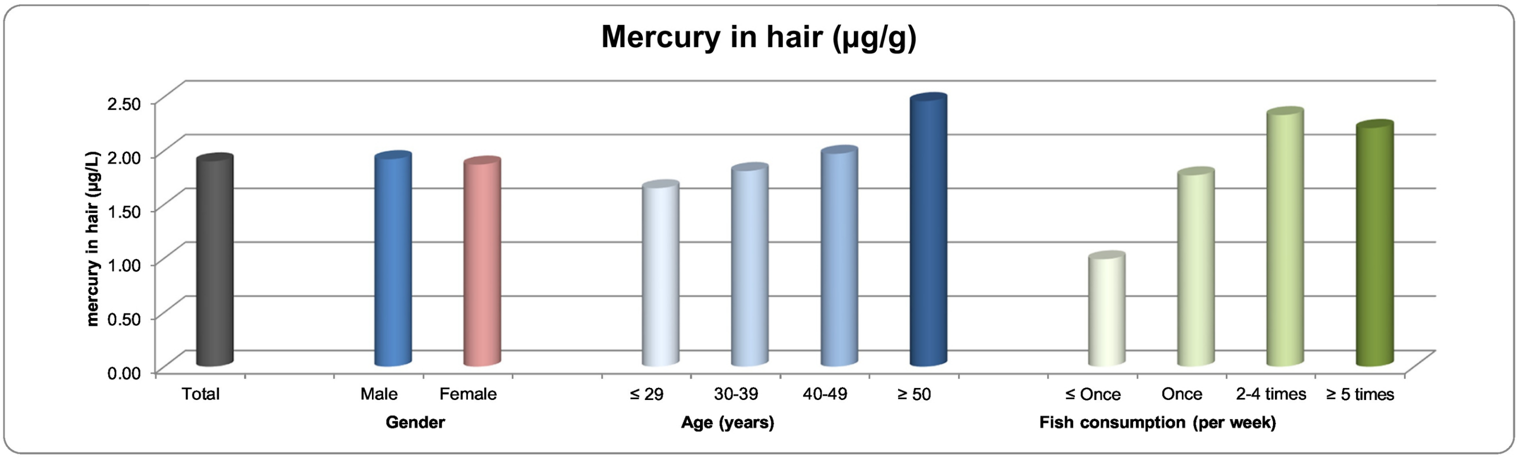
Figure 1

A



Mercury in urine (creatinine adjusted) (µg/g)
mercury in urine (µg/g)
1.20
1.00
0.80
0.60
0.40
0.20
0.00
Total
Male
Female
≤ 29
30-39
40-49
≥ 50
≤ Once
Once
2-4 times
≥ 5 times
Gender
Age (years)
Fish consumption (per week)

B



Mercury in hair (µg/g)
mercury in hair (µg/L)
2.50
2.00
1.50
1.00
0.50
0.00
Total
Male
Female
≤ 29
30-39
40-49
≥ 50
≤ Once
Once
2-4 times
≥ 5 times
Gender
Age (years)
Fish consumption (per week)

Figure 2

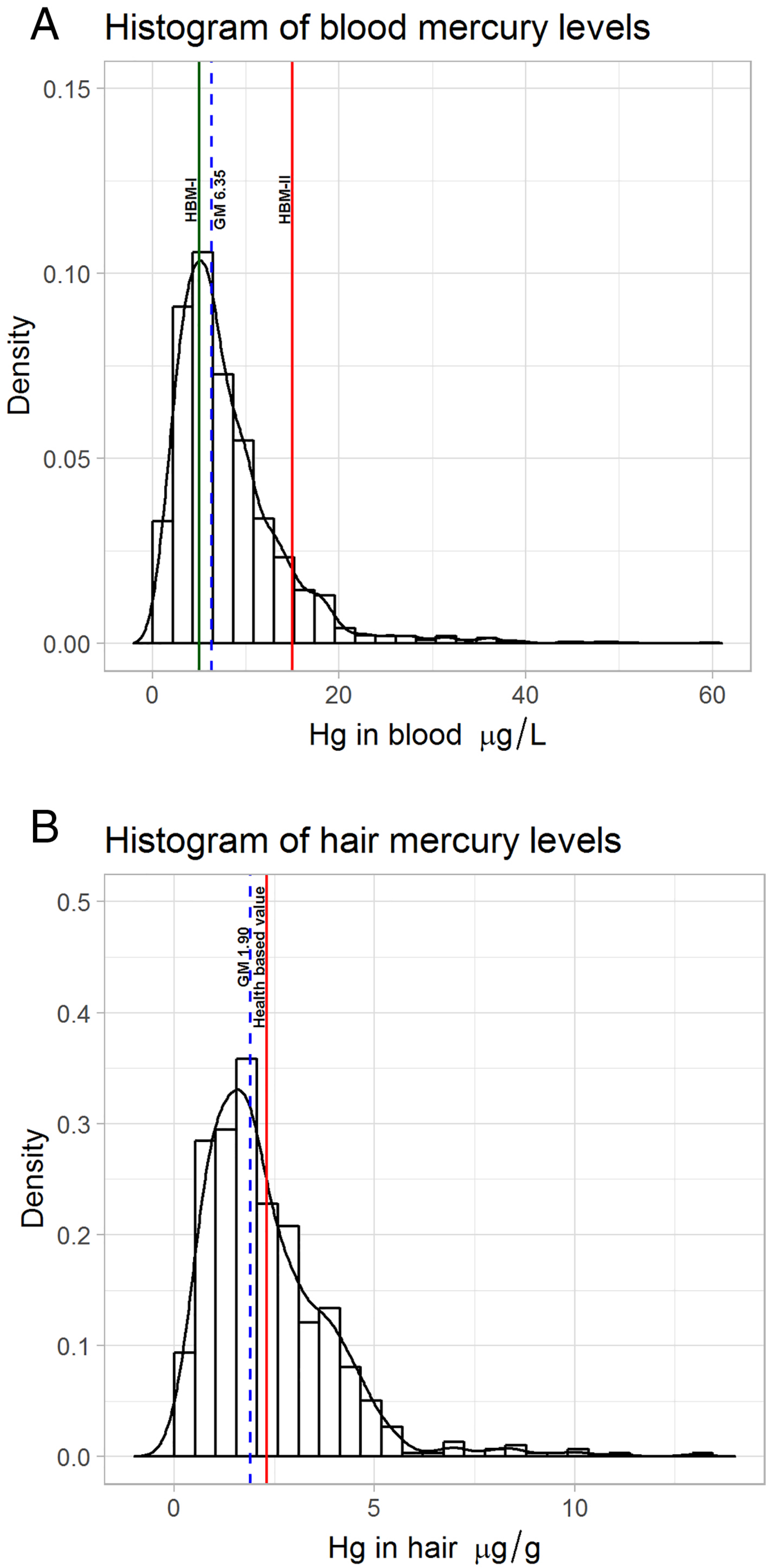

A
Histogram of blood mercury levels
HBM-I
GM 6.35
HBM-II
Density
0.15
0.10
0.05
0.00
0
20
40
60
Hg in blood μg/L
B
Histogram of hair mercury levels
GM 1.90
Health based value
Density
0.5
0.4
0.3
0.2
0.1
0.0
0
5
10
Hg in hair μg/g

Figure 3