



## HBM4EU-MOM: Prenatal methylmercury-exposure control in five countries through suitable dietary advice for pregnancy – Study design and characteristics of participants

Andromachi Katsonouri<sup>a,\*</sup>, Catherine Gabriel<sup>b,c,\*\*</sup>, Marta Esteban López<sup>d</sup>, Sonia Namorado<sup>e,f,g</sup>, Thorhallur I. Halldorsson<sup>h</sup>, Janja Snoj Tratnik<sup>i</sup>, Laura Rodriguez Martin<sup>j</sup>, Achilleas Karakoltzidis<sup>b,c</sup>, Anthonia Chatzimpaloglou<sup>b,c</sup>, Despina Giannadaki<sup>a</sup>, Elena Anastasi<sup>a</sup>, Anthi Thoma<sup>a</sup>, Noelia Domínguez-Moruco<sup>d</sup>, Ana I. Cañas Portilla<sup>d</sup>, Eva Jacobsen<sup>h</sup>, Ricardo Assunção<sup>k</sup>, Maria Peres<sup>e</sup>, Susana Santiago<sup>e</sup>, Carla Nunes<sup>e</sup>, Susana Pedraza-Díaz<sup>d</sup>, Ivo Iavicoli<sup>l</sup>, Veruscka Leso<sup>l</sup>, Marina Lacasaña<sup>m,n,o,p</sup>, Beatriz González-Alzaga<sup>m,n,o</sup>, Milena Horvat<sup>i</sup>, Ovnair Sepai<sup>q</sup>, Argelia Castano<sup>d</sup>, Marike Kolossa-Gehring<sup>r</sup>, Spyros Karakitsios<sup>b,c</sup>, Dimosthenis Sarigiannis<sup>b,c,s</sup>

<sup>a</sup> State General Laboratory, Ministry of Health, Nicosia, Cyprus

<sup>b</sup> HERACLES Research Center – KEDEK, Aristotle University of Thessaloniki, Thessaloniki, Greece

<sup>c</sup> Environmental Engineering Laboratory, Department of Chemical Engineering, Aristotle University of Thessaloniki, Thessaloniki, Greece

<sup>d</sup> Environmental Toxicology Unit, National Centre for Environmental Health. Instituto de Salud Carlos III (CNSA-ISCIII), Madrid, Spain

<sup>e</sup> Department of Epidemiology, National Institute of Health Dr. Ricardo Jorge (INSA), Lisbon, Portugal

<sup>f</sup> NOVA National School of Public Health, Public Health Research Centre, Universidade NOVA de Lisboa, Lisbon, Portugal

<sup>g</sup> Comprehensive Health Research Center (CHRC), Lisbon, Portugal

<sup>h</sup> University of Iceland, Reykjavik, Iceland

<sup>i</sup> Jozef Stefan Institute, Ljubljana, Slovenia

<sup>j</sup> VITO, Mol, Belgium

<sup>k</sup> Egas Moniz School & Health Science, Campus Universitário, Quinta da Granja, 2829-511, Monte de Caparica, Almada, Portugal

<sup>l</sup> Department of Public Health University of Naples Federico II, Naples, Italy

<sup>m</sup> Andalusian School of Public Health (EASP), Granada, Spain

<sup>n</sup> Instituto de Investigación Biosanitaria, Ibs.GRANADA, Granada, Spain

<sup>o</sup> CIBER Epidemiology and Public Health (CIBERESP), Madrid, Spain

<sup>p</sup> Andalusian Health and Environment Observatory (OSMAN), Granada, Spain

<sup>q</sup> UK Health Security Agency, Radiation, Chemicals and Environment Division, Harwell, UK

<sup>r</sup> German Environment Agency (UBA), Berlin, Germany

<sup>s</sup> Environmental Health Engineering, School for Advanced Study IUSS, Pavia, Italy

### ABSTRACT

**Background:** Seafood is a major source of vital nutrients for optimal fetal growth, but at the same time is the main source of exposure to methylmercury (MeHg), an established neurodevelopmental toxicant. Pregnant women must be provided with dietary advice so as to include safely fish in their diet for nutrition and mercury control. The aim of this work is to present the design of a multicentre randomized control trial (RCT), which combines human biomonitoring (HBM) with dietary interventions using seafood consumption advice to pregnant women for MeHg control, and to collect information about other possible sources of exposure to mercury. It also presents the materials developed for the implementation of the study and the characteristics of the study participants, which were self-reported in the first trimester of pregnancy.

**Methods:** The “HBM4EU-MOM” RCT was performed in the frame of the European Human Biomonitoring Initiative (HBM4EU) in five coastal, high fish-consuming European countries (Cyprus, Greece, Spain, Portugal and Iceland). According to the study design, pregnant women ( $\geq 120$ /country,  $\leq 20$  weeks gestational age)

\* Corresponding author. Head of the Human Biomonitoring and Control of Industrial Products Laboratory, State General Laboratory, Ministry of Health, P.O. Box 28648, 2081 Nicosia, Cyprus.

\*\* Corresponding author. Environmental Engineering Laboratory, Department of Chemical Engineering Aristotle University of Thessaloniki, and HERACLES Research Center – KEDEK, BALKAN CENTER, Building B, B.1.0.1, 10th km Thessaloniki - Thermi Rd, GR 57001, Greece.

E-mail addresses: [akatsounouri@sgl.moh.gov.cy](mailto:akatsounouri@sgl.moh.gov.cy) (A. Katsonouri), [katerinagabriel@cheng.auth.gr](mailto:katerinagabriel@cheng.auth.gr), [katerinagabriel79@gmail.com](mailto:katerinagabriel79@gmail.com) (C. Gabriel).

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provided a hair sample for total mercury assessment (THg) and personal information relevant to the study (e.g., lifestyle, pregnancy status, diet before and during the pregnancy, information on seafood and factors related to possible non-dietary exposures to mercury) during the first trimester of pregnancy. After sampling, participants were randomly assigned to “control” (habitual practices) or “intervention” (received the harmonized HBM4EU-MOM dietary advice for fish consumption during the pregnancy and were encouraged to follow it). Around child delivery, participants provided a second hair sample and completed another tailored questionnaire.

**Results:** A total of 654 women aged 18–45 years were recruited in 2021 in the five countries, primarily through their health-care providers. The pre-pregnancy BMI of the participants ranged from underweight to obese, but was on average within the healthy range. For 73% of the women, the pregnancy was planned. 26% of the women were active smokers before the pregnancy and 8% continued to smoke during the pregnancy, while 33% were passive smokers before pregnancy and 23% remained passively exposed during the pregnancy. 53% of the women self-reported making dietary changes for their pregnancy, with 74% of these women reporting making the changes upon learning of their pregnancy. Of the 43% who did not change their diet for the pregnancy, 74% reported that their diet was already balanced, 6% found it difficult to make changes and 2% were unsure of what changes to make. Seafood consumption did not change significantly before and during the first trimester of pregnancy (overall average ~8 times per month), with the highest frequency reported in Portugal ( $\geq 15$  times per month), followed by Spain ( $\geq 7$  times per month). During the first-trimester of pregnancy, 89% of the Portuguese women, 85% of the Spanish women and  $< 50\%$  of Greek, Cypriot and Icelandic women reported that they had consumed big oily fish. Relevant to non-dietary exposure sources, most participants ( $> 90\%$ ) were unaware of safe procedures for handling spillage from broken thermometers and energy-saving lamps, though  $> 22\%$  experienced such an incident ( $> 1$  year ago). 26% of the women had dental amalgams. ~1% had amalgams placed and ~2% had amalgams removed during peri-pregnancy. 28% had their hair dyed in the past 3 months and 40% had body tattoos. 8% engaged with gardening involving fertilizers/pesticides and 19% with hobbies involving paints/pigments/dyes.

**Conclusions:** The study design materials were fit for the purposes of harmonization and quality-assurance. The harmonized information collected from pregnant women suggests that it is important to raise the awareness of women of reproductive age and pregnant women about how to safely include fish in their diet and to empower them to make proper decisions for nutrition and control of MeHg, as well as other chemical exposures.

## 1. Introduction

Seafood contains beneficial nutrients that are essential for foetal growth and development, as well as infant neurodevelopment during lactation. However, it is also a significant source of exposure to mercury that poses risks to the developing nervous system. Accordingly, controversial discussions and dissenting opinions are wide spread within the scientific community and also in public discourse.

Foetal neurodevelopment relies on important nutrients, which can be obtained through proper dietary habits (Hibbeln et al., 2007; Oken et al., 2008a). These nutrients include docosahexaenoic acid (3-DHA) for visual and cognitive development, n-3 long-chain polyunsaturated fatty acids (omega 3-PUFAs) for optimal brain development (Aparicio et al., 2021; Stratakis et al., 2020; Tressou et al., 2019), vitamin D for growth and development and selenium which plays a protective role against the neurotoxicity of methylmercury (Hibbeln et al., 2007; Oken et al., 2003, 2008a, 2008b, 2013; Davidson et al., 2010; Strain et al., 2012). While seafood is a primary source of these nutrients, it is also the primary source of exposure to methylmercury (MeHg) in Europe (Maulvault et al., 2015; Nguetseng et al., 2015; Višnjevec et al., 2014). Therefore, both the frequency of seafood consumption and the types of species consumed are critical factors in the risk/benefit balance (Hellberg et al., 2012; Li et al., 2020; Vilavert et al., 2017; Becker et al., 2007; Maulvault et al., 2015; Nguetseng et al., 2015; Višnjevec et al., 2014). Mercury (Hg) is a global pollutant due to its high persistence, long range transport, bioaccumulation and biomagnification (Driscoll et al., 2013; Liu et al., 2021; Pavithra et al., 2023). Despite the recent stringent Regulation 2017/852 of the European Parliament and of the Council on Hg and the entry into force of the United Nations (UN) Minamata Convention on Hg in 2017 (Minamata, 2019), Europeans remain exposed - primarily to legacy Hg and to Hg originating from sources outside the Union (Karel Houessionon et al., 2021; Li et al., 2022; Liu et al., 2021; Mng'ong'o et al., 2021; Ren et al., 2022; Višnjevec et al., 2014). Even low exposures to Hg may cause severe health effects including irreversible damage to the central nervous system during foetal development (Dórea, 2021; Re et al., 2022; Roe, 2022) (Bjørklund et al., 2022; Boucher et al., 2012; Garf et al., 2022) (Di Ciaula, 2021; Feng et al., 2020; Zhao et al., 2023; Zheng et al., 2023; Basu et al., 2018, 2023). Hg is a matter of concern to the global society, including in Europe, where the main source of exposure for the general population is seafood.

The feasibility study of the COPHES project/DEMOCOPHES (2010–2012) provided the first harmonized cross-border HBM data in Europe by measuring total mercury (THg) in scalp hair of women of

reproductive age and of children aged 6–11 years. This study confirmed an association between Hg exposure and fish consumption, revealing that exposure is higher in countries where residents have a higher fish consumption. Notably, in DEMOCOPHES, high fish-consumers from Spain and Portugal had the highest Hg exposures out of 17 countries (5–7 times above the European average) (Hond et al., 2015; Castaño et al., 2015).

Many people in Europe remain unaware of effective ways to balance the risks/benefits of fish consumption. This applies also to vulnerable groups like pregnant women or women intending to get pregnant, as well as health professionals who care for/consult these women (e.g., obstetricians/gynaecologists, midwives and dietitians). Moreover, risk communication must be carefully balanced due to the well-known nutritional benefits of seafood consumption. Due to the presence of MeHg and other contaminants in seafood, inappropriate dietary advice may lead to the reduction of seafood consumption and compromise its nutritional value. A study from the US showed that a federal advisory recommending pregnant women to limit consumption of certain fish due to mercury resulted in reduced overall fish intake. This result highlights the importance of tailored dietary advice (Oken et al., 2003). A later US pilot randomized controlled trial to promote healthful fish consumption during pregnancy increased consumption of fish and DHA but not mercury (Oken et al., 2013). Though several European countries have developed official guidelines for pregnant women's seafood consumption, they are often not adequately communicated to the stakeholders' consumers, even in countries where the risk is high (Nunes et al., 2014; Taylor et al., 2018). Furthermore, several countries also lack suitable advice.

Therefore, exposure management knowledge is of utmost importance - especially for pregnant women in vulnerable regions of Europe. At the same time, the simultaneous assurance of the intake of nutrients prevailing through suitable dietary advice and concerning the consumption of seafood is also important (Hibbeln et al., 2019). This thesis is in agreement with the recommendation of the European Food Safety Authority (EFSA), which in 2015 called for Member States to examine their national situation and issue advice for vulnerable groups (EFSA, 2016). Recent reports in the scientific literature show that Human Bio-monitoring in combination with the provision of suitable dietary advice to pregnant women is a powerful tool for controlling prenatal exposure to Hg while assuring the nutritional benefits provided by seafood. Such studies have been performed successfully in the United States and in Denmark (Kirk et al., 2017; Oken et al., 2013, Turyk et al., 2019).

The HBM4EU-MOM study (“Methylmercury-contrOl in expectant Mothers through suitable dietary advice for pregnancy”) was conducted

in the context of the European Joint Programme HBM4EU (<https://www.hbm4eu.eu/>) (Ganzleben et al., 2017; Kolossa-Gehring et al., 2023). The study was operated at the science-policy interface to generate knowledge, which can directly be used for chemicals' policy and the improvement of environmental health (Kolossa-Gehring et al., 2023; Ganzleben et al., 2017). HBM4EU-MOM was designed and implemented in five coastal seafood-consuming European countries (Cyprus, Greece, Iceland, Portugal, Spain) and presents the main objectives: (a) to collect information about the basic characteristics of pregnant women in the five countries and lifestyle factors relevant to Hg exposures, (b) to evaluate the exposure of pregnant women to Hg during the first trimester of pregnancy and to investigate the associated factors with emphasis on seafood consumption, (c) to investigate the practices, attitudes and preferences of the women with regard to seafood consumption and to receive dietary information for pregnancy, (d) to design dietary recommendations for healthy seafood consumption during pregnancy emphasizing nutrition and Hg control, (e) to test the effect of the recommendations in a Randomized Control Trial, (f) to engage with the health-care providers of pregnant women, and (g) to communicate the results of the study to the participants, policy makers, health professionals, the wider public and the scientific community.

This work presents the study design, the harmonized procedures and materials developed in the frame of the study, as well as the characteristics of the participants, based on information collected during the first trimester of pregnancy. The results of Hg biomonitoring measurements and the impact of the dietary intervention will be presented in a different manuscript.

## 2. Methods

### 2.1. Study design

**HBM4EU-MOM** is a multicentre two-armed randomized controlled interventional trial implemented under the HBM4EU European partnership, with the overall aim to support “Methylmercury-contrOl in expectant Mothers through suitable dietary advice for pregnancy” (Fig. 1). The study was conducted between October 2020 and March 2022, involving research organisations from 11 European countries and recruited pregnant women (N = 654, gestational age  $\leq 20$  weeks) from 5 countries (described below). At baseline (Phase 1), each participant had to provide a hair sample for total mercury assessment (THg) assessment and complete a standardized questionnaire with personal information relevant to the study aims. Afterwards, the participants were randomized distributed 1:1 to a “control group” and an “intervention group” and were blinded to the allocation. The control participants received the standard pregnancy care provided in their country of residence, whereas the intervention participants additionally received the HBM4EU-MOM

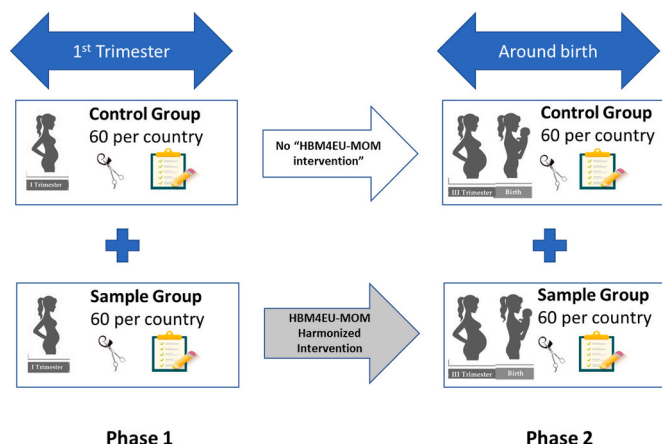


Fig. 1. The HBM4EU-MOM study design in a “nutshell”.

seafood consumption advice (which was tailored to the seafood species consumed nationally) and were encouraged to follow it. A second round of sampling (Phase 2) took place post-intervention, at the end of the pregnancy, with each participant providing a second hair sample and completing another standardized questionnaire. The questionnaires are described below and are provided in the Supplementary material.

### 2.2. Geographic coverage

HBM4EU-MOM harmonized and integrated five national cohorts from the following coastal countries in Europe: in the Southeast: Cyprus (CY) and Greece (EL); in the Southwest: Portugal (PT) and Spain (ES); in the Arctic: Iceland (IS) (Fig. 2). The participating countries were selected to represent different coastal geographic regions, with hypothesized high and distinct seafood consumption patterns.

### 2.3. Study population

Inclusion criteria for participation were: women in singleton pregnancy; aged between 18 and 45 years; gestational age of up to 20 weeks; absence of health problems or conditions; sufficient fluency in the language of the national cohort (alternatively in Greek or English in the case of Cyprus); residency in the country of the national cohort for at least three years prior to recruitment; not excluding seafood from their diet; and willingness and ability to provide two hair samples.

Based on power calculations, the minimum estimated sample size at European level was 600 participants, equally distributed among the control and intervention groups, which amounted to a minimum of 120 participants in each of the five countries.

### 2.4. Ethics and personal data protection

The study was approved by the competent national ethics committees prior to the initiation of the recruitment of participants in the study (Table S1). All five countries completed internal declarations of ethics (Knudsen et al., 2023) within the HBM4EU project and submitted copies of the national ethics approvals to the coordinators for uploading on the European Commission Participant Portal. All study procedures and

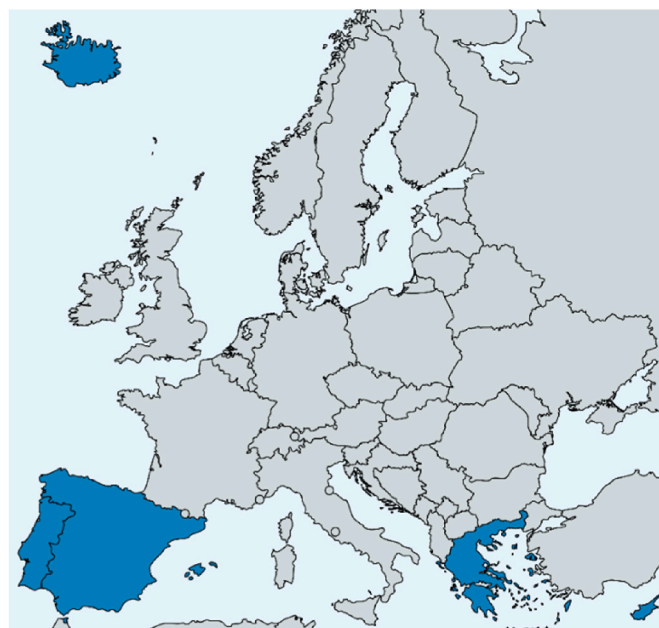


Fig. 2. HBM4EU-MOM harmonized and integrated national cohorts from five coastal, fish-consuming European countries (Cyprus and Greece in the Southeast; Portugal and Spain in the Southwest; Iceland in the Arctic).

materials (Table 1) complied with the General Data Protection Regulation of the European Union (GDPR). In IS, the study was further approved by the Icelandic Data Protection Agency. Informed consent was provided by all the pregnant women prior to fieldwork (e.g., completion of questionnaires and provision of hair samples). The certificates of informed consent included the provision to keep the hair samples in a biobank for a minimum period of two years.

## 2.5. Harmonization of procedures

A detailed protocol was developed based on the HBM4EU concepts (Fiddicke et al., 2015) and described the study characteristics (e.g., hypothesis, objectives, target population, recruitment strategy, biomarkers and analytical considerations, data management and statistical analysis, communication aspects, timeline). It is presented below and the harmonized materials developed for its implementation are presented in the supplement.

## 2.6. Questionnaires and communication materials

Tailored questionnaires were developed in the frame of the HBM4EU-MOM study for the purpose of collecting personal information from participants relative to the study aims. The questions assessed sociodemographic factors, pregnancy status, dietary practices before and during pregnancy with a dedicated section on seafood consumption (e.g., seafood species consumed, portions – standardized as “small”, “medium” or “large” with the aid of photographs, frequency, seasonality, preferences and barriers to eating seafood). Moreover, they assessed preferences related to receiving dietary advice for pregnancy, lifestyle, dental amalgams, as well as possible occurrences of incidents of breakage of mercury-containing products (thermometers, energy-saving lamps) and whether participants knew how to properly deal with such occurrences.

To ensure the correct correspondence of seafood species across the five countries, the Latin names and photographs of different species were examined. While the master files of the questionnaires included all the seafood species from all five countries, the targeted questionnaire of each country was adapted according to the species of relevance to their population (e.g., the ones that are commonly consumed at country level) (Table 1 and Supplementary material B\_Questionnaires).

The questionnaires were validated at European level through detailed discussions on each question among the partners. After verifying the harmonization of the national questionnaires in English, each country translated them into the corresponding national language(s) and validated them at national level through pilot testing. Instructions were elaborated for national fieldworkers, who received proper training. The countries were free to choose if they conduct the assisted interviews by completion of paper or electronic versions of the questionnaires.

Several tailored communication products were developed (Pack et al., 2023) to facilitate recruitment of participants and collection of

informed consent (e.g., information leaflets, invitation letters, certificates of informed consent, reply cards, withdrawal forms, personal reports, etc). These materials were elaborated in a harmonized way at European level, based on HBM4EU templates (Pack et al., 2023). Limited flexibility was allowed for country adaptations according to the national situation and needs, while ensuring that the harmonization and comparability of the results would not be compromised. After verifying the harmonization of the national materials in English, each country translated them into the corresponding national language(s). Examples of the communication materials in English are presented in Tables 1 and in the Supplementary material B\_Communication Material. The communication materials included a harmonized framework for reporting personal results to the participants. All participants were provided with a personalized letter at the end of the study with their results, unless they explicitly declared in their certificate of informed consent that they did not wish to be informed. The personalized reporting of results included the THg measurements of each participant with interpretative information, the HBM4EU-MOM advice on safe seafood consumption during pregnancy and breastfeeding and guidelines for the safe handling of a potential Hg spillage at home from broken items. The materials developed and used in the frame of the study for the dietary intervention will be presented in another manuscript (under preparation).

## 2.7. Recruitment

Pregnant women who fulfilled the predefined inclusion criteria were invited to participate in the study according to the procedures described in Table S2. The preferable model was to recruit via health care providers of the women during routine antenatal visits in clinics. Each country adjusted its recruitment procedures as necessary to achieve the recruitment targets within the short timelines and the complications presented by the COVID-19 pandemic (e.g., lockdowns, social distancing, precautionary isolation of pregnant women).

## 2.8. Sampling and chemical analysis

A ‘Train-the-Trainers’ model was applied to ensure harmonized, standardized and quality-assured sampling of hair in all countries.

Study leaders and designated trainers of each country participated in an online training workshop due to the restrictions imposed by the COVID-19 pandemic. A video and a standard operating procedure (SOP) with detailed instructions for the hair sampling were created by CNSA-ISCI team and made available to the national teams to use at country level. Subsequently, national training sessions of health care providers/fieldwork team members were organized as necessary in each country, to train the fieldwork team members and the engaged health-care providers. The national teams also received a SOP for hair sampling collection (Supplementary material B\_Hair Sampling SOPs) together with a harmonized ‘Hair Sampling Questionnaire’ (Table 1), to collect

**Table 1**  
Study material developed and used in the frame of the HBM4EU-MOM study.

No.	Title	Supplementary materialB
1	HBM4EUmom_Invitation LetterGeneral(1a) and ControlGroup (1 b)	Communication Material
2	HBM4EUmom_InformationLeaflet	Communication Material
3	HBM4EUmom_ReplyCardYes (a) and No(b)	Communication Material
4	HBM4EUmom_ReminderLetter	Communication Material
5	HBM4EUmom_Letterof ThanksIneligible	Communication Material
6	HBM4EUmom_PreVisitLetter	Communication Material
7	HBM4EUmom_InformedConsent	Communication Material
8	HBM4EUmom_WithdrawalDocument	Communication Material
9	HBM4EUmom_PersonalResults	Communication Material
10	HBM4EUmom_1stPhasedQuestionnaire	Questionnaires
11	HBM4EUmom_2ndPhasedQuestionnaire	Questionnaires
12	HBM4EUmom_HairSamplingQuestionnaire	Questionnaires
13	HBM4EUmom_StandardOperatingProcedureforHairSamplingCollection	Hair Sampling SOP

relevant information in a uniform way (e.g., hair colour, length, treatment with products, etc). Hair samples were stored in dark, at room temperature, with no other specific conditions prior to analysis.

The laboratory chosen to perform the chemical analyses of all HBM4EU-MOM hair samples was CNSA-ISCIII in Spain. For this purpose, the countries and the laboratory co-signed a bilateral Material-Data Transfer Agreement (MDTA), using an internal HBM4EU template (Knudsen et al., 2023; Pack et al., 2023). An accredited method was applied, using direct Hg analysis via thermal decomposition-gold amalgamation atomic absorption spectroscopy (DMA-80 Direct Mercury Analyzer, Milestone, USA) (Esteban et al., 2015). The laboratory personnel were unaware of the allocation of samples to control/intervention groups during their processing and analysis.

### 2.9. Data management and statistical analysis

All samples and data were pseudonymised before or immediately after their collection and before any other action by awarding a unique code to each participant according to the harmonized guidelines for coding developed. The personal identification information of the participants (name and contacts) was only accessible to the coordinators of each national cohort and specific researchers.

For each of the five HBM4EU-MOM cohorts, national databases were constructed using a harmonized methodology of encoding the data from the chemical analysis and questionnaires on excel data sheets. The national databases were encrypted, submitted via a dedicated web platform (developed in-house at VITO, Belgium) and subjected to quality control (QC). Each cleaned national database was pooled to a central European database, managed by VITO. The corresponding authorized data users were provided with individual encrypted extracts, including specific variables needed for the analysis, from the central European database. These export “views” were provided in excel format, together with the appropriate sample metadata in a separate worksheet.

Descriptive statistics included the calculation of percentages of women by various groups referring to the general characteristics of the study population (e.g., education level, pregnancy-related variables, smoking, having amalgam fillings and tattoos, diet-related variables, information on the diet during pregnancy, etc). General characteristics of the study population were stratified by country. The implementation of the statistical analysis was performed in R programming environment (R-Project, 2009).

## 3. Results and discussion

### 3.1. Harmonized approach and materials

To our knowledge, HBM4EU-MOM is the first international, multi-centre randomized control trial combining harmonized dietary interventions for safe seafood consumption in pregnancy with mercury biomonitoring of pregnant women. HBM4EU-MOM demonstrated the importance of a network of experts working closely together, developing and using standardized tools, which are fit for the purposes of quality assurance and harmonization of study protocols. The HBM4EU-MOM study was implemented in less than a year-and-a-half, which required highly effective time management and overall coordination, but still exerted a lot of pressure on the researchers. Because all the study materials needed to be available for submission to ethics committees, the time management for the preparatory phase was crucial for preventing unacceptable delays in the initiation of the fieldwork. A central unit to oversee the harmonization of the study was essential, so as to ensure the comparability of the results. At the same time, it was extremely important for the countries to have flexibility to adjust the implementation according to the national situations. The frame developed under HBM4EU (e.g. template communication materials and consent forms, sample transfer agreements, analytical and quality assurance tools, data transfer agreements, data management and statistical tools)

served as the basis for developing tailored tools. The HBM4EU-MOM approach and materials (Table 1, Supplementary material B) served their purpose well and they may contribute to the collection of comparable information in future studies. The feasibility of assessing the exposure of European women of reproductive age to mercury using harmonized assessment of THg in hair in different countries, was first demonstrated in the frame of the DEMOCOPHES pilot study (Castaño et al., 2015; Hond et al., 2015). Three of the five HBMEU-MOM countries (ES, PT, CY) participated in DEMOCOPHES and transferred valuable expertise to the study (Castaño et al., 2015; Hond et al., 2015). In fact, the lessons-learned and materials developed (e.g., SOP, sampling questionnaire and training material) in DEMOCOPHES were highly valuable, which proves the usefulness of this kind of harmonized and validated materials in the European HBM arena. Those materials also contributed to the development of the WHO protocol for human biomonitoring surveys for the assessment of prenatal exposure to Hg using THg in maternal hair of women who just gave birth (WHO, 2020). In the HBM4EU-MOM study design, the second phase of maternal hair samplings at the time of child delivery, resembles the WHO approach so that the results from the control participants could be comparable to those collected globally using the WHO protocol.

### 3.2. Recruitment, samplings and retainment of participants

The population of pregnant women is vulnerable and difficult to engage in research trials (Mary Dawn Koenig et al., 2022; Van Delft et al., 2022; MacLachlan et al., 2021). The HBM4EU-MOM study employed successful recruitment and retention strategies to achieve the recruitment targets within tight timelines during the COVID-19 pandemic, and to control attrition so that the intervention and the second sampling phase could be completed. Overall, 654 participants were recruited between January and September 2021, with all five countries managing to reach or exceed the recruitment target of 120 participants (Table 2).

The recruitment procedures applied by each country are presented in Table S2. Our study plan envisioned the engagement health care providers of pregnant women for the recruitment and hair samplings, so as to build on the relationship of trust and health care between the pregnant women and their providers, to create learning opportunities for the health professionals and to facilitate more sustainable use of the results. Despite the pressures exerted by the pandemic on the health care systems, four of the five countries (CY, EL, ES and IS) succeeded in involving health professionals in the study and the national study teams provided to them training, support, encouragement and supervision. The type of engaged health-care providers depended on the structure of the health-care systems of the countries and they were primarily medical doctors and/or midwives. This model of recruitment was very successful. In the case of PT, the direct engagement of health professionals in the study was not possible and this caused delays and the need to explore different strategies.

To various degrees, two more approaches were used for recruitment in the countries: Researcher-led recruitment, where the research team directly engaged with potential participants in the clinics or other venues, and self-referrals of interested pregnant women in response to study advertising in social and other communication media.

With regard to the geographic distribution of the samples within the countries, CY had a national geographical coverage, EL and PT had national representation, but with 63–64% of participants recruited in a specific area, and ES and IS had a regional geographical coverage (Table S2). None of the geographical areas were known hotspots for mercury. When several health professionals in different clinics and geographic regions could be employed, the recruitment and samplings were achieved faster, but the burden on the national study team was greater due to more complicated management requirements.

Because HBM4EU-MOM was designed as a RCT with a dietary intervention phase and a second phase of post-intervention samplings,

**Table 2**  
Basic characteristics of the study population.

	CY	EL	ES	IS	PT	All
<b>Characteristics</b>						
<b>Country/Region</b>	Cyprus	Greece	Spain/Fuenlabrada	Iceland/Reykjavik	Portugal	NA
<b>Number of participants (n)</b>	133	130	136	120	135	654
<b>Sampling Period</b>	February–May	January–July	March–August	May–October	June–November	January–November
<b>Age (mean, min-max)</b>	31(18–45)	33(24–43)	34(19–43)	30(20–42)	34(24–45)	32(18–45)
<b>BMI, mean (SD)</b>	23.7 (18.2, 39.7)	23.4 (18.6, 35.8)	24.7 (16, 41.1)	26.4 (17.2, 43)	23 (17.4, 38.5)	24.1 (16, 43)
<b>Residential degree of urbanization N(%)</b>						
Cities	45.9	86.9	83.8	0	75.6	58.4
Towns/Suburbs	16.5	4.6	0	100	20	28.2
Rural area	37.6	8.5	14.7	0	4.4	13.0
No Information	0	0	1.5	0	0	0.3
<b>Education level of the Participant (%)</b>						
Low (ISCED 0–2)	1.5	0	25	3.3	0.7	6.3
Medium (ISCED 3–4)	11.3	10.8	32.4	27.5	8.2	17.9
High (ISCED ≥5)	86.5	88.5	42.7	52.5	91.1	72.5
No Information	0.8	0.8	0	16.7	0	3.4
<b>Education level of the Participant's partner (%)</b>						
Low (ISCED 0–2)	5.3	3.9	32.4	8.3	3	10.7
Medium (ISCED 3–4)	34.6	15.4	30.2	25.8	23.7	26
High (ISCED ≥5)	59.4	77	35.3	43.3	73.3	57.8
No Information	0.8	3.9	2.2	22.5	0	5.5
<b>First-time pregnant (%)</b>	54.1	60.8	51.5	35	51.9	50.9
No Information	0.8	0	0	16.7	0	3.2
<b>Prior pregnancies (%)</b>						
one	31.6	28.5	36	30	31.9	31.7
two	12	10.8	8.1	10.8	9.6	10.2
three	0	0	0.7	2.5	0	0.6
four	0	0	0.7	0.8	0	0.3
five	0.8	0	0	0	0	0.2
six	0.8	0	0	0	0	0.2
<b>Planned pregnancy (%)</b>						
No	39.8	26.2	16.2	14.2	8.9	21.1
Yes	58.6	62.3	81.6	68.3	91.1	72.6
No Information	1.5	11.5	2.2	17.5	0	6.3
<b>Active smoker before pregnancy (%)</b>						
No	57.9	63.8	62.5	81.7	85.2	70
Yes	41.4	35.4	35.3	2.5	14.8	26.3
No Information	0.8	0.8	2.2	15.8	0	3.7
<b>Passive smoker before pregnancy (%)</b>						
No	47.4	77.7	64	80.8	49.6	63.5
Yes	51.9	21.5	34.6	2.5	50.4	32.9
No Information	0.8	0.8	1.5	16.7	0	3.7
<b>Active smoker during pregnancy, %</b>						
No	89.5	89.2	83.1	82.5	96.3	88.2
Yes	9.8	9.2	16.2	1.7	3.7	8.3
No Information	0.8	1.5	0.7	15.8	0	3.5
<b>Passive smoker during pregnancy (%)</b>						
No	74.4	83.8	66.2	83.3	60.7	73.4
Yes	24.8	15.4	32.4	0.8	39.3	23.1
No Information	0.8	0.8	1.5	15.8	0	3.5
<b>Amalgam fillings %</b>						
No	71.4	59.2	75	57.5	76.3	68.2
Yes	24.1	40.8	22.8	18.3	22.2	25.7
No Information	4.5	0	2.2	24.2	1.5	6.1
<b>Last placed %</b>						
Less than 4 months	0	1.9	3.2	0	0	1.2
4–12 months	0	0	3.2	0	0	0.6
more than a year	100	98.1	93.5	100	100	98.2
<b>Last removed %</b>						
Less than 4 months	0	0	6.5	0	3.3	1.8
4–12 months	3.1	1.9	0	0	0	1.2
more than a year	43.8	98.1	38.7	59.1	46.7	62.5
<b>Tattoo, %</b>						
No	61.7	73.8	38.2	40	68.9	56.7
Yes	37.6	26.2	61.8	41.7	31.1	39.8
No Information	0.8	0	0	18.3	0	3.5

measures needed to be proactively employed to ensure that a sufficient number of participants would remain engaged with the study until its completion. To begin with, the national research teams tried to recruit more participants than the recruitment goal of 120 pregnant women per country. To retain the interest and engagement of participants and to

encourage intervention participants to consume seafood according to the provided dietary advice, the countries used different approaches, such as free webinars and other means of providing information and guidance to participants related to pregnancy, lactation, nutrition and parenting. An attrition of less than 8% was observed and it was

attributed to occasional incidents of miscarriages and health-issues or loss of interest in the study. A very small number of participants did not provide the second hair sample because of time considerations and precautions due to the spread of COVID-19. This attrition is small and does not cause concerns for bias (Dumville et al., 2006; Close et al., 2016; Babic et al., 2019). The reasons for the observed attrition are comparable to those reported in other studies (Close et al., 2016).

### 3.3. General characteristics of the participants at baseline

The general characteristics of the study population are described in Table 2. The average age of the participants was 32 years and was similar across the five countries [range: 31–34]. The youngest participant was 18 years old and the oldest one was 45. Most participants (59%) resided in cities, 28% lived in towns and suburbs, while 13% lived in rural areas.

#### 3.3.1. Educational level

Most participants (72%) were highly educated (attained high level tertiary education of ISCED  $\geq 5$ ), while 18% attained upper secondary to post-secondary non-tertiary education (ISCED 3–4). Only 6.3% had a low education level (ISCED 0–2) (Table 2, Fig. 3). The distribution of the educational levels of the participants' partners was wider, with 58% having a high education level (ISCED  $\geq 5$ ), 26% a medium level (ISCED 3–4) and 11% a low education level (ISCED 0–2). The spread of educational levels of HBM4EU-MOM participants and their partners was compared to that of the general European population aged 18–45 years, as reported by EUROSTAT for the year 2021 (EUROSTAT, 2022). Overall, the HBM4EU-MOM population is skewed to higher educational levels. The overrepresentation of highly educated participants was most pronounced in PT, EL and CY. In the case of ES, there was better representation of low and medium education levels than in the other countries. Previous studies have shown that mercury exposures are higher in people of higher educational levels, putatively because of higher seafood consumption (WHO, 2019). Such possible associations will also be explored for HBM4EU-MOM participants.

#### 3.3.2. Diet

Maternal pre-pregnancy body mass index is a measure of maternal health and nutrition, which is the key to meeting the nutrient demands of pregnancy and vital for foetal development and infant health. Both high and low pre-pregnancy body mass index (BMI) have been associated with suboptimal foetal growth and risk of pregnancy complications (Gudipally et al., 2023; Zong et al., 2022; Tang et al., 2021). Several studies of European women show an increasing trend in maternal obesity incidence over time (Heslehurst et al., 2007). Maternal pre-pregnancy BMI is classified (Zong et al., 2022) as underweight ( $<18.5 \text{ kg/m}^2$ ), normal weight ( $18.5\text{--}24.9 \text{ kg/m}^2$ ), overweight

( $25.0\text{--}29.9 \text{ kg/m}^2$ ) or obese ( $\geq 30 \text{ kg/m}^2$ ). The average pre-pregnancy BMI of HBM4EU-MOM participants was within the healthy range and so were the national averages of CY, EL, ES and PT (Table 2), but the average BMI value in IS was within the overweight category. It is also noteworthy that in all countries except for EL, there were participants who were underweight and that in all five countries there were obese participants. These results indicate a need in all the participating countries to better inform women about the importance of maintaining a healthy BMI when planning for pregnancy.

Because the maternal diet during the periconceptional period and pregnancy is vital for the health of both the mother and the child (Rodríguez-Bernal et al., 2013), the WHO (WHO, 2001) and many national governments issue dietary guidelines for pregnant women (WHO, 2001; Linou, 2014). More than half of the HBM4EU-MOM participants reported that they made dietary changes for their pregnancy (Fig. 4). Despite the fact that most of the women said that their pregnancy was planned (Table 2), the timing of the changes for most women was when they found out that they were pregnant (Table 3). Very few women said that they made dietary changes before they got pregnant. The women who did not make any dietary changes for the pregnancy mostly believed that their diet was already balanced, while a few found it difficult to make changes or did not know what changes to make. These results indicate that more attention should be given to awareness raising and education of women about the importance of their diet for both nutrition and exposure control. Ideally, women should prepare themselves for a healthy pregnancy before conception. Several food contaminants are eliminated very slowly from the body and therefore changing the diet at the onset of pregnancy will not prevent the embryo from getting exposed during a critical developmental phase. This is the case for MeHg, whose half-life in seafood-eating humans is estimated to be between 39 and 70 days (Gad, 2014; WHO, 1990).

According to data of the Food and Agriculture Organization of the United Nations (FAO, 2022), three of the five countries participating in HBM4EU-MOM are among the top per-capita consumers of seafood in the world. In 2019, the estimated average per-capita consumption of aquatic food worldwide was 20.5 kg/capita/year and in Europe was 21.1 kg/capita/year. High- and low-income countries exceeded these averages (26.5 and 28.1 kg/capita/year, respectively), whereas lower-middle and low-income countries were below the averages (15.2 and 5.4 kg/capita/year, respectively). IS was the top consumer at 91.2 kg/capita/year, PT was third at 57.2 kg/capita/year and ES was sixth at 42.3 kg/g. EL and CY have lower consumption than the other three countries, but remain among the top seafood consumers in Europe.

HBM4EU-MOM results showed that seafood was consumed by the participating women at almost constant frequency (7.9 times/month or 1.8 times/months) both before and during pregnancy, in all five countries. Mild changes are noted in the before/during pregnancy average consumption frequencies within each country, but at the level of individual participants, a range of behaviours is observed (Fig. 5). As

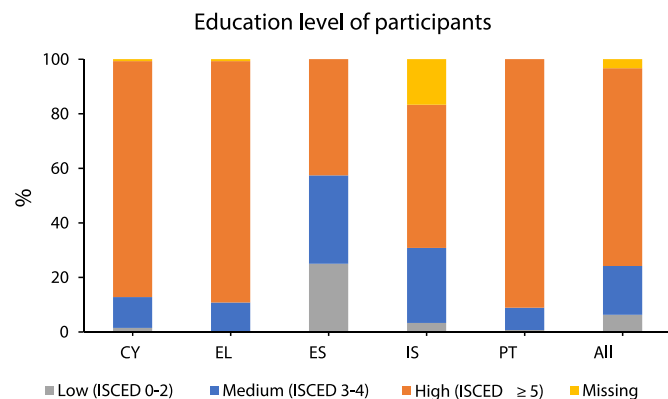


Fig. 3. Educational level of the participants, showing that most of the recruited women are highly educated (ISCED  $\geq 5$ ).

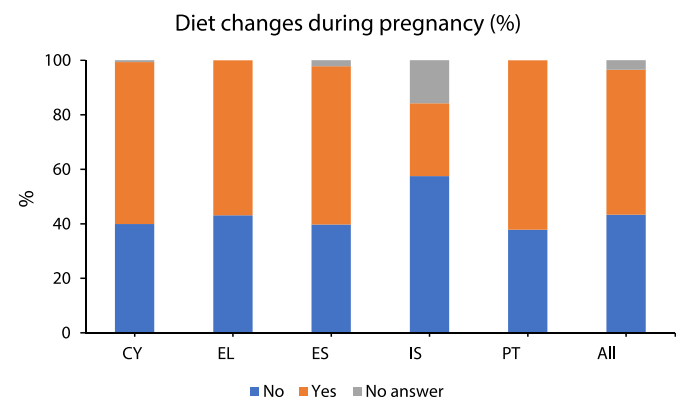
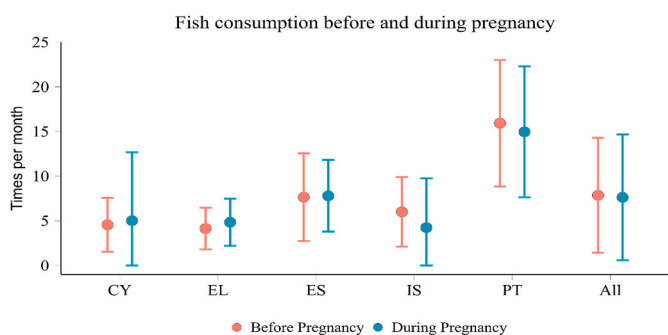


Fig. 4. Dietary changes made by the participants for the pregnancy.

**Table 3**  
Dietary changes made for the pregnancy by the study population (N = 654).

	CY	EL	ES	IS	PT	All
<b>Made dietary changes for this pregnancy (%)</b>						
No	39.9	43.1	39.7	57.5	37.8	43.3
Yes	59.4	56.9	58.1	26.7	62.2	53.2
No answer	0.8	0.0	2.2	15.8	0.0	3.5
<b>If yes, when (%)</b>						
3 months before the pregnancy	3.8	1.4	3.8	6.3	2.4	3.2
1–3 months before the pregnancy	2.5	14.9	2.5	6.3	1.2	5.2
Just before the pregnancy	2.5	9.5	11.4	0.0	11.9	8.1
When I learned I was pregnant	70.9	68.9	76.0	84.4	77.4	74.4
Refuse to answer	0.0	1.4	0.0	0.0	0.0	0.3
Other	20.3	4.1	6.3	3.1	7.1	8.9
<b>If no, why (%)</b>						
It is difficult to make changes	3.9	8.9	9.3	–	2.0	6.1
My diet was already balanced	59.6	71.4	83.3	–	82.4	74.2
I did not think I had to make any changes	23.1	16.1	5.6	–	13.7	14.6
I did not know what changes to make	5.8	0.0	0.0	–	2.0	1.9
Refuse to answer	1.9	0.0	0.0	–	0.0	0.5
Other	5.8	3.6	1.9	–	0.0	2.8



**Fig. 5.** Self-declared frequency of seafood consumption (in times per month) before and during the pregnancy, for each country. ‘All’ refers to the pooled samples from all countries. The circles present the mean consumption frequency, the vertical lines present the range of values and the T-bars indicate the minimum and maximum frequencies.

hypothesized, the frequency of consumption presents high geographic variability. Portuguese participants reported the highest average frequency of consumption both before (15.9 times/month or 3.7 times/week) and during pregnancy (15.0 times/month or 3.5 times/week) and some of the Portuguese women consumed seafood more than 5 times per week. Spanish participants presented the second highest average consumption, at 1.8 times per week both before and during pregnancy. It is noteworthy that despite the very high per capita seafood consumption in IS, Icelandic women consumed seafood at 1.4 times per week before the pregnancy and this dropped to an average consumption of 1.0 times per week during the pregnancy. The Greek and Cypriot participants had lower and similar consumption frequencies (around 1 time/week), both before and during the pregnancy.

The concentration of MeHg can vary considerably across different species of seafood and their habitats. Likely, the nutritional value of seafood presents high variability. Smaller fish, at the lower level of food webs, usually contain more unsaturated fatty acids and lower MeHg concentrations. Large and predatory fish usually contain less nutritional value and higher concentrations of MeHg. For this reason, suitable advice for seafood consumption must contain recommendations of species to prefer or avoid, in addition to frequency of consumption. The HBM4EU-MOM dietary intervention was based on this premise and in agreement with EFSA’s statement on the benefits of seafood consumption compared to the risks of methylmercury in seafood (EFSA, 2015). According to EFSA, pregnant women would have to consume 1–4

servings of seafood to reach the nutritional benefits based on the dietary reference value (DRV) of n-3 long-chain polyunsaturated fatty acids (PUFAs). However, if seafood species with high methylmercury content are consumed, the tolerable weekly intake (TWI) for MeHg can be reached with <1–2 servings and before attaining the DRV. Because of the variety of seafood species consumed across Europe, EFSA recommended that each country considers the national species consumed for the development of appropriate recommendations.

The types of seafood consumed by the pregnant women participating in HBM4EU-MOM over the four months before joining the study (e.g., right before conception and at early gestation) are presented in Table 4. It is notable that big oily fish are consumed in all countries and collectively by 62% of the participants. The highest prevalence is in PT (consumed by 89%), followed by ES (85%), with the other three countries trailing at <50%. The most consumed category in all countries was that of the white fish. These results indicate that women of childbearing age need to be better informed about including seafood in their diet in a safe way.

### 3.3.3. Non-dietary sources of exposure to Hg

Pregnant women and their fetuses may get exposed to other forms of Hg from non-dietary sources, such as accidental spills from mercury-containing products (Sarigiannis et al., 2012) or placement/removal of dental amalgams. The occurrence of dental amalgam fillings in the pregnant women are presented in Table 2. The highest prevalence presented was in EL (41%), followed by a range of 22–24% in CY, ES, PT, and the lowest one in IS (18%). In line with the commitments to the Minamata Convention on Mercury, the Regulation (EU) 2017/852 (Minamata, 2019) on mercury states that amalgam should not be used on pregnant women except when deemed strictly necessary by the dental practitioner based on the specific medical needs of the patient. It is noteworthy however, that a small number of participants had dental amalgams placed (1%) or removed (2%) during the peri-pregnancy period (within 4 months from their participation in HBM4EU-MOM). This indicates a need to raise the awareness of dentists and pregnant women.

Citing Hg toxicity, the European Union is phasing out general-purpose fluorescent lighting across Europe in 2023 in the frame of the Restriction of Hazardous Substances (RoHS) Directive. Thermometers containing Hg have been banned in the European Union since 2009 (REACH, 2009). Our findings, however, show that several European pregnant women still experience occurrences of breakage of these products and that they are not aware of safe procedures to respond to such an incident (Table 5). Because people may have both types of products stocked away or in use. Thus, it is important to continue raising awareness to safety guidelines for proper disposal and response to accidental spillage.

### 3.3.4. Other possible chemical exposures

Maternal exposure to tobacco smoke during pregnancy has been

**Table 4**  
Categories of seafood consumed by women during the 4 months preceding baseline sampling, which took place in the first-trimester of pregnancy.

	CY	EL	ES	IS	PT	ALL
<b>White Fish Consumption %</b>						
Yes	83.5	94.6	96.3	75.8	100	90.4
No	15.8	5.4	3.7	6.7	0	6.3
<b>Small oily Fish Consumption %</b>						
Yes	51.1	89.2	83.8	55.8	93.3	75.1
No	48.1	10.8	15.4	25.8	6.7	21.3
<b>Big oily Fish Consumption %</b>						
Yes	48.1	43.8	85.3	42.5	88.9	62.4
No	51.1	56.2	14.7	38.3	11.1	33.9
<b>Other Sea Food Consumption %</b>						
Yes	89.5	90	92.6	0	94.1	74.8
No	9.8	10	7.4	0	5.2	6.6



**Table 5**

Occurrences of broken mercury thermometers and energy-saving bulbs within participants' residences and self-reported knowledge of appropriate response measures (data collected at baseline sampling, which took place in the first-trimester of pregnancy).

	CY	EL	ES	IS	PT	All
<b>Broken mercury-containing thermometer (%)</b>						
Do not know	1.5	0	1.5	29.2	5.9	7.2
No	87.2	60.8	75.7	62.5	66.7	70.8
Yes	11.3	39.2	22.8	8.3	27.4	22
Yes/Less than 1 year ago	0	4.6	0.7	0.8	0.7	1.4
Yes/More than 1 year ago	11.3	33.8	22.1	7.5	26.7	20.5
Able to react	9	24.6	2.9	0.8	8.1	9.2
<b>Broken energy-saving lamp (%)</b>						
Do not know	4.5	0	4.4	37.5	14.8	11.8
No	85	96.2	83.4	59.2	674	78.4
Yes	10.5	3.8	12.5	3.3	17.8	9.8
Yes/Less than 1 year ago	1.6	1.6	2.9	0	1.5	1.5
Yes/More than 1 year ago	9	2.3	9.6	3.3	16.3	8.3
Able to react	6	0	0.7	0.8	16.3	4.9

linked to several adverse health effects for both the fetus and the mother. As a result of awareness raising campaigns and regulations, a general reduction in cigarette was achieved worldwide. Surprisingly, however, many pregnant women continue to smoke and many more remain exposed to second hand smoke (Beck et al., 2023; Lange et al., 2018). Overall, 26% of HBM4EU-MOM participants were smokers before the pregnancy and 8.3% continued to smoke during the pregnancy, with the highest prevalence observed in Spain (16%). This prevalence is higher than the 8.1% reported in Europe by Bednarczuk et al. (2020). A significant percentage of women (23%) remained exposed to second-hand smoke during pregnancy, ranging from 39% in Portugal, 32% in Spain, 25% in Cyprus and 15% in Greece to only 0.8% in Iceland. These data suggest that further efforts are needed in the four Southern European countries to prevent fetal and maternal exposure to tobacco smoke.

The HBM4EU-MOM study provided an opportunity to collect information from pregnant women about lifestyle practices, which may lead to various chemical exposures.

Many participants (40% overall) reported that they had a tattoo, ranging from 26% in EL to 62% in ES. This is a relatively underexplored exposure source, which may be linked to exposures to various chemical substances, such as polycyclic aromatic hydrocarbons, primary aromatic amines and metals (Negi et al., 2022). Collectively, 28% of the women had their hair dyed within 3 months from sampling (Table S3). This practice varied geographically, ranging on average from 0% in IS to 47% in ES. A natural hair dye was used most frequently in CY and EL (77% and 82%, respectively), whereas in ES and PT a chemical one was often used (70% and 81%, respectively). Only few participants from CY (2.3%), ES (1.5%) and PT (0.7%) underwent a chemical hair structure treatment within three months from the sampling of hair. The safety of many of the substances used in hair dyes is assessed by the expert panel's Cosmetic Ingredient Review. Although many substances are considered safe for users at the levels used in hair dyes, there are conflicting data on a large number of formulations (He et al., 2022).

Participants from all five countries engaged with gardening involving the use of fertilizers or pesticides (overall mean: 8%; range of national means: 2–12%) and with activities involving paints, pigments or dyes (overall mean: 19%; range of national means: 9–24%). Activities such as leather tanning, recycling of electrical parts and smelting, welding and soldering were performed by only a negligible number of participants (Table S4).

HBM studies can support policy decisions for safer chemicals management and public health protections. The engagement of pregnant women and their health care providers in studies such as HBM4EU-MOM also creates opportunities for disease prevention and improved health through personalized actions. However, our experiences show that the public and the medical sector should be better educated about

human biomonitoring as a force in citizen empowerment and health promotion. Enhancing this understanding will also facilitate enrolment in HBM studies.

#### 4. Conclusions

The HBM4EU-MOM study developed and made available validated harmonized procedures and materials for the assessment of prenatal exposures to mercury and the associated factors in different countries. The harmonized information collected regarding the basic characteristics of pregnant European women and their lifestyle practices, suggests the following: (a) it is important to raise the awareness of women of reproductive age and pregnant women about how to safely include fish in their diet and to empower them to make proper decisions for nutrition and control of MeHg, (b) prenatal and women's exposure to tobacco smoke remains a concern in Europe and targeted awareness campaigns are necessary for prevention and control, (c) more generally, women of reproductive age and pregnant women must be better informed about lifestyle choices to prevent harmful chemical exposure and about the importance of maintaining a healthy BMI when planning for pregnancy.

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#### Author contributions

Conceptualization: A.K., C.G., D.S., M.E.L., T.I.H., S.N., Methodology: A.K., C.G., D.S., S.K., M.E.L., A.C., T.I.H., S.N., R.A. Formal analysis: A.K., C.G., J.S.J., A.K. (Achilleas Karakoltzidis), Writing-Original draft: A.K., C.G., A.C. Writing—review and editing: A.K., C.G., M.E.L., S.N., T. I.H., L.R.M., J.S.J., A.K. (Achilleas Karakoltzidis), A.C., D.G., N.D-M., M. L., B.G.-A., I.I., M.H., A.C. (Argelia Castano), M.K.-G. Visualization: A.K., C.G., J.S.J., A.K. (Achilleas Karakoltzidis), Supervision: A.K.

All authors have read and agreed to the published version of the manuscript.

#### Data availability statement

Not applicable.

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#### Abbreviations

BMI	Body Mass Index
BE	Belgium
BP	Before pregnancy
CNSA-ISCI	National Centre for Environmental Health-Instituto de Salud Carlos III
CY	Cyprus
DRV	Dietary Reference Value

DP	During pregnancy
3-DHA	3-docosaehaenoic acid
EC	European Commission
EFSA	European Food Safety Authority
EL	Greece
ES	Spain
GM	Geometric Mean
HBM	Human Biomonitoring
HBM4EU-MOM	“Methylmercury-control in expectant mothers through suitable dietary advice for pregnancy”
IS	Iceland
ISCED	International Standard Classification of Education
MOH-CY	Ministry of Health -Cyprus – State General Laboratory
PT	Portugal
PUFA	Polyunsaturated Fatty Acids
RCT	Randomized control trial
RoHS	Restriction of Hazardous Substances
QA/QC	Quality Assurance/Quality Control
SOP	Standard Operating Procedure
THg	Total mercury
TWI	Tolerable Weekly Intake
VITO	Vlaamse Instelling voor Technologisch Onderzoek- Flemish Institute for Technological Research
WHO	World Health Organization

## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ijh.2023.114213>.

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