Fish consumption patterns and hair mercury levels in children and their mothers in 17 EU countries

Argelia Castaño [a,*], Francisco Cutanda [a], Marta Esteban [a], Peter Pärt [b], Carmen Navarro [a],
Silvia Gómez [a], Montserrat Rosado [a], Ana López [a], Estrella López [c], Karen Exley [d],
Birgit K. Schindler [e], Eva Govarts [f], Ludwine Casteleyn [g], Marike Kolossa-Gehring [h],
Ulrike Fiddicke [h], Holger Koch [e], Jürgen Angerer [e], Elly Den Hond [f], Greet Schoeters [f],
Ovnair Sepai [d], Milena Horvat [i], Lisbeth E. Knudsen [j], Dominique Aerts [k], Anke Joas [l],
Pierre Biot [k], Reinhard Joas [l], José A. Jiménez-Guerrero [a], Gema Diaz [a], Catherine Pirard [m],
Andromachi Katsonouri [n], Milena Cerna [o], Arno C. Gutleb [p], Danuta Ligocka [q],
Fátima M Reis [r], Marika Berglund [s], Ioana-Rodica Lupsa [t], Katarína Halzlová [u],
Corinne Charlier [m], Elizabeth Cullen [v], Adamos Hadjipanayis [w], Andrea Krsková [o],
Janne F Jensen [j], Jeanette K Nielsen [j], Gerda Schwedler [h], Michael Wilhelm [x], Peter Rudnai [y],
Szilvia Középesy [y], Fred Davidson [z], Mark E. Fischer [aa], Beata Janasik [q], Sónia Namorado [r],
Anca E. Gurzau [t], Michal Jajcaj [u], Darja Mazej [i], Janja Snoj Tratnik [i], Kristin Larsson [s],
Andrea Lehmann [ab], Pierre Crettaz [ab], Giagkos Lavranos [n], Manuel Posada [c]

[a] Environmental Toxicology, Centro Nacional de Sanidad Ambiental (CNSA), Instituto de Salud Carlos III(ISCIII), 28220 Majadahonda, Madrid, Spain
[b] European Commission, Joint Research Centre (JRC), Institute of Environment and Sustainability, 21027 Ispra, Italy
[c] Instituto de Investigacion de Enfermedades Raras (IIER) Instituto de Salud Carlos III (ISCIII), Madrid, Spain
[d] Public Health England, Centre for Radiation, Chemical and Environmental Hazards, Chilton, United Kingdom
[e] Institute for Prevention and Occupational Medicine of the German Social Accident Insurance-Institute of the Ruhr-Universität Bochum (IPA), Germany
[f] Environmental Risk and Health, Flemish Institute for Technological Research (VITO), Mol, Belgium
[g] University of Leuven, Leuven, Belgium
[h] Federal Environment Agency (UBA), Dessau-Rosslau, Berlin, Germany
[i] Department of Environmental Sciences, Jožef Stefan Institute, Ljubljana, Slovenia
[j] Departament of Public Health, University of Copenhagen, Copenhagen, Demark
[k] DG Environment, Federal Public Service Health, Food Chain Safety and Environment, Brussels, Belgium
[l] BiPRO, Munich, Germany
[m] CHU of Liege, Laboratory of Clinical, Forensic and Environmental Toxicology, Liege, Belgium
[n] State General Laboratory, Nicosya, Cyprus
[o] National Institute of Public Health, Prague, Czech Republic
[p] Luxembourg Institute of Science and Technology (LIST) Louxembourg
[q] Nofer Institute of Occupational Medicine, Lodz, Poland
[r] Lisbon Faculty of Medicine, Lisbon, Portugal
[s] Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden
[t] Environmental Health Center, Cluj-Napoca, Romania
[u] Public Health Authority of the Slovak Republic (UVZ SR), Bratislava, Slovak Republic
[v] Department of Community of Health, Health Service Executive, Kildare, Ireland
[w] Larnaca General Hospital, Larnaca, Cyprus
[x] Department of Hygiene, Social and Environmental Medicine, Ruhr-University Bochum, Germany
[y] National Institute of Environmental Health, Budapest, Hungary
[z] Public Analyst's Laboratory Health Service Executive, Cork, Ireland
[aa] Laboratoire National de Santé, Dudelange, Luxembourg
[ab] Federal Office of Public Health (FOPH), Berne, Switzerland

A B S T R A C T

The toxicity of methylmercury (MeHg) in humans is well established and the main source of exposure is via the consumption of large marine fish and mammals. Of particular concern are the potential neuro-developmental effects of early life exposure to low-levels of MeHg. Therefore, it is important that pregnant women, children and women of childbearing age are, as far as possible, protected from MeHg exposure.

Within the European project DEMOCOPHES, we have analyzed mercury (Hg) in hair in 1799 mother–child pairs from 17 European countries using a strictly harmonized protocol for mercury analysis. Parallel, harmonized questionnaires on dietary habits provided information on consumption patterns of fish and marine products. After hierarchical cluster analysis of consumption habits of the mother–child pairs, the DEMOCOPHES cohort can be classified into two branches of approximately similar size: one with high fish consumption (H) and another with low consumption (L). All countries have representatives in both branches, but Belgium, Denmark, Spain, Portugal and Sweden have twice as many or more mother–child pairs in H than in L. For Switzerland, Czech Republic, Hungary, Poland, Romania, Slovenia and Slovakia the situation is the opposite, with more representatives in L than H.

There is a strong correlation ($r=0.72$) in hair mercury concentration between the mother and child in the same family, which indicates that they have a similar exposure situation. The clustering of mother–child pairs on basis of their fish consumption revealed some interesting patterns. One is that for the same sea fish consumption, other food items of marine origin, like seafood products or shellfish, contribute significantly to the mercury levels in hair. We conclude that additional studies are needed to assess and quantify exposure to mercury from seafood products, in particular. The cluster analysis also showed that 95% of mothers who consume once per week fish only, and no other marine products, have mercury levels 0.55 µg/g. Thus, the 95th percentile of the distribution in this group is only around half the US-EPA re-commended threshold of 1 µg/g mercury in hair. Consumption of freshwater fish played a minor role in contributing to mercury exposure in the studied cohort.

The DEMOCOPHES data shows that there are significant differences in MeHg exposure across the EU and that exposure is highly correlated with consumption of fish and marine products. Fish and marine products are key components of a healthy human diet and are important both traditionally and culturally in many parts of Europe. Therefore, the communication of the potential risks of mercury exposure needs to be carefully balanced to take into account traditional and cultural values as well as the potential health benefits from fish consumption. European harmonized human biomonitoring programs provide an addi-tional dimension to national HMB programs and can assist national authorities to tailor mitigation and adaptation strategies (dietary advice, risk communication, etc.) to their country's specific requirements.

1. Introduction

Lifestyles and particularly the diet play a crucial role in personal exposure to environmental chemicals. Exposure to methylmercury (MeHg) is a clear example as the general population is exposed to this metal compound through consumption of fish and other pro-ducts from the aquatic environment (Schoeman et al., 2009).

Mercury is a ubiquitous heavy metal, naturally present in the environment but human activities have increased its concentra-tion in the environment about three-fold over the last century (Mason et al., 2012; Lamborg et al., 2014). In aquatic ecosystems, mercury is transformed to its organic form, MeHg, which is more bioavailable and bioaccumulates in aquatic food chains to reach the highest concentrations in the upper trophic levels. In October 2013, the Minamata Convention under the auspices of the United Nations Environment Program (UNEP), a global action to protect human health and the environment from anthropogenic emissions and releases of mercury and mercury compounds, was signed (http://www.mercuryconvention.org). Although mercury emis-sions will be reduced when this treaty becomes fully operative, old mercury releases in deposits (e.g. in soil, sediments, ice) will still be mobilized and become biologically available. Therefore, old "legacy" contamination will contribute to the current mercury levels in the environment and consequently determine human exposure for many years to come (Selin, 2009).

The neurotoxicity of MeHg in humans is well established. The vulnerability of the developing fetus to MeHg exposure resulted in extreme fetal neurotoxicity as first described for Minamata, Japan, in 1956 (Kurland et al., 1960; Yorifuji et al., 2013). In Minamata, the exposure levels were high. Later, several large-scale epidemiolo-gical studies (New Zealand, the Seychelles and Faroe Islands) have shown that even low-level exposure to MeHg during early life stages could interfere in neural development leading to cognitive malfunction (reduction in IQ, attention deficit disorder) later in life (Kjellstrom et al., 1986; Julshamn et al., 1987; Myers et al., 2003). On the other hand, negative effects of contaminants could be counter-balanced by the positive effects of healthy nutrients in fish and marine products (Karagas et al., 2012; Choi et al., 2014). This highlights the importance of balancing the risks and benefits of fish consumption.

Since the symptoms of methylmercury exposure are subtle and multi-causal, there is still no consensus on a heath-based guidance value for MeHg exposure despite the large number of recent studies trying to connect low exposure levels to actual risk (Schoeman et al., 2009; Karagas et al., 2012; Valent et al., 2013). However, there is a general recommendation that pregnant women, children and women of childbearing age should be protected as much as possible from mercury exposure. Therefore, it is important to know what the actual exposure is to MeHg in the general population and what the sources of exposure are in order to formulate adequate mitigation strategies and recommendations.

* Corresponding author.
  E-mail address: castano@isciii.es (A. Castaño).

Fig. 1. Map with the urban (●) and rural (X) sampling locations in the 17 European implementing countries of DEMOCOPHES.

The European project DEMOCOPHES (DEMOnstration of a study to COordinate and Perform Human biomonitoring on a European Scale) was initiated to demonstrate how harmonized HBM tools developed in the European project COPHES (Consortium to Perform Human biomonitoring in a European Scale) could be applied in Europe to provide baseline information on selected contaminant levels in the European population. Mercury was one of the contaminants included and here we give an insight into mercury levels, based on hair measurements, of children and their mothers in 17 European countries measured under strictly standardized and harmonized conditions (Esteban et al., 2015). Our results build on analysis of total mercury in hair, which is significantly cheaper than analysis of methylmercury. For hair analysis, total mercury is generally accepted as a good proxy for MeHg exposure (Harkins and Susten, 2003).

As shown before, DEMOCOPHES results confirmed the clear correlation between consumption of fish and marine products and mercury levels in hair (Den Hond et al., 2015). However, since the habits and frequency of fish and marine products consumption show great variation among the participating European countries, and since the study applied harmonized questionnaires and analytical procedures, we used this unique opportunity for a more detailed analysis of the relationship between dietary patterns and mercury exposure. In addition, the fact that the study included mother–child pairs enabled us to analyze how adult food habits and related exposure influences children's exposures in the same family. This type of information is pivotal for health and food safety authorities when developing dietary recommendations for young families, and managing the delicate balance between the assumed health benefits of a diet rich in fish and the potential negative effects of an increased burden of environmental contaminants including mercury.

2. Materials and methods

DEMOCOPHES was a pilot study involving 17 European countries (Fig. 1). The aim of the project was to test the feasibility of a harmonized human biomonitoring approach; testing protocols developed in the EU project COPHES (Joas et al., 2012).

2.1. Harmonized tools

2.1.1. The European protocol

Based on the European study protocol, countries developed their national protocols with only minor adaptations so as not to jeopardize the comparability of the results. The study design has been described previously by Becker et al. (2014). In summary, 120 mother–child pairs, up to 45 years and from 6 to 11 years, respectively, in each country (except Cyprus and Luxembourg which were expected to contribute with 60 pairs per country because of their smaller population size) were to be recruited from two locations (Fig. 1) representing the upper and lower degree of urbanization (urban and rural), via inhabitant registries or schools. Participants were recruited between September 2011 and February 2012 and had been living for the 5 last years or more in the same location. Volunteers living in hospitals or institutions, who were homeless, or presenting metabolic disturbances (e.g. diabetes, nephritic syndrome or porphyria) or abnormal urine excretions (creatinine values < 0.3 g/L or > 3 g/L) were excluded (WHO, 1996).

2.1.2. Samples and data collection

Hair samples were collected according to the instructions detailed in the Standard Operation Procedure (SOP) included in the study protocol (Esteban et al., 2015). A trained field worker conducted the interviews of the mothers based on validated structured questionnaires. Using different reverse/independent translators the quality of translations of the questionnaire into national languages was controlled. The questions were categorized into following sections: residence environment, nutrition, smoking behavior, occupation and socio-demography. The mothers answered all questions including those concerning the consumption of fish and marine products for both herself and her child (Fig. 2). Each question was asked separately for the mother and for her child, so that personalized data could be collected.

Fig. 2. Image of the harmonized DEMOCOPHES questionnaire on consumption habits of fish and marine products.

Additionally, data on the sampling procedure and basic information about the samples were collected e.g. whether the hair had been dyed or tinted within the previous 6 months.

2.1.3. Chemical analysis

Mercury determinations in the DEMOCOPHES pilot study were done in laboratories which successfully passed the COPHES/DEMOCOPHES external quality assurance program (Esteban et al., 2015 and Table S1 as Supplementary material). To assess mercury in hair, the closest centimeters (maximum 3 cm) to the scalp of the hair samples were analyzed.

2.1.4. Databases management

The analytical results and the information gathered with the questionnaire were recorded in the national databases using a common format and coding. Identical quality controls were applied at national level, using a centrally developed script (written in R statistical software, R Development CoreTeam, 2012). The data from all the countries were merged into the European database (Den Hond et al., 2015).

2.1.5. Training

In support of the study protocol and the Standard Operating Procedures, various training sessions were organized covering the following tasks: sampling, sample transport and preservation, interview/questionnaire conduct, statistical analysis, ethics and communication (Fiddicke et al., 2015).

2.1.6. Ethical approval

The ethical committees of each involved European country approved the study protocol and country specific requirements were followed. All mothers gave written informed consent on her and her child's behalf (Casteleyn et al., 2015) (www.eu-hbm.info/democophes).

2.2. Statistical analysis of data

2.2.1. Cluster analysis of diet-patterns in the European sample

The classification procedure (cluster analysis) was based on the seven possible distinct answers to questions B, C, D, and E (SEA FISH; SHELLFISH; FRESHWATER FISH and SEAFOOD PRODUCTS) (Fig. 2) from the mother–child pair, totaling eight variables with seven levels.

DEMOCOPHES data including answers to the questionnaire on fish consumption, and number of amalgam fillings of mothers and children along with area of residence, gender, age, and parental educational levels were taken from the central database (Den Hond et al., 2015).

Hierarchical cluster analysis, and the complete linkage algorithm of cluster building, was used, with a Manhattan distance as dissimilarity measure (Johnson and Wichern, 1988). Manhattan dissimilarity would be zero if two questionnaires answers were identical (all eight variables). It would be one if just *one* of the eight variables differs in *one step* in the sequence "several times per day" - "daily" - "several times per week" - "once per week" - "several times per month" - "once per month" - "almost never". It would be two if that happens in *just two items* in the questionnaire, *or* if there is a *two steps* difference in just *one* item, and so on. In general, the dissimilarity will be the total number of steps of difference between two questionnaires there are for all items (Fig. S1 in Supplementary material, shows an example for two items).

The analysis of combined consumption in mother–child pairs was based on the lowest reported consumption in the pair.

Table 1

Number of mother–child pairs recruited in each DEMOCOPHES implementing country and number of pairs with complete fish questionnaires (valid for analysis).

Country	Number of pairs	Number of valid pairs
Belgium	129	126
Cyprus	60	59
Czech Republic	120	120
Denmark	145	138
Germany	120	120
Hungary	120	120
Ireland	120	101
Luxembourg	60	52
Poland	120	116
Portugal	120	120
Romania	120	120
Slovakia	129	126
Slovenia	120	118
Spain	134	133
Sweden	100	94
Switzerland	120	115
United Kingdom	21	21

2.3. Analysis of mercury levels in hair in relation to diet patterns

Once mother–child pairs were classified, and consumption patterns were described, mercury concentrations in hair for the different groups and diets were compared. Hair mercury levels of mothers and children were taken from the central database (Den Hond et al., 2015). Mercury levels have been validated and stored in the central database after imputation of values below the limits of quantification (LOQ) as LOQ/2. Medians and 90th percentiles of mercury in hair were calculated for the different groups. Wilcoxon–Mann–Whitney rank sum tests were used in order to compare consumption of food types between groups, and Kendall's tau-*b* correlation was utilized to analyze the association of each type between mothers and children. It was also used to measure the association of mercury levels between mother and child.

The clustering of the European mother–child pairs on the dietary answers was the basis for the analysis of mercury exposure (from hair analysis) in relation to consumption of food items from the marine or freshwater environments. All pairwise comparisons between cluster groups were tested for differences in diet and mercury (Wilcoxon–Mann–Whitney test). When comparing groups, two kinds of results were of interest for the discussion of mercury exposure factors in this work:

1. Equal SEA FISH consumption/significantly different Hg hair levels.
2. Significantly different SEA FISH consumptions/equal Hg hair level.

The confidence level for hypothesis testing was 0.05. All statistical analyzes were carried out using Stata 12 (Stata Corp LP, USA). Automated searches, written as Stata do scripts, were used in order to find diet-patterns of high frequency in the European sample, and within countries.

3. Results

Most of the 17 DEMOCOPHES implementing countries met the minimum required sample size of 120 mother child pairs (60 in Luxembourg and Cyprus because of their smaller population size). Exceptions were UK (21 pairs) and to a minor extent Sweden (100 pairs). Some countries extended their sample sizes beyond the minimal DEMOCOPHES requirements (Table 1). The grand total was 1858 pairs.
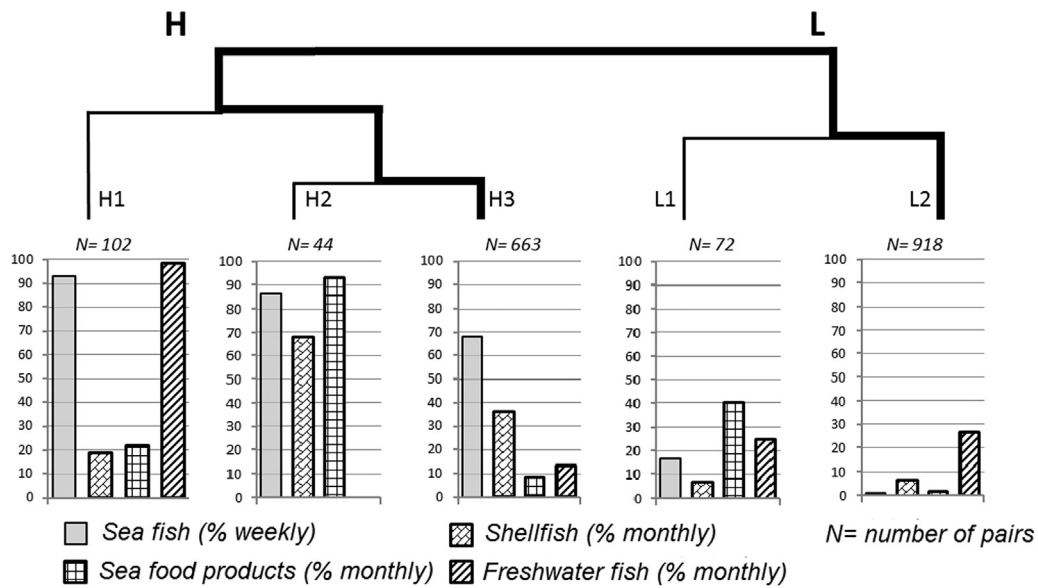
Fig. 3. Dendrogram of the hierarchical cluster analysis for fish and marine product consumption of participants in the European sample DEMOCOPHES with the two main branches H (high consumption) and L (low consumption) and the secondary groups at the third level of dendrogram: H1, H2, H3 and L1, L2. Bar graph below each group, showing percentages of pairs consuming SEA FISH more than weekly, SHELLFISH more than monthly, SEAFOOD PRODUCTS more than monthly and FRESHWATER FISH more than monthly.

A total of 59 pairs were excluded from the classification of fish products consumption because of their incomplete questionnaires. In Ireland, 19 pairs were excluded, because SEA FISH was consistently reported, but there were missing answers for the rest of the items. The rest of the exclusions are scattered among eleven countries. Thus a final set of 1799 pairs was considered in the present analysis (Table 1).

Mercury levels in the hair of participants showed large differences, up to a factor of 40 in national medians, among the 17 participating countries. Analysis of the data clearly indicates that differences in mercury levels for the European participants are associated with diet (Den Hond et al., 2015 and Tables S2 and S3 as Supplementary material).

3.1. Cluster analysis of diet

Fish consumption reported in the DEMOCOPHES questionnaires can be classified in two main branches and in secondary groups. Fig. 3 and Fig. S2 show the dendrogram of the hierarchical cluster analysis considering the eight variables (questions B, C, D, and E for each member of the pair). First, as a result of the classification procedure, two broad branches were identified: "high fish consumption" **(H)** branch containing 809 pairs (45% of the total) and "low fish consumption" **(L)** branch with 990 pairs (55% of the total). All the top scoring pairs for any of the four questions are in **H**. Moreover, considering a family habit of once per week as a reference for SEA FISH (both members of the pair) and once per month as a reference for SHELLFISH, FRESHWATER FISH and SEAFOOD, it was found that pairs reporting higher frequencies than the reference were all in **H** for SEA FISH (except three), they were ten times more abundant in **H** than in **L** for SHELLFISH and they were twice as many in **H** than in **L** for SEAFOOD. Although FRESHWATER FISH presented just 1.4 more pairs above the reference in **H**, their consumption levels were higher, even above one per week. Pairs with frequencies above the reference for more than one question were mostly in **H**.

All countries had participants in both branches, but it can be pointed out that Belgium, Denmark, Spain, Portugal and Sweden had twice as many or more pairs in **H** (high fish consumption) than in **L** (the low fish consumption) groups, and vice versa for Switzerland, Czech Republic, Hungary, Poland, Romania, Slovenia and Slovakia (Table S4).

There are no significant differences between the assignment of pairs to **H** or **L** between the rural and urban locations of each country ($p > 0.110$), with the exceptions of Hungary ($p=0.024$) and Slovenia ($p=0.005$) where rural locations show lower consumption levels.

Table 2
Recurrent diet patterns in pairs (both mother and child) of groups H3 and L2, according to the answers given to the DEMOCOPHES fish and marine product consumption questionnaire. SHELLFISH and SEAFOOD PRODUCTS answers for both members of the pair are "almost never" in all six diet patterns. Mercury in hair median, 90th percentile and percentage of subjects above the limit of quantification (LOQ) are also shown.

		I	II	III	IV	V	VI
Group[a]		L2	L2	L2	L2	H3	H3
N (pairs)		128	37	80	113	106	44
SEA FISH Mother/Child		(Almost) never	(Almost) never	Once a month	2–3 Times per month	Once a week	Several times/week
FRESH WATER FISH Mother/Child		(Almost) never	Once a month	(Almost) never	(Almost) never	(Almost) never	(Almost) never
Hg in hair (µg/g) Mother	P50	0.069	0.069	0.118	0.127	0.149	0.808
	P90	0.243	0.345	0.387	0.479	0.550	2.391
	> LOQ %	53.5	49.5	59.8	74.3	75.0	84.9
Hg in hair (µg/g) Child	P50	0.061	< LOQ	0.078	0.087	0.094	0.766
	P90	0.168	0.178	0.285	0.472	0.473	1.918
	> LOQ %	52.3	49.4	38.0	68.0	73.5	74.5

N=number of pairs. P50=50th percentile. P90=90th percentile.

[a] Group according to cluster classification shown in Fig. 2.

Table 3
Correlation (Kendall's tau-b) between mothers' and children's frequency of consumption of SEA FISH, SHELLFISH, SEAFOOD PRODUCTS and FRESHWATER FISH and mercury in hair (μg/g). Correlation within each of the main groups according to cluster classification represented in Fig. 2 (H1,H2, H3, L1 and L2).

Group	N	SEA FISH	SHELLFISH	SEAFOOD PRODUCTS	FRESHWATER FISH	Hg in hair
H1	102	0.599*	0.465*	0.621*	0.649*	0.655*
H2	44	0.693*	0.445*	0.531*	−0.048	0.592*
H3	663	0.667*	0.536*	0.509*	0.716*	0.535*
L1	72	0.283*	0.185*	−0.010	0.427*	0.442*
L2	918	0.424*	0.589*	0.455*	0.699*	0.429*
Total	1858	0.678*	0.598*	0.610*	0.748*	0.545*

* $p < 0.05$.

The two big branches (**H** and **L**) were further divided in a main classification of three (**H1; H2; H3**) and two groups (**L1; L2**) of unequal size but optimal dissimilarity (Fig. 3).

The high consumption branch (**H**) includes a big mainstream group (**H3**, 663 pairs) in addition to two smaller extreme groups (**H1** and **H2**) with high consumption (Fig. 3). **H1** (102 pairs) and **H2** (44 pairs) stand out from **H3** because of their higher consumption rates of SEA FISH and, additionally, FRESHWATER FISH (**H1**) or SHELLFISH and SEAFOOD PRODUCTS (**H2**). Therefore, **H1** and **H2** gathered pairs with the highest scores in SEA FISH, SHELLFISH, SEAFOOD PRODUCTS and FRESHWATER FISH consumption.

Likewise, the low fish consumption branch **L** was divided into a small group (**L1**, 72 pairs) and a mainstream group (**L2**, 918 pairs).

Within the **L** branch, the group **L1** includes pairs with an intermediate range of SEA FISH consumption. None of the pairs answered "almost never" to all B, C, D and E questions in **L1**. The most prominent feature within the **L** groups is the difference in consumption of SEAFOOD PRODUCTS: in **L1**, 21 pairs out of 72 (29%) had consumption levels above the reference of once per month, whereas there were none in **L2**.

L2 grouped participants with the lowest consumption in the whole European sample. Half the pairs in the group answered "2 or 3 times per month" or less for SEA FISH AND "almost never" for consumption of SHELLFISH, SEAFOOD PRODUCTS and FRESHWATER FISH.

3.2. Group comparison and analysis.

A distinct pattern could be observed in the analysis of questionnaire replies. In the European sample, six recurring questionnaire answers for mother and child pairs (Table 2) were dominating. Given that the theoretical number of answers is 5.7 million, six recurrent diet patterns show that there is a clear preference in consumption patterns of fish and marine products in the studied European cohort. The six recurring answers are in core groups **H3** and **L2** and account for little less than a third of the total number of replies. Thus, 358 pairs in **L2** (39%) and 150 pairs in **H3** (23%) replied just one of the six recurring fish patterns

Table 4
Results for mercury in hair (μg/g) for mothers and children in each of the groups, and selected subgroups utilized for comparisons. Groups were obtained from cluster analysis of fish and marine products diet. Median (P50), 90th percentile (P90) and percentage of samples above the limit of quantification (LOQ).

Group (N)	Subgroup (N)	Mothers				Children			
		P50 95% CI	P90 95% CI	Max	> LOQ %	P50 95% CI	P90 95%CI	Max	> LOQ %
H1 (102)		0.630 0.442–0.877	2.770 2.000–3.400	8.90	97.0	0.370 0.280–0.494	1.560 1.164–2.764	6.60	93.1
	H1a (88)	0.501 0.331–0.770	2.100 1.515–3.592	8.90	96.6	0.310 0.209–0.406	1.300 0.875–2.390	6.60	92.0
	H1a1 (71)	0.400 0.233–0.683	1.671 1.305–2.094	4.50	95.7	0.232 0.168–0.345	0.898 0.702–1.791	2.80	90.0
	H1b (14)	1.724 1.211–3.217	3.400 2.871–3.400	3.40	100	0.950 0.645–1.433	3.700 1.400–4.200	4.20	100
H2 (44)		1.335 1.003–1.797	3.200 2.600–5.732	6.70	100	0.979 0.600–1.156	2.103 1.593–5.841	7.10	100
H3 (663)		0.404 0.352–0.453	1.649 1.504–1.892	9.66	97.9	0.263 0.229–0.289	1.200 1.078–1.335	6.40	94.5
	H3a (506)	0.347 0.311–0.395	1.619 1.444–1.800	8.90	97.3	0.220 0.205–0.259	1.188 1.016–1.300	6.40	93.6
	H3a1 (291)	0.347 0.282–0.413	1.818 1.539–2.294	8.90	96.9	0.220 0.198–0.284	1.266 1.171–1.681	6.40	98.8
	H3b (107)	0.587 0.455–0.790	2.434 1.606–3.362	9.66	100	0.356 0.304–0.469	1.553 1.102–2.009	5.07	97.2
	H3c (50)	0.605 0.440–0.737	1.449 0.987–2.154	2.70	100	0.297 0.228–0.348	0.967 0.531–2.538	2.90	98.0
L1 (72)		0.333 0.241–0.451	1.470 0.997–2.731	3.90	95.8	0.151 0.116–0.199	0.700 0.381–1.063	1.30	88.7
	L1a (63)	0.340 0.226–0.451	1.320 0.915–2.472	3.90	95.2	0.137 0.095–0.176	0.700 0.385–1.090	1.30	87.1
	L1b (9)	0.250 0.186–1.432	2.822 0.610–2.822	2.82	100	0.205 0.156–0.279	0.490 0.263–0.490	0.49	100
L2 (918)		0.132 0.120–0.148	0.520 0.457–0.601	3.90	83.7	0.076 0.069–0.088	0.355 0.310–0.404	3.20	78.3
	L2a (848)	0.130 0.118–0.146	0.518 0.449–0.597	3.90	84.4	0.074 0.069–0.087	0.352 0.310–0.414	3.20	79.0
	L2b (64)	0.143 0.081–0.196	0.518 0.380–0.871	1.21	73.0	0.080 0.069–0.140	0.341 0.254–0.972	1.82	66.7
	L2c (6)	0.339 0.161–0.969	1.000 0.457–1.000	1.00	100	0.208 0.130–0.848	0.900 0.274–0.900	0.90	100

N=number of pairs, P50= 50th percentile, P90=90th percentile, and CI=confidence interval.

shown in Table 2. The common denominator in these groups is the reply "almost never" to the consumption of SHELLFISH and SEAFOOD PRODUCTS. This makes it possible to compare mercury levels just on the basis of fish (seawater or fresh water) consumption without interference of SHELLFISH or SEAFOOD PRODUCTS.

None of the recurring fish patterns identified in the questionnaires seems to be characteristic for a particular country. There are some countries with up to four prevalent reply patterns, while in other countries they are rare or even absent, but considering the lack of country representativeness of the samples in general, the information should be considered cautiously. For example, pattern I (Table 2) is prominent in Romania and Hungary but absent in Cyprus, Spain, Luxembourg, Portugal and United Kingdom. Looking at Romanian and Hungarian pairs, there is a strong incidence of patterns I, II and III with pattern II (FRESHWATER FISH) being characteristic for these two countries. Patterns I, III, and IV of low consumption are frequent in Switzerland, Czech Republic, Poland and Slovenia. In Germany and Slovak Republic patterns I, II, IV and V make up half of the total DEMOCOPHES sample. Pattern VI applies only to Portugal. Pairs in Belgium, Denmark, Spain and Sweden rarely provide any recurrent combination of answers to the questionnaire and even less frequently agree in any combination (i.e. four repetitions at most in each country) (Table S4).

The five main groups (**H1**, **H2**, **H3**, **L1** and **L2**) differ significantly ($p < 0.05$) in each of the four questions, when comparing every two groups, except in the low consumption branches (**L1** and **L2**) that have the same consumption of SHELLFISH ($p = 0.862$) and FRESHWATER FISH ($p = 0.725$).

Comparing the questionnaire replies of mothers and their children, about half of the pairs in DEMOCOPHES report identical consumption patterns (880 pairs). Still, the mother–child replies are positively correlated for all except two items FRESHWATER FISH in the **H2** group and SEAFOOD PRODUCTS in **L1** (Table 3). In fact **L1**-the highest consumer group in the lowest branch-has the lowest positive correlation (Kendall's tau-b) between mothers' and children's replies. The largest differences between mother and child answers to the same item occurred in this group (**L1**). In general, mothers eat fish and marine products more frequently than their children, particularly SEAFOOD PRODUCTS with 15.3% of mothers in **L1** exceeding their children's frequency of consumption by more than three levels.

When comparing questionnaires replies for mothers and questionnaires replies for children independently, the five main groups (**H1**, **H2**, **H3**, **L1** and **L2**) also differ significantly ($p < 0.05$) in each of the four questions when comparing every two groups, as was shown for the pairs. But in this case the exceptions increases to five cases for mothers (SEA FISH for **H1**–**H2**; SHELLFISH for **H1**–**L1**; SEAFOOD PRODUCTS for **H2**–**L1** and FRESHWATER FISH for **H2**–**H3** and **L1**–**L2**) and in two more for children (SHELLFISH and FRESHWATER FISH for **L1**–**L2**). In other words, mothers in the two extreme high consumption **H** branches reported the same frequency of SEA FISH consumption ($p = 0.072$); and, children in the low consumption branches (**L1** and **L2**) replied the same for SHELLFISH ($p = 0.898$), 93% of them reported "almost never" to question C.

When levels of mercury in hair were compared to the classification based on fish consumption (Fig. 3), the dendrogram matched differences in mercury levels both in mothers and children. The main five groups (level 2 on Fig. 3) differed in their mercury values in hair, which are shown in Table 4. **H1**, with a high consumption of SEA FISH and FRESH FRESHWATER FISH, showed extreme values of up to 8.9 μg/g mercury in one mother and 6.6 μg/g mercury in one child. Group **H2**, with a high consumption of SHELLFISH and SEAFOOD PRODUCTS, shows the highest median levels

Table 5

Comparison of subgroups H2 and H3b (A); H3a1 and H3b (B) and H1a1 and H3a1 (C) with results of the tests of equality of distribution of SEA FISH, SHELLFISH, SEAFOOD PRODUCTS, FRESHWATER FISH and mercury in hair between both subgroups (Wilcoxon–Mann–Whitney rank sum test). Percentages of mothers or children having amalgam fillings, consuming SEA FISH and SHELLFISH more than weekly, or SEAFOOD PRODUCTS and FRESHWATER FISH more than monthly, along with median values of mercury in hair (μg/g) are shown to illustrate the differences between subgroups.

A

		Subgroups		
		H3b (N=107)	H2 (N=44)	p
Amalgam fillings	Mothers	67.7%	66.7%	0.910
	Children	4.7%	11.4%	0.139
SEA FISH	Mothers	44.9%	61.3%	0.201
(>1/week)	Children	43.0%	59.1%	0.137
SHELLFISH	Mothers	89.7%	68.2%	< 0.001*
(>1/month)	Children	43.0%	45.5%	0.674
SEAFOOD PRODUCTS	Mothers	3.7%	100%	< 0.001*
(>1/month)	Children	0.0%	88.6%	< 0.001*
FRESH WATER FISH	Mothers	0.0%	6.8%	0.806
(>1/month)	Children	0.0%	0.0%	0.099
Mercury	Mothers	0.587	1.335	< 0.001*
(median μg/g)	Children	0.356	0.979	< 0.001*

B

		Subgroups		
		H3a1 (N=291)	H3b (N=107)	p
Amalgam fillings	Mothers	69.7%	67.7%	0.702
	Children	9.6%	4.7%	0.118
SEA FISH	Mothers	46.7%	44.9%	0.737
(>1/week)	Children	45.7%	43.0%	0.383
SHELLFISH	Mothers	4.1%	89.7%	< 0.001*
(>1/month)	Children	0.7%	43.0%	< 0.001*
SEAFOOD PRODUCTS	Mothers	3.1%	3.7%	0.012*
(>1/month)	Children	0.3%	0.0%	0.325
FRESH WATER FISH	Mothers	0.3%	0.0%	0.357
(>1/month)	Children	2.4%	0.0%	0.168
Mercury	Mothers	0.347	0.587	< 0.001*
(median μg/g)	Children	0.220	0.356	0.003*

C

		Subgroups		
		H3a1 (N=291)	H1a1 (N=71)	p
Amalgam fillings	Mothers	69.7%	73.2%	0.558
	Children	9.6%	5.6%	0.289
SEA FISH	Mothers	46.7%	26.8%	0.018*
(>1/week)	Children	45.7%	25.4%	0.001*
SHELLFISH	Mothers	4.1%	12.7%	0.508
(>1/month)	Children	0.7%	2.8%	0.476
SEAFOOD PRODUCTS	Mothers	3.1%	12.7%	0.019*
(>1/month)	Children	0.3%	0.0%	0.173
FRESH WATER FISH	Mothers	0.3%	98.6%	< 0.001*
(>1/month)	Children	2.4%	100.0%	< 0.001*
Mercury	Mothers	0.347	0.400	0.598
(median μg/g)	Children	0.220	0.232	0.664

N = number of pairs.

  * $p < 0.05$.

(1.3 μg/g for mothers and 0.98 μg/g for children) and the highest 90th percentiles (3.2 μg/g for mothers and 2.1 μg/g for children). **H** groups have higher medians (P50) and 90th percentiles (P90) than **L** ones, although the highest exposure pairs in **L1** clearly keep up with typical mercury levels in **H3**.

H3 group, representing the 37% of the studied population, consumed fish and/or marine products (B, C, D, E questions) on a weekly basis and its median mercury values are 0.4 μg/g. In fact except for the **H2** group, the P50 values for the five main groups were below 1 μg/g mercury in hair.

Median as well as P90 values are higher for mothers than for children. The correlation between mother and child mercury levels was a bit lower in low consumption groups than in higher consumption ones (Table 3). Correlation between mother and child in mercury shows wider variation by country (range 0.195–0.494) than by diet group.

Mercury levels increased in relation to the frequency of SEA FISH consumption in study participants reporting no consumption of SHELLFISH and SEAFOOD PRODUCTS (Table 2). 90% of the women consuming only SEA FISH once per week (pattern V) (independently of the species or the size) had mercury levels of 0.55 μg/g and 50% of the mothers who consume SEA FISH several times per week (pattern VI) had mercury levels of 0.80 μg/g. 89% of the mothers in the high fish consumer group (pattern VI) are at the benchmark value set up by JECFA/WHO 1.9 μg/g (EFSA, 2012) (Table 2).

All differences in mercury levels between pairs of the five main groups are significant ($p < 0.05$) except for mothers in **H3**–**L1** (the lowest high consumer group and the highest low consumer group) ($p = 0.153$).

H1 and **H2** have significantly different levels of mercury in hair despite having no significant differences in SEA FISH consumption and this was the driver to investigate the influence of other dietary items on mercury levels.

For that aim, some subgroups of the main five have been selected from the lower branches of the dendrogram of our cluster analysis (Table 4). These subgroups show either significantly different mercury levels although the same SEA FISH consumption frequency, or the opposite way, there were significant differences in SEA FISH consumption but with no differences in mercury in hair. The possible contribution of amalgam fillings, their occurrence and their number, have been tested. Both have been found to be non-significant with respect to the mercury levels measured in hair (Fisher exact test for the proportion of mothers with amalgam fillings $p > 0.142$, Wilcoxon–Mann–Whitney for their number $p > 0.057$).

Significant differences in Hg levels although similar SEA FISH, intake were observed in a number of group-pair comparisons. This is exemplified when a subgroup of **H3** (**H3b**) is compared with **H2**. Both groups are described in Table 5A, in which the proportion of mothers and children having each of the food items more frequently than the reference (once per week for SEA FISH, once per month for SHELLFISH, SEAFOOD PRODUCTS and FRESHWATER FISH), are shown along with median values of mercury in hair. Incidence of amalgam fillings is also shown for reference. The consumption of SEA FISH is high in both groups (50% of the members consume more than once per week) with no significant differences between the two subgroups ($p = 0.201$ for mothers and 0.137 for children). However, mercury in hair is more than twice in participants from **H2** with regard to those from **H3b**. The consumption frequency of SEAFOOD PRODUCTS is what separates these groups and it seems that SEAFOOD PRODUCTS is the potential determinant for the increase in the mercury levels.

Similar results have been observed in many other pairs of groups, for example between groups **H2**, and **H3a1** with a significantly ($p < 0.001$) wide gap in their monthly SEAFOOD PRODUCTS consumption (almost everyone in **H2** and almost nobody in **H3a1** having SEAFOOD PRODUCTS monthly), or between groups **H1b** and **H1a** also with significantly ($p < 0.001$) different SEAFOOD PRODUCTS

consumption (almost everyone in **H1b** and almost nobody in **H1a** having SEAFOOD PRODUCTS monthly) while the consumption frequency of SEA FISH is the same, and 3 times more mercury in hair both in mothers and their children of **H2** with regard to **H3a1** or of **H1b** with regard to **H1a** (Table 4).

Consumption of SHELLFISH also shows a certain influence on the increase of Hg levels as was observed in a number of pair-wise group comparisons. To exemplify this, two subgroups of **H3** (**H3a1** and **H3b**) were compared (Table 5B). When comparing **H3a1** and **H3b** SHELLFISH consumption is significantly different, and SEA FISH and FRESHWATER FISH are consumed at the same level. Mercury in hair was significantly higher in the subgroup with higher SHELLFISH consumption (Table 5B).

Therefore consumption of SHELLFISH and SEAFOOD PRODUCTS need to be further investigated, in addition to already established contribution of SEA FISH with respect to mercury exposure.

There are also examples in the material of groups that, although having significant differences in SEA FISH and FRESHWATER FISH consumption, showed no significant differences in mercury levels, for example when comparing subgroups **H1a1** and **H3a1** (Table 5C). In this case SEA FISH was consumed almost twice as frequently in **H3a1** compared to **H1a1**, with no visible difference in mercury level between the two groups. The consumption of FRESHWATER FISH (98% more frequent) had no influence on mercury levels.

The lack of association of FRESHWATER FISH consumption with the levels of mercury was confirmed in **H2** and **H1b** comparisons. In this case, SEA FISH and SHELLFISH were equally frequent in the diets of members of both subgroups but there was a significant difference in the frequency of FRESHWATER FISH in the diet (92% more frequent in **H1b** both in mothers and children), and no significant effect was seen on mercury levels in hair for mothers neither for children ($p = 0.131$ and $p = 0.313$, respectively) (Table 4).

4. Discussion

In this paper we have compared results both for dietary habits and mercury in hair for the first time in a harmonized way in mother–child pairs in 17 European countries. Data are based on European dietary habits and on fish marketed and consumed in Europe. This was possible thanks to the use of a commonly developed protocol (target population and questionnaires), a full training scheme for field work as well as stringent quality control programs for chemical and data analysis.

In the overview of results from the DEMOCOPHES project (Den Hond et al., 2015), we report that mercury levels in hair of participants (mother–child pairs) varied with more than a factor of 50 between the lowest (Hungary, geometric mean (GM) 0.02 μg/g hair) and the highest (Portugal, GM 1.03 μg/g hair) with an overall geometric mean in the DEMOCOPHES material of 0.14 μg/g, based on values from the children. The mothers had higher mercury levels than their children but followed the same pattern. Fish consumption and social status were identified as important and independent determinants of mercury levels, both in mothers and their children, which confirms results from other studies (Mahaffey, 2004). Although France, Greece and Italy did not participate in the DEMOCOPHES project, recent biomonitoring studies on hair levels of mercury in women from France (0.60 μg/g), Greece (1.12 μg/g) and Italy (0.77 μg/g) show that they fall well within the range of the DEMOCOPHES countries in which fish and other marine products constitute an important part of the diet (Frery et al., 2010; Miklavčič et al., 2013). The wide-spread difference in mercury exposure in the European population can be related to dietary habits and in particular to consumption of fish and other products from the aquatic environment. With respect to mother–

child pairs, there is a strong correlation ($r=0.72$) in hair mercury concentration between the mother and child (Den Hond et al., 2015) which shows that there is a common source of exposure in the studied families. Most likely this is the diet since mercury levels in hair reflect primarily exposure to MeHg from food sources (Sherman et al., 2013). We also found that mercury from amalgam fillings is of minor importance (Table 5) with respect to hair levels as also reported by others (Sherman et al., 2013).

An important part of the study was the questionnaire data on the participants' consumption frequencies of fish and other products from the aquatic environment. The weakness of these types of questionnaires is that they are a retrospective survey in which the mothers have to remember what and when they ate the different items covered and to what extent their children ate them. Probably the most certain reply is when the answer is "never" – in this case the respondents probably know for sure. Therefore, the questionnaire provided only approximate information on fish consumption and was not a precise measure of the fish ingested by an individual. Despite this limitation, the material still allows for analysis of consumption patterns across the DEMOCOPHES countries and of items that may have an additional influence on the levels of mercury measured in hair.

There is a clear pattern in consumption of fish and other aquatic products across Europe, with relatively higher frequency in the Mediterranean and North European countries and a lower frequency in Central European countries. Based on questionnaire answers we could separate mercury exposure due to SEA FISH consumption from FRESHWATER FISH consumption. In the results there was no evidence that consumption of FRESHWATER FISH contributes to mercury levels in mothers and their children. This finding is not surprising since the market for FRESHWATER FISH is very limited in Europe with 5.3% of total sales of aquatic products while seawater fish represents 73.5% and the remaining 21.2% is other types of aquatic products (EUMOFA, 2014). In some European countries FRESHWATER FISH could be a significant source of mercury exposure. Swedish FRESHWATER FISH (pike, *Esox lucius*, perch, *Perca fluviatilis*) contain significant amounts of methylmercury, in the range of 0.5–1.0 mg/kg, because of historical mercury contamination (Åkerblom and Johansson, 2008). This exposure was not reflected in the Swedish data from the DEMOCOPHES study probably because consumption of FRESHWATER FISH is small and concentrated to local populations, which were not captured in the DEMOCOPHES sample.

In the DEMOCOPHES material, the group **H3**, with 37% of the studied sample, reported consumption of fish and marine products once a week or more. The mercury levels in this group are below 1.65 µg/g for 90% of the mothers. When analyzing the contribution of the different food items, we found that 90% of those who ate fish but no other marine products had mercury values below 0.55 µg/g, and half of the group (P50) had values below 0.15 µg/g. Furthermore, 50% of the mothers consuming SEA FISH several times per week had mercury values below 0.81 µg/g (Table 2). This is valuable information for public health authorities when developing dietary recommendations. For example, the US-EPA recommended level for women in childbearing age is 1 µg mercury per gram hair (US-EPA, 2001) which means that the whole group studied here (eating only SEA FISH once per week independently of the species and size) and half of the group of frequent consumers would be on the "safe side". Furthermore, if we consider the JECFA/WHO recommended levels of 1.9 µg/g, 89% of the higher consumers (eating only SEA FISH) fall below this limit (Table 2).

A very clear, additional pattern comes out from the analysis. SEAFOOD PRODUCTS, and to a lesser extent SHELLFISH, contributes significantly to the mercury exposure, both in mothers and their children. The SEAFOOD PRODUCTS group includes algae, seaweed and surimi that are quite common in "modern diets". Surimi is a food

product based on several fish species such as Alaska Pollock, Pacific whiting, Tilapia and other species of less market value and it forms the bases for food items such as imitation crab meat, satsumi-age/tenpura, hanpen, and fish sausage (Tina et al., 2010). Depending on the origin of surimi products, they may contribute to the body burden of mercury. There could be a risk that they originate from environments with elevated mercury levels or that some of the fish species used are top predators in the marine food chain. Eurostat data from 2011 and 2012 showed an increase of 69% in volumes of miscellaneous aquatic products imported from extra-EU countries, and it should be mentioned that no imports of seaweed and other algae were reported in 2011, but they totalled 53.000 tonnes in 2012 (EUMOFA, 2014). Clearly, there is a need for more extensive monitoring of these products to obtain a better picture of their role as potential sources of mercury exposure.

There were also groups of consumers that had lower mercury levels than expected on basis of their consumption of SEA FISH and SEAFOOD PRODUCTS. The species of fish consumed were not reported in the questionnaires and therefore it is difficult to make exact correlations. However, it is well known that type of fish, the size, age and the position in the food chain are important with respect to mercury content. Top predators accumulate the highest levels since mercury is concentrated along the food chain (NRC, 2000). The metal is bioaccumulated over a lifetime and larger and older specimens will have higher concentrations than younger and smaller ones. Therefore active pelagic top predators like tuna, swordfish or long-lived species that such as sharks attain high mercury levels. The mercury values reported can be assumed to represent exposure from fish landed in Europe by EU fishing fleets. The majority of the fish marketed in Europe comes from North-East Atlantic (71.5%) and the Eastern Central Atlantic (13.4%) (EUROSTAT, 2014). According to the European Commission Regulation (EC) No. 78/2005 of January 19, 2005 the maximum allowed level of mercury in the species anglerfish, swordfish and tuna is 1 mg/kg. For other fishery products and fish muscle the maximum allowed level of mercury is 0.5 mg/kg.

Individual susceptibility can also play a role in mercury accumulation. In fact, inter-individual variation in mercury biomarkers may be partly explained by genetic variability. This may explain some of the exceptional cases that were identified in the present material. For example one mother in the L1 cluster reported that she "almost never" consumed fish or marine products. She reported that she ate fish and shellfish "once per month or less" and "almost never" other SEAFOOD PRODUCTS. As mentioned, a "never" answer in the questionnaire could be considered quite reliable. She had 2.8 µg/g mercury in the hair which is the range of high SEA FISH consumers. These exceptions in single cases could be associated with individual susceptibility or a genetic polymorphism (Basu et al., 2014; Julvez and Grandjean, 2013).

Mercury contamination of the human food chain is a significant problem for human health and wellbeing. Fish and shellfish are important components in a healthy diet and have a high cultural and social value in many parts of Europe. They are central ingredients in the so-called "Mediterranean diet", which today is recommended to mitigate obesity and cardio-vascular disease. Unfortunately these products can be a significant source of mercury exposure. There is a risk that people stop eating fish and shellfish because of the perceived health risks of mercury, which would be highly undesirable from a public health point of view. The first recommendation is to focus the advice on the vulnerable groups, which in the case of mercury, is pregnant women. Well-designed communications strategies are therefore important in order to correctly communicate both the risks and benefits to human health. An example of this comes from the Bermuda

Islands where a successful communication strategy and public health interventions resulted in an 80% reduction of blood mercury levels in a population of pregnant women following the provision of dietary advice of local versus global market fish intake (Dewailly et al., 2012).

It is important to reduce anthropogenic mercury emissions as far as possible. The ratification of the Minamata Convention will be an important step in this direction. These kinds of strategies become useless if it is not possible to show that they work. Human biomonitoring, based on analysis of mercury in hair, is relatively cheap and a very sensitive tool to follow the implementation of this strategy and determine its effectiveness. It will be able to clearly demonstrate whether the strategy works and human exposure to mercury is reduced or not.

5. Conclusions

There is a widespread difference in mercury exposure in the European population and the difference is very likely related to dietary habits and in particular to consumption of fish and other products from the marine environment. The frequency of fish and aquatic products consumption shows a clear pattern across Europe with a relatively higher frequency in the Mediterranean and North European countries and a lower frequency in Central European countries. The hair mercury levels also show the same general pattern. The mercury levels in the mothers and their children are strongly correlated indicating a common source of exposure, most likely the diet. There was no significant contribution from dental amalgams to hair mercury levels. The mercury exposure is related to consumption of marine fish and seafood and the contribution from freshwater fish was minor in the studied sample. The majority (a 95%) of those consuming fish once a week or more have mercury levels (0.55 µg/g hair) well below the health based limit values recommended by US-EPA (1 µg/g hair) and by WHO (1.9 µg/g hair) for the most vulnerable population group. Seafood products and shellfish were found to significantly contribute to mercury exposure and this potential exposure source should be further monitored. As mercury will be present in the environment for many years to come, human biomonitoring programs, like DEMOCOPHES, are important tools in assessing current population exposure and in discovering trends and patterns related to mercury mitigation policies (Minamata convention), life style and food consumption. This information is essential for assessing the effectiveness of policies and for advisory authorities in developing relevant consumer recommendations with respect to products from the aquatic environment.

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.envres.2014.10.029.

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