MERCURY EXPOSURE ASSESSMENT IN THE SLOVENIAN POPULATION

Ana Miklavčič

Doctoral Dissertation
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MEDNARODNA PODIPLOMSKA ŠOLA JOŽEFA STEFANA
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IZPOSTAVLJENOST SLOVENSKE POPULACIJE ŽIVEMU SREBRU

Doktorska disertacija

Supervisor: Prof. Dr. Milena Horvat

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Abstract

Mercury (Hg) is one of the most toxic metals. Its toxicity depends on the route of exposure, on its chemical form and the dose to which a person is exposed. The most susceptible population are pregnant women and children. Epidemiological studies suggest neuropsychological dysfunctions in children who were prenatally exposed to monomethyl mercury (MeHg). Even at low levels of MeHg exposure subtle neurobehavioural deficits were observed. The general population is mainly exposed to MeHg by fish consumption and other seafood. Therefore, our aim was to assess the Hg exposure of the most susceptible Slovenian population of pregnant women and their foetuses through fish consumption. Hg and its species were determined in fish and other seafood consumed by the population involved in this study. Data on the frequency of fish consumption and the number of amalgam fillings obtained by questionnaires were used. Exposure was also assessed through different biomarkers of exposure such as total mercury (THg) and MeHg determined in hair, cord blood and breast milk. In addition, selenium (Se) and arsenic (As) levels were determined in the same biological samples as THg and MeHg, because seafood can represent a common source of exposure. Furthermore, Se can affect the bioavailability of Hg. In addition, our aim was to assess the Hg exposure of the population living in a specific contaminated area (Idrija mercury mine area) through consumption of local food items. This study was mainly part of the prospective cohort study of the EU FP7 PHIME project, which includes 4 recruitment areas in Italy, Slovenia, Croatia and Greece involving together 2202 pregnant women. Compared to pregnant women from Italy (n=900), Croatia (n=234) and Greece (n= 484), Slovenian pregnant women (n=584) have the lowest MeHg exposure levels, due to their low fish consumption. Moreover, most fish consumed by these women are probably farmed ones with lower levels of Hg than those from wild fisheries. At these low levels of exposure a number of factors can influence the strength of the correlation obtained between biomarkers of exposure and intake of MeHg through fish consumption. These important factors are the accuracy of the data contained in the questionnaire, the high variability of MeHg in fish, the presence of inorganic mercury from sources other than fish and intraindividual differences. THg and MeHg determined in cord blood or THg determined in hair are suitable biomarkers for MeHg exposure. Based on our results, it was concluded that THg level in hair is the most appropriate biomarker for exposure assessment for large epidemiological studies, because of the simple and practical collection and storage of the samples, and the relatively simple, fast and precise analytical determination of THg in hair and its relatively high concentration. Significant linear correlations were found between log transformed levels of Hg and Se, As and Se, and As and Hg in cord blood, mother's blood and breast milk. In addition, significant correlations were found between the frequency of fish consumption and various biomarkers of exposure. Therefore, fish consumption, the possible common source of As, Hg and Se exposure, could explain the correlations between the elements determined in cord blood, mother's blood or breast milk. While in general Hg levels in foodstuffs are much lower compared to levels in fish, levels of Hg in food from the contaminated site of the Idrija mine area are elevated. Moreover, the levels of Hg in vegetables, mushrooms, fish and deer have not diminished

significantly during the past years, in spite of closure of the mine. In addition, up to 10 times higher levels of MeHg in some vegetables were found compared to those previously determined in earlier studies. However, the exposure levels assessed through biomarkers of exposure in this area during pregnancy were on the level of the population that live sin uncontaminated area.

Povzetek

Živo srebro (Hg) se uvršča med najbolj toksične kemijske elemente, njegova toksičnost pa je odvisna od kemijske zvrsti, odmerka in načina izpostavljenosti. Najbolj občutljiv segment populacije so nosečnice in otroci. Dosedanje epidemiološke študije kažejo na oslabljene psiho-fizične sposobnosti otrok, ki so bili prenatalno izpostavljeni monometil živemu srebru (MeHg). Že pri izpostavljenosti nizkim koncentracijam Hg so se pokazali negativni vplivi na razvoj živčnega sistema zarodka. Najpogosteje so ljudje izpostavljeni organskemu živemu srebru predvsem preko uživanja rib in druge morske hrane. Zato smo se v doktorski disertaciji osredotočili predvsem na izpostavljenost organskemu živemu srebru preko uživanja rib. Analizirali smo vsebnosti živega srebra in njegovih spojin v ribah in drugi morski hrani, ki jo uživa preiskovana populacija in uporabili podatke o frekvenci uživanja morske hrane ter številu amalgamskih zalivk iz vprašalnikov. Izpostavljenost smo ocenili tudi preko merjenja koncentracij celokupnega živega srebra (THg) in MeHg v različnih telesnih tkivih kot so lasje, popkovna kri in materino mleko. V povezavi s Hg smo v naštetih bioloških tkivih določili vsebnost selena in arzena, saj morska hrana predstavlja pomemben vir izpostavljenosti tem elementom. Določitev selena v teh vzorcih je pomembna, ker selen lahko vpliva na biološko uporabnost živega srebra. Poleg tega smo izpostavljenost ljudi Hg ocenili tudi na kontaminiranem območju. Da bi ugotovili obremenjenost hrane s Hg z območja Idrije in s tem pripomogli k oceni tveganja lokalnega prebivalstva, smo ribam, vrtninam, gobam in srnjadi določili vsebnost živega srebra in njegovih spojin. Večji del raziskave je bil del kohortne študije obširnega Evropskega projekta EU FP7 PHIME, ki je vključevala 4 vzorčevalna področja iz Italije, Slovenije, Hrvaške in Grčije, v kateri je sodelovalo skupaj 2202 žensk. V primerjavi z izpostavljenostjo nosečnic in doječih mater iz Italije (n=900), Hrvaške (n=234) in Grčije (n=484), smo v naši raziskavi potrdili, da so nosečnice in doječe matere iz centralne Slovenije (n=584) izpostavljene nižjim koncentracijam MeHg. Najverjetnejši razlog je redko uživanje rib. Poleg tega so ribe na slovenskem trgu večinoma gojene in vsebujejo nižje koncentracije živega srebra v primerjavi s prostoživečimi ribami. Šibka statistično značilna povezava med biološkimi kazalci in frekvenco uživanja rib je lahko posledica zelo grobe ocene frekvence uživanja rib preko vprašalnikov, raznolikosti koncentracij MeHg v ribah ter relativno visokega in variabilnega deleža anorganskega živega srebra v popkovni krvi pri nizki izpostavljenosti, ki izvira iz drugih virov kot so ribe. Iz naših rezultatov je razvidno, da je THg v laseh glede na enostavnost vzorčenja, shranjevanja in enostavnosti analize najbolj primeren biološki kazalec in zadovoljiv pokazatelj izpostavljenosti pri tovrstnih epidemioloških študijah, če ga primerjamo s koncentracijami THg ali MeHg v popkovni krvi. Linearne korelacije med logaritmiranimi koncentracijami živega srebra in selena, arzena in selena, in arzena in živega srebra v popkovni krvi, krvi in materinem mleku so bile statistično značilne. Med drugim so bile povezave med biološkimi kazalci izpostavljenosti in frekvenco uživanja rib tudi statistično značilne. Iz tega sledi, da so korelacije med biološkimi kazalci izpostavljenosti elementov posledica skupnega izvora izpostavljenosti in sicer uživanja rib. Medtem, ko so ponavadi koncentracije živega srebra v ostali hrani dosti nižje v primerjavi z morsko hrano, so na kontaminiranem območju Idrije koncentracije živega srebra v hrani večinoma povišane. Glede na podatke, ki so na valjo, lahko trdimo, da se v zadnjih letih koncentracije živega

srebra v ribah, gobah, vrtninah in divjačini niso značilno znižale. Poleg tega smo v nekaterih vzorcih vrtnin izmerili tudi do 10 krat višje koncentracije MeHg v primerjavi z že izmerjenimi koncentracijami MeHg. Vendar pa ocena izpostavljenosti živemu srebru preko bioloških kazalcev izpostavljenosti kaže, da nosečnice iz tega območja niso bistveno bolj izpostavljene živemu srebru kot prebivalstvo, ki ne živi na kontaminiranem območju.

Abbreviations

As = arsenic
BSID-II = Bayley Scales of Infant Development-II
Cd = cadmium
CONTAM = EFSA Panel on Contaminants on the Food Chain (CONTAM)
DHA = docosahexaenoic acid
DPA = docosapentaenoic acid
EFSA = European Food Safety Authority
EPA = eicosapentaenoic acid
GSHPx = plasma glutythion peroxidase activity
Hg = mercury
Ln = logarithmic transformation
JECFA = The Joint FAO/WHO Expert Committee on Food Additives
LOAEHC = Lowest Observable Adverse Effect Hair Concentration
Med = median
MeHg = monomethylmercury
Pb = lead
PCBs = polychlorinated biphenyls
PDI = probable daily intake
PHIME = Public Health Impact of Long-term Low-level Mixed Element Exposure in Susceptible Population Strata
PTWI = provisional tolerable weekly intake
PUFAs = polyunsaturated fatty acids
Se = selenium
THg = total mercury
TWI = tolerable weekly intake
UNEP = United Nations Environment Programme
US EPA = United States Protection Agency

1. Introduction

This study consists first by of an introductory section which provides some basic facts and knowledge on mercury (Hg) related to environmental health. In addition, the introduction provides the selting of this doctoral disertation study in its various aspects, particularly concerning exposure assessment and its uncertainties. In this section we also refer to work already published, as well as what will be covered in articles in preparation.
The second part of this doctoral disertation consists of published scientific papers and those submitted for publication.
This study was part of a prospective cohort study of the Public Health Impact of Long-term Low-level Mixed Element Exposure in Susceptible Population Strata (PHIME) project. The aim of this project was to improve the integrated health risk-assessment of long-term, low-level environmental exposure to toxic and essential metals *via* food. The study design was established within the project and included 4 recruitment areas: (1) the coastal Province of Trieste, Italy; (2) the city of Ljubljana, Slovenia and its surroundings (number of samples (n) =513) and Izola, Koper, Piran, Idrija and Kočevje (n=72); (3) the coastal city of Rijeka and its county, Croatia; and (4) the Greek islands of Lesvos, Chios, Samos and Leros in the Eastern Aegean. At recruitment, eligible women were approached for consent following their routine morphological ultrasound scan between the 20^{th} and 22^{nd} weeks of gestation (Italy), at their 34^{th}-38^{th} week of gestation visit (Croatia), or during their hospital stay for delivery (Slovenia, Croatia and Greece). For logistic reasons, the timing of the data and sample collection was different among the 4 centres and some biological samples were not collected in all countries (see Miklavčič et al., 2013a; Valent et al., 2013). Importantly however, the data collection protocol and sampling methods were the same in all 4 countries and all analyses of biological samples were performed by the same laboratory at the "Jožef Stefan" Institute in Ljubljana.
The part of this study referring to Hg exposure assessment in the Idrija Hg mine area was not part of the PHIME project. However, by including many foodstuff samples (see Miklavčič et al., 2013b), this part contributes importantly to the major aim of the doctoral study and gives a focus to this exposure assessment research that initially was missing.

1.1 Mercury

Hg is a naturally occurring element found ubiquitously in air, water and soil. It is distributed throughout the environment by both natural and anthropogenic (human) processes. It is found in various inorganic and organic forms such as mercuric sulfide (cinnabar ore), mercuric chloride and methyl mercury (MeHg). Hg species are broadly classified into three categories: (1) elemental or metallic mercury (Hg^0), (2) inorganic Hg: mercurous mercury (Hg_2^{2+}), mercuric mercury (Hg^{2+}) and (3) organic forms: MeHg and ethyl mercury (NRC, 2000; Horvat et al., 2011a), having different physical and chemical properties (Table 1).

Table 1: Physical and chemical properties of some toxicologically relevant mercury compounds (NRC, 2000).

chemical name	elemental mercury	mercuric chloride	mercurous chloride	methyl mercuric chloride	dimethyl mercury
molecular formula	Hg^0	$HgCl_2$	Hg_2Cl_2	CH_3HgCl	C_2H_6Hg
molecular structure		Cl-Hg-Cl	Cl-Hg-Hg	CH_3-Hg-Cl	CH_3-Hg-CH_3
molecular weight	200.59	271.52	427.09	251.1	230.66
aqueous solubility	5.6 x 10^{-5} g/L (25 ºC)	69 g/L (20 ºC)	2.0 x 10^{-3} g/L (25 ºC)	0.100 g/L (21ºC)	1 g/L (20 ºC)
density	13.534 g/cm^3 (25 ºC)	5.4 g/cm^3 (25 ºC)	7.15 g/cm^3 (19 ºC)	4.06 g/cm^3 (20 ºC)	5.4 g/cm^3 (20 ºC)
oxidation state		+2	+1	+2	+2

1.1.1 Elemental mercury

Elemental Hg is the uncombined or pure form of Hg. It is the only metal liquid at standard conditions of temperature and pressure, with a melting point of -38,84 ºC and a boiling point of 356.58 ºC. At room temperature, some of the elemental Hg will evaporate and form Hg vapour, which is colourless and odourless. Elemental Hg forms amalgams with gold, zinc and many metals, but not with iron. Hg does not react with most acids. It dissolves only in acids such as concentrated sulfuric acid and nitric acids.

1.1.2 Inorganic mercury species

In inorganic Hg species, Hg exists as mercurous mercury (Hg_2^{2+}) and mercuric mercury (Hg^{2+}). Hg_2^{2+} is most commonly found as calomel or mercurous chloride, Hg_2Cl_2. In previous centuries it was widely used in medicine (Suzuki et al., 1991). It is sparingly soluble in water (Table 2). Hg^{2+} forms a large number of stable chemical compounds. Many salts of Hg^{2+} are readily soluble in water, such as mercuric chloride ($HgCl_2$), sometimes called “corrosive sublimate”. In the past it was commonly used as a disinfectant and is well known for its acute toxicity to humans (Suzuki et al., 1991). For example the first symptoms were shown at the ingestion of an amount sufficient to reise blood Hg concentration to 14.3 mg/L (Magos and Clarkson, 2006). In contrast to $HgCl_2$, the water solubility of HgS (cinnabar) is extremely low (NRC, 2000; Horvat et al., 2011a; Suzuki et al., 1991).

1.1.3 Organic mercury

The mercuric ion can form a number of organic Hg compounds also called organomercurial compounds in which the Hg atom is covalently bonded to at least one carbon atom. The most common organic Hg compounds in the environment are monomethyl Hg compounds. To represent these compounds the term “methyl mercury” (MeHg) is usually used in the literature. Therefore, it is also used in this text, since in many cases, the complete identity of these compounds is not known, except for the monomethyl Hg cation, CH_3Hg^+ (MeHg) which is associated either with a simple anion, like chloride, or a large charged molecule (e.g. a protein) (Horvat et al., 2011a; Suzuki et

al., 1991). Monomethylmercury chloride is commonly available from chemical suppliers and is lipid soluble; this has given rise to the erroneous impression that all forms of MeHg are lipid soluble. In contrast, many water soluble compounds are formed in nature (Suzuki et al., 1991; NRC, 2000). Another organic Hg compound called dimethyl mercury is an uncharged, lipophilic compound that is highly volatile. It is a reference standard for some chemical tests and it is very harmful to humans and animals (Horvat et al., 2011a; Suzuki et al., 1991). Ethyl mercury is used as a preservative in vaccines in the thimerosal molecule, for which only limited toxicological information is available. This is based on the assumption that ethyl mercury is toxicologically similar to its close chemical relative, MeHg (Clarkson, 2002; Ball et al., 2001). It is used primarily in developing countries and its presence has been detected in children`s hair (Dorea et al., 2011), and in adults (Barregard et al., 2011).

1.1.4 Mercury in the environment

Hg is a global pollutant. Its global cycling begins with evaporation of Hg from the land and sea surface. Volcanoes can be an important natural source of Hg and the burning of fossil fuels is a major anthropogenic source of Hg to the atmosphere. Hg vapour is a chemically stable monoatomic gas, which is oxidized in the upper atmosphere to water-soluble ionic Hg. The process is not yet fully understood. Water-soluble ionic Hg is returned to the earth's surface in rainwater. Hg is transported worldwide before being deposited in different ecosystems. Therefore, this global cycling of Hg can result in its long range transport to the most remote regions of the planet such as Artic waters (Fitzgerald and Clarkson, 1991; Clarkson, 2002; Selin, 2009). Microorganisms in the aquatic environment are capable of converting inorganic Hg to MeHg, which bioaccumulates in higher organisms *via* plankton, herbivores fish, and in the top fish predators such as sharks and fish-eating marine mammals (EPA, 1997a, 1997b, 2003a; UNEP, 2002; 2013).

Because Hg is mostly deposited in the environment in its inorganic form Hg^{2+} and MeHg is more toxic than other stable forms of Hg, the biogeochemical cycling of Hg in the environment (Figure 1) plays a key role in modulating Hg toxicity. Direct transformation processes involving MeHg in the environment are the methylation of Hg^{2+} and demethylation of Hg^{2+}. The reduction of Hg^{2+} to Hg^{0} and the oxidation of Hg^{0} to Hg^{2+} affect MeHg formation indirectly by controlling levels of Hg^{2+}, the substrate for methylation (Barkay and Wagner-Döbler, 2005; Selin, 2009, Parks et al. 2013). The biogeochemical cycling of Hg and its transformation processes in the marine environment are shown in the Figure 1.

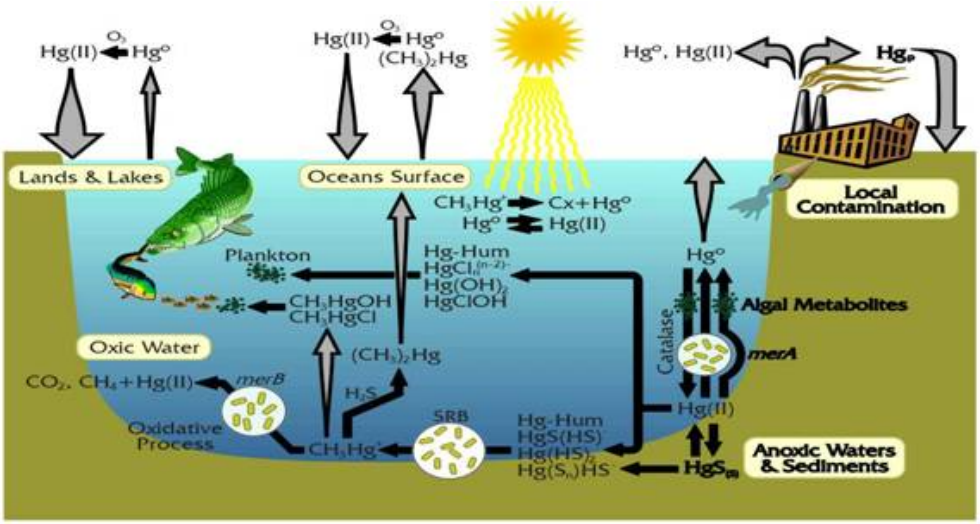


Figure 1: The geochemical cycle of Hg (Barkay and Wagner-Döbler, 2005).

Due to its high chemical activity, global spread, geochemical cycling, its chemical transformations in the environment, its bioaccumulation in the food chain and its toxic properties, Hg is still a very important issue in Europe and worldwide. Estimates of the total annual Hg release resulting from human activities range from one-third to two-thirds of total Hg releases (Horvat et al., 2011a). The United Nations Environment Programme (UNEP) is dealing with this issue on the global level, and in 2002 prepared a report "Global mercury assessment" which was the basis for the development of an action plan to reduce emissions of Hg into the air and control Hg exposure of people and other living organisms. An updated report has been prepred in 2013 (UNEP, 2013). Negotiations are currently underway at the global level, with the aim of managing the problem on a legal basis in the framework of global conventions to be adopted in this year.

1.2 Impact of mercury on human health

Nowadays, the general population is exposed to MeHg mainly through the consumption of fish or other seafood, and to elemental Hg from dental amalgams (UNEP and WHO, 2009; UNEP, 2002, EFSA 2012). Moreover, occupational Hg exposure is still important. Although concerns about Hg toxicity have led to reduction in the widespread use of Hg, because of its unique properties elemental Hg remains in use in artisanal and small-scale mining of gold and silver, chlor-alkali production, vinyl chloride monomer production and in products such as manometers for pressure measurement and control, thermometers, electrical switches, fluorescent lamb bulbs and dental amalgam fillings. Hg compounds are used in some batteries, pharmaceuticals, paints and as laboratory reagents and industrial catalysts (WHO, 1991; WHO, 2008).
In the biogeochemical cycling of Hg following subchapter the main exposure pathways of Hg will be first adressed. Moreover, in order to understand the impact of Hg on human health, the toxicokinetic properties of Hg will be summarized and finally the impact of Hg on human health will be discussed.

1.2.1 Exposure pathways

People may be exposed to Hg^0 or inorganic Hg species from dental amalgams. This amalgam is a mixture of several metals including silver and copper, but Hg is the important component, usually accounting for about 50% by weight. Dental amalgams release Hg vapour that may be inhaled. Although concentrations of Hg vapour in the air in the oral cavity were shown to exceed occupational health standards, the vapour retained is much less than that when inhaled under conditions of occupational exposure (Clarkson, 2002). In this, people may be exposed to Hg^0 or inorganic Hg species through inhalation of ambient air during occupational activities in which Hg and Hg compounds are produced or used. Exposure to elemental Hg or inorganic Hg forms can also occur due to use of some skin-lightening creams and soaps, the presence of Hg in some traditional medicines, use of Hg in cultural practices, and due to various accidental Hg spills in homes, schools or other locations (UNEP, 2002; WHO, 2008).
People are exposed to MeHg mainly through their diet, especially through the consumption of freshwater and marine fish, and the consumption of other animals that consume fish (such as marine mammals). The highest levels are found in fish that are apical predators of older age such as king mackerel, pike, shark, swordfish, walleye, barracuda, large tuna, scabbard, marlin and fish-consuming mammals such as seals and toothed whales (EPA, 1997a, 1997c, 2003a; UNEP, 2002; Miklavčič et al., 2011). Trimming, skinning and cooking the mercury-contaminated fish does not reduce the Hg content of the fillet portion. However, people who consume moderate amounts of a variety of fish are not at risk (UNEP, 2002). Minor exposure to other forms of Hg may result from the use of thimerosal (ethylmercury thiosalicylate) as a preservative in some vaccines and other pharmaceuticals (WHO, 2008, Dorea et al., 2011, Barregard et al., 2011).
In the present work exposure to MeHg during pregnancy was assessed in Slovenian women and compared to those in Northern Italy, the Croatian coast and Eastern Aegean Greek islands. In addition, the exposure of a smaller number of pregnant women from the contaminated area in Idrija was assessed from their cumulative exposure to Hg(0) in air, and Hg(II) and MeHg in food.

1.2.2 Toxicokinetics

The toxicokinetics of Hg is dependent on the dose, route of exposure (inhalation, oral or dermal exposure) and on the form of Hg to which a person is exposed. The toxicokinetics of different Hg species was described in detail in Horvat et al. (2011a).

Absorption

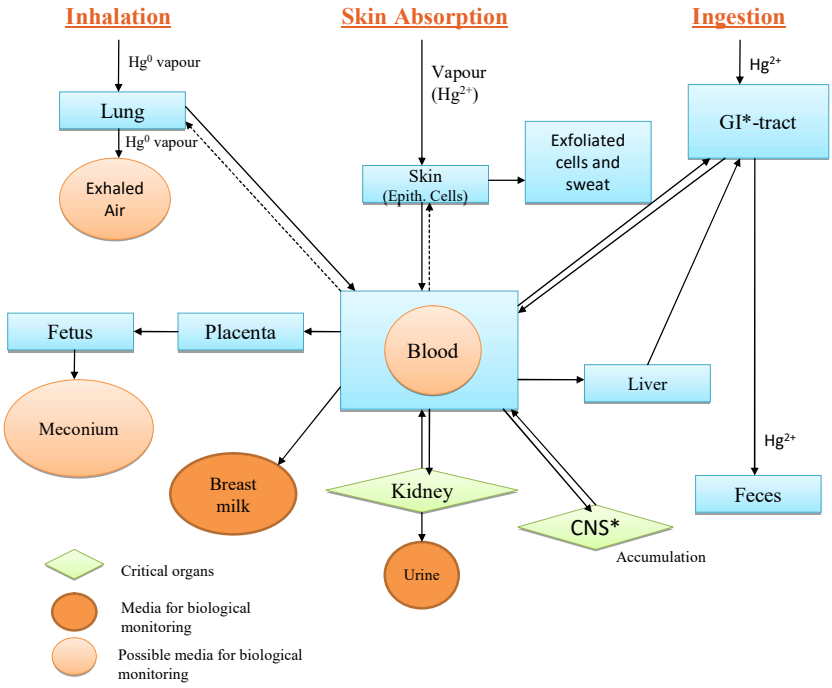
Elemental Hg is highly lipophilic (Table 2). Therefore, inhaled Hg vapour can easily cross the alveolar membranes of the lungs. Approximately 70-80 % of inhaled Hg vapour can be absorbed through the lungs (Hursh et al., 1976; Teisinger and Fiserova-Bergova, 1965; Sundborgh-Englung et al., 1998). Small amounts of elemental Hg such as that contained in a standard thermometer do not produce symptoms of intoxication if swallowed, because the absorption of ingested elemental Hg is negligible (Sue, 1994;

Wright et al., 1980). Animal studies indicate that the absorption of inorganic Hg compounds such as mercuric chloride is approximately 10-30 % (Piotrowski et al., 1992, Morcillo and Santamaria, 1995). The rate of oral absorption of Hg^{2+} compounds in rats is dependent on the intestinal pH, compound dissociation, age and diet (Endo et al. 1990). Importantly, MeHg is more readily absorbed in the gastrointestinal tract than inorganic Hg compounds. About 95 % of MeHg ingested is thought to be absorbed by the oral route. However, the absorption and bioavailability of MeHg may be affected by dietary components in food such as dietary selenium (Se) (ASTDR, 1999; Chapman and Chan, 2000; Syversen and Kaur, 2012). From the literature it is still not clear whether the acidic, high chloride conditions in the human stomach convert MeHg cysteine or other MeHg S-conjugates of MeHg present in seafood (EFSA, 2012). While MeHg and elemental Hg most likely cross cell membranes by passive diffusion, the methylmercury L-cystein complex (MeHgCys) is believed to be transported *via* amino acid transporters by mimicking L-methionine. MeHg L-cysteine and glutathione complexes might also be transported by organic anion transporters (EFSA, 2012; Bridges and Zalupes, 2012)

Distribution

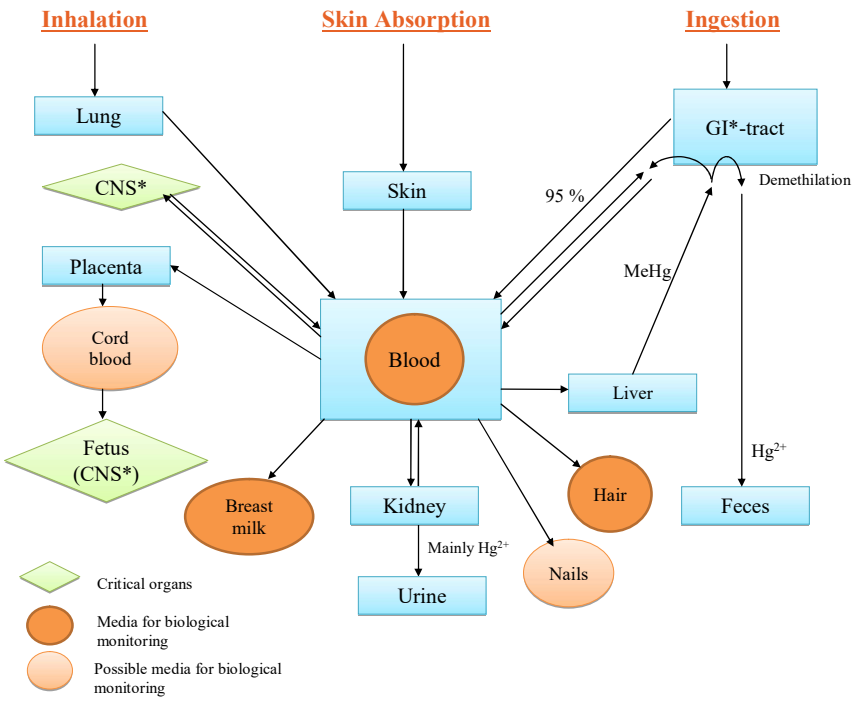
Because of its lipophilic nature, elemental Hg is distributed throughout the body and can cross the blood-brain and placental barriers very easily (Clarkson, 1989). The kidney is the major organ of Hg deposition after inhalation of elemental Hg vapour (Rothstein and Hayes, 1964; Eide and Wasenberg, 1993). In contrast to elemental Hg and MeHg, Hg^{2+} hardly crosses placental barriers (Dencker et al., 1983) due to its limited lipophilicity. After oral exposure to mercuric chloride, animal studies showed the highest level in the kidneys (Nielsen and Andersen, 1990; Sin et al, 1983). Renal uptake of Hg salts occurs from luminal membranes in the renal proximal tubule in the form of cysteine S-conjugates (Cys-S-Hg-S-Cys), or from the basolateral membrane through organic anion transporters (Bridges and Zalupes, 2005).
MeHg is distributed readily to all tissues, including the brain and foetus (ATSDR, 1999; EFSA, 2012). It is bound to thiol-containing molecules such as cysteine, which mimic methionine to cross the blood brain barrier and placenta through the neutral amino acid carrier (Bridges and Zalupes, 2005). Foetal distribution is similar to maternal distribution, with the exception of higher MeHg levels in erythrocytes (Sakamoto et al., 2004, 2008, 2010). MeHg is also excreted in breast milk. However, unlike the placenta, where MeHg moves more easily across the placental barrier than inorganic Hg, inorganic Hg is more readily eliminated in breast milk than MeHg (Sundberg and Oskarsson, 1992).
The distribution in red blood cells and in plasma is dependent on Hg species. After exposure to Hg vapour Hg levels in red blood cells are twice those measured in plasma (Cherian et al., 1978), after exposure to MeHg 90% of Hg is found in red blood cells (Kershew et al., 1980; Berglund et al., 2005), while after administration of mercuric salts the level of mercuric ions in plasma is similar to that of mercuric ions in red blood cells (ASTDR, 1999).
In general exposure can be estimated by measuring pollutant levels in various body tissues and fluids. These measurements are also known as biomarkers of exposure. Selection of media depends on the Hg compound, exposure pattern (e.g. chronic, acute) and time of sampling after exposure (Pirrone et al., 2002). As indicators of exposure to MeHg from diet generally the concentrations of total mercury (THg) and MeHg in blood and hair are used (NRC, 2000). The urinary Hg concentration is another commonly used biomarker that reflects exposure to inorganic Hg (Mason et al., 2001). The distribution, metabolism, appropriate or possible media for biological monitoring in the case of exposure to elemental Hg or inorganic Hg compounds, and in the case of exposure to

MeHg, are shown in Figures 2 and 3, respectively. From these figures it is seen that the media for biological monitoring of elemental Hg or inorganic Hg compounds are breast milk and urine, while in the case of exposure to MeHg the media are blood and hair. Different biological media in use are due to different toxicokinetic properties of inorganic and organic forms of Hg that have been already described.



*GI: gastrointestinal; CNS: Central Nervous System

Figure 2: Distribution, metabolism, and media for biological monitoring in the case of exposure to elemental Hg or inorganic Hg compounds (Adopted from Elinder et al., 1988).



*GI: gastrointestinal; CNS: Central Nervous System

Figure 3: Distribution, metabolism, and media for biological monitoring in the case of exposure to MeHg (Adopted from Elinder et al., 1988).

Metabolism

When elemental Hg enters the bloodstream, it can undergo rapid oxidation to the Hg^{2+} form by hydrogen peroxide-catalase (Clarkson 1989; Halbach and Clarkson, 1978). Because ethanol is a competitive substrate for hydrogen peroxide catalase, the oxidation of metallic Hg can be inhibited by ethanol (Nielsen-Kudsk, 1965). Oxidation of metallic Hg may occur in the brain, liver, lungs and probably other tissues to some degree (Magos et al., 1978; Hursh et al., 1976; Clarkson, 1989). In the literature there is no evidence for the synthesis of methylated Hg species in human tissue (EFSA, 2012; Barregard et al., 1994).

After absorption, MeHg can be converted into Hg^{2+} (Dunn and Clarkson, 1980) in the presence of reactive oxygen species, which may be formed in liver through the involvement of nicotinamide adenine dinucleotide phosphate (NADPH) cytochrome P450 reductase (Suda and Hirayama, 1992). The intestinal flora can also convert MeHg into inorganic Hg species (Nakamura et al., 1977; Rowland et al., 1980). Besides the liver and intestinal tract, demethylation can occur in the spleen and to a lesser extent in phagocytic cells and brain (EFSA, 2012). Mercuric mercury found in the brain could be a result of dealkylation of organic Hg species (Rodrigues et al., 2010), or oxidation of elemental Hg (EFSA, 2012; ASTDR, 1999). Demethylation also cannot be excluded in other tissues (EFSA, 2012).

Elimination and excretion

The main excretory pathways of elemental Hg and inorganic Hg compounds in humans are the urine and faeces, with a half-life of approximately 1-2 months (Clarkson, 1989). It must be pointed out that excretion of Hg depends on the duration of exposure (Sallsten et al., 1995; Hursh et al., 1976; Rice et al., 1989). The predominant excretory route for MeHg is the faecal (biliary) pathway, with less than one-third of total Hg excretion occurring through the urine (Norseth and Clarkson, 1971; EFSA, 2012). In a study that included four Japanese people, the excretion of MeHg into faeces was confirmed, but similar concentrations of MeHg were found in the urine compared to concentrations in faeces (Ishihara, 2000). Animal studies showed that MeHg is secreted in the bile after conjugation with liver glutathione-S-transferase and can be reabsorbed in the intestine (Berlin et al., 1975; Norseth and Clarkson, 1971; Tsutomu et al., 1990; Ballatori and Clarkson, 1985; EFSA, 2012). Interestingly, an association between certain GST genotypes and the retention of Hg has been established (Barcelos et al., 2012).
Clearance half-times are longer for MeHg (approximately 70-80 days) compared to inorganic Hg compounds (ATSDR, 1999; EFSA. 2012).

1.2.3 Health impacts

The toxicity of Hg is highly dependent on the dose, the route of exposure (inhalation, oral or dermal exposure) and the form of Hg to which the person has been exposed. In general it is necessary to distinguish between low level long-term chronic exposure, short-term acute exposure and occupational exposure. Moreover, in terms of evaluating the health impacts of Hg, the susceptibility to Hg exposure in different life stages (in-utero, postnatal, early life, adolescence, later in life) should be considered.

Elemental and inorganic Hg compounds

Effects on the nervous system appear to be the most sensitive adverse consequences following exposure to inorganic Hg. Symptoms include tremour, initially affecting the hands and sometimes spreading to the other parts of the body, emotional lability, insomnia, headaches, memory loss and performance deficits in tests of cognitive function (ATSDR, 1999; EPA 1997c, UNEP, 2002; Kobal and Grum, 2010).
In general in occupationally non-exposed people, dental amalgams are the main source of exposure to Hg vapour, which can be inhaled when released. It was estimated that 10 amalgam surfaces would raise urinary levels by 1 µg Hg/L. These are far below toxic levels or even those causing subtler-neurobehavioural and renal effects. However, excessive chewing may lead to urine levels in excess of 20 µg Hg/g creatinine, thereby approaching safe limits for health (Kingman et al., 1996; Sallsten et al., 1996). The mechanism of action of inhaled Hg vapour on brain function is not known. It is assumed that the vapour is first oxidized to inorganic divalent Hg which can become attached to thiol groups present in most proteins (Clarkson, 2002).
The most sensitive adverse effect observed following exposure to Hg^{2+} is the formation of autoimmune glomerulonephritis (inflammation of the kidney). Even in intermittently exposed miners with increased absorption of Hg vapour, only a slight glomerular and tubular effect of elemental Hg was found (Kobal et al., 2000).
In terms of identifying the effects of low level elemental Hg exposure, no definitive data

on the toxic effects of Hg° on children's mental development have been documented so far (WHO, 2003; EFSA, 2013). Some studies suggest an association of occupational exposure with reproductive effects in the female, such as toxicosis, spontaneous abortion, stillbirth and congenital malformations. However, these effects were not confirmed in some other studies (WHO, 1991, 2003). Moreover, few data are available on the health effects of long term exposure to low doses, and on the effects of elemental Hg on other organs, the heart and blood vessels. A study done on workers from Hg mines and mills from Spain, Slovenia, Italy and the Ukraine suggest an association between employment in Hg mining or refining and mortality from cardiovascular diseases (Boffetta et al., 2001). However, exposure to inorganic Hg in mines and mills does not seem strongly associated with cancer risk, with the exception of liver cancer (Boffetta et al., 1998).
Accidental ingestion of mercuric chloride by children resulted in cardiac effects (ATSDR 1999; EPA 1997c). Accidental drinking or ingestion of inorganic Hg can also cause considerable damage to the digestive tract and kidney, even with its limited absorption rate. Dermal exposure to ionic Hg can lead to adverse effects to the skin such us dermatitis (ASTDR, 1999; MPP and NRDC, 2005).
Elemental Hg represents the main health problem in people exposed through inhalation of ambient air during occupational activities such as artisanal and small-scale mining of gold and silver, chlor-alkali production or dental care (UNEP, 2002; WHO, 1991; WHO, 2008). Artisanal small-scale mining is a major source of gold production and the world's largest employer in gold mining, representing around 15% of the gold supply (around 400 tonnes) and 90% of the gold mining workforce worldwide. This is especially of concern because of the large number of miners involved, estimated to to be around 10-15 million in 70 countries (including approximately 3 million women and children), who are irresponsibly using Hg, causing helath and evironmental problems (UNEP. 2011).
In contrast to occupationally exposed people working in the chlor-alkali industry, exposure levels in the general population living near chlor-alkali plants are at the level of the normal population (Barregard et al., 2006; Gibičar et al., 2009).

Methylmercury

The major toxic effects of MeHg are on the central nervous system. Its toxic action on the developing brain differs in both mechanism and outcome from its action on the mature organ. The latent period between exposure and onset of symptoms in adults can be several weeks or even months, depending on the dose and exposure period. The mechanism underlying this long latent period is still unknown. The first symptom to appear at the lowest dose is paresthesia (numbness or a "pins and needles" sensation). This can lead to cerebellar atexia (dysfunction of the region of the brain that plays an important role in the integration of sensory perception), dysarthria (a motor speech disorder), and constriction of the visual fields and loss of hearing. These symptoms are caused by the loss of neuronal cells in specific anatomical regions of the brain. The mechanism is still not established with any certainty (Bakir et al., 1973; Clarkson, 2002). Cellular defence may be decisive in determining the toxic outcome and deserves further study. Thiol compounds probably play a key role. Resistant cells have higher levels of the thiol-containing peptide glutathione, which also plays a key role in the excretion of MeHg (Sarafian et al., 1996; Miura et al., 1994; Miura and Clarkson, 1993). MeHg is converted to Hg^{2+} in the brain. It could be that Hg^{2+} is responsible for brain damage. However, Magos et al. (1985) suggested that the intact MeHg radical is the toxic agent.
Salonen et al. (2000) found a significant correlation between Hg exposure levels and accelerated progression of carotid atherosclerosis. However, stress, which is believed to be a major risk factor, was not directly measured. Moreover, outlying and "influential

points" may play an important role in this study and it is questionable if the correlation would persist if very high Hg levels were excluded (Clarkson, 2002). Another study found a correlation between Hg exposure and pulse pressure, indicating that Hg intake from marine food might be involved in cardiovascular disease. However, in this case genetic factors could be responsible to some extent for the differences in pulse pressure between Greenlanders and Danes (Pedersen et al., 2005).

The foetal brain is more sensitive than the mature organ. The threshold level for neurologic effects in adults was estimated at about 50 mg/kg of Hg in hair. This level may be compared with an estimated threshold as low as 10 mg/kg for prenatal effects. Furthermore, MeHg consumed in diet might represent a risk of prenatal damage (Bakir et al., 1973; Cox et al., 1987; Clarkson, 2002). Opposed to the focal lesions seen in adult tissue, widespread damage to all areas of the foetal brain was evident. From experimental work in animals and in vitro it was suggested that MeHg in foetal brain binds to thiol ligands (-SH) groups on the tubulin and block the assembly process. The disassembly continues unchanged, thus leading to the complete loss of a tubule. Moreover, this inhibits both cell division and migration (Miura and Imura, 1987; Rodier et al., 1984; Philbert et al., 2000).

In low-level Hg exposure Cheuk and Wong (2006) showed that an increased Hg level is associated with attention-deficit hyperactivity disorder. However, at these levels of exposure other epidemiological studies have found more subtle adverse effects (Davidson et al., 1998). In a study that took place in Greenland, neuropsychological tests showed possible exposure-associated deficits, though reaching statistical significance only in a few cases (Weihe et al., 2002). Another study found that a Hg cord blood of level 0.9 μg/L is associated with delayed neurocognitive and psychomotor status of children at 12 months of age, but the association was less visible in subsequent examinations at 24 and 36 months of age. In this study the authors suggested that the cognitive and psychomotor deficits ascertained at 12 months of age that are possibly attributable to prenatal Hg exposure are subtle and rather easily reversible over the next 2 years of life (Jedrychowski et al., 2007). In addition, Cace et al., 2011 detected the influence of low-level MeHg exposure on foetal brain development as a decreased size of the newborn`s cerebellum.

Overall, a considerable body of literature exists focusing on the effects of prenatal Hg exposure through fish consumption on neurodevelopment. The findings have been inconsistent, particularly when assessing the effects of exposures to low Hg levels (Schoeman et al., 2009; Karagas et al., 2012). Possible reasons for the inconsistent results are different endpoints, the use of different biomarkers (cord blood vs. maternal hair Hg concentrations), choice of covariates for the statistical models, differences in the study populations and different concentrations of Hg (and other contaminants, e.g. PCBs) in fish (Schoeman et al., 2009). More comprehensive studies involving the same data collection approach and the same sampling methods in all the selected countries are needed to improve comparisons of Hg exposure and its effects. In studies evaluating the effects of low level Hg exposure, proper adjustment for potential negative confounding factors seems to be very important. In an overview study on the evidence for the human health effects of low level Hg exposure, Karagas et al. (2012) concluded that recent evidence suggests that low level MeHg exposure might affect foetal growth, and evidence exists that low levels of prenatal MeHg exposure may cause neurocognitive effects in early childhood. Evidence of Hg effects on cardiovascular disease have been inconsistent, and only few studies exist about the immunological effects of Hg exposure without any clear pattern (Karagas et al., 2012).

Table 2: Summary of toxic properties of different forms of Hg in humans (Horvat and Gibičar, 2006).

Form	Exposure	Effects	Biological indication	Disposition and mechanism of action
Hg^0	**Occupational**: Chlor-alkali industry, production of thermometers, thermostats and fluorescent bulbs, Hg mining, dentistry **Non-occupational**: Dental amalgam fillings	**Severe exposure**: Tremour, gingivitis, erethism, loss of memory, emotional and psychological disturbance, damage to kidneys. **Lower exposure**: Cognitive deficits, mild proteinuria, insomnia, loss of appetite, immunological disturbances. Damages are reversible.	**Hg in Urine**: chronic exposure, indication of Hg levels in kidney **Hg in Blood**: indicator of short term exposure **Exhaled air**: indicator of short term exposure. No good indicators for brain.	Inhaled elemental Hg is absorbed in the lung and enters the blood stream, high lipid solubility allows it to cross the blood/brain and placenta barriers easily, dissolved elemental Hg is oxidised in red cells, brain, liver, lung, and other tissues where it may inhibit the activity of some enzymes that contain –SH groups, denaturate proteins, damage cell membranes. At high concentrations it causes cell death and destruction of tissue. Mechanism of damage to the central nervous system is still not well understood.
Hg(I)	Removed from medical use. Rarely used.	Acrodynia (pink disease). Damage is reversible.	-	Inhibition of enzymes, its action is poorly understood.
Hg(II)	Antiseptic, leather industry, production of batteries, fungicides, use in bleaching soaps and creams	Chronic toxicity: neurological disorders similar to the effects of Hg^0. Repeated exposure to low doses effects the immune systems. Acute exposure may cause irreversible damage of kidney and indirectly cardiovascular collapse.	**Hg in Urine**	Similar to Hg^0, except direct passage through the above mentioned biological barriers is more difficult.
MeHg	Fungicide, food – mainly fish and other marine products	Immediate damage of neuronal cells and delayed symptoms of sensory disturbance, constriction of visual field, deafness, motor aberrations, mental disorders, cramps, paralysis	**Hg in blood** **Hg in hair**	MeHg is distributed in all tissues including brain. Prenatal damage occurs in all parts of the brain while in adults the damage is local. Inhibition of protein synthesis. It affects cell division and abnormal neuronal migration. It causes the destruction of microtubules in neuronal and astrocystic cells. MeHg damage to the central nervous system is still unexplained.

1.2.4 What is safe?

In relation to exposure assessment it is necessary to take into account recommended doses indicating what is safe. The reference levels set by different organizations and issues relating to this area are discussed below. In addition, maximum allowed levels of Hg in food set by Slovenian legislation are also given.

Based on risk assessment and other considerations, several countries and international organizations have established daily or weekly MeHg or THg intakes estimated to be safe or without appreciable risk to health, based on available information (WHO, 2008; EFSA, 2013). The US EPA (United States Environmental Protection Agency) reference dose estimated in 1997 is 0.1 μg MeHg per kg body weight per day and corresponds to a hair Hg concentration of about 1μg/g (NRC, 2000). This reference dose was based on data collected after the mass MeHg poisoning in Iraq. The defined threshold toxicological level associated with adverse effects to the foetus amount is as low as 10 μg/g in maternal hair (Clarkson et al., 2003). However, Schoeman et al., 2009 criticized extrapolation of results from the past mass poisoning environmental disaster and stressed that the reference dose obtained on this basis does not address the risk-benefit ratio of fish consumption by specific components of the general population, especially pregnant women. Based on evaluation of cross-sectional studies on the neurodevelopmental effects of prenatal MeHg exposure through maternal fish consumption, they suggested the Lowest Observable Adverse Effect Hair Concentration (LOAEHC) as 0.3 μg/g of maternal Hg. Longitudinal studies yielded an LOAEHC of 0.5 μg/g (Schoeman et al., 2009). Likewise, another study adjusted the exposure limit about 50% below the recommended level set by US EPA (Grandjean et al., 2007). However, it must be pointed out that the findings of different studies have been inconsistent (Schoeman et al., 2009), particularly when assessing the effects of exposures to low Hg levels and therefore it is difficult to provide a sound basis for risk assessment. Moreover, in line with the Joint FAO/WHO Expert Committee on Food Additives (JECFA), the European Food Safety Authority (EFSA) Panel on Contaminants in the Food Chain (CONTAM) has recently established a higher tolerable weekly intake (TWI) for MeHg. They decided to use the mean of the apparent NOEL from the Seychelles Nutrition Cohort at 9 and 30 months (11 mg/kg maternal hair) and the $BMDL_{05}$ from the Faroese Cohort 1 at age seven years (12 mg/kg in maternal hair), giving 11.5 mg/kg maternal hair as the basis for derivation of a health-based guidance value. Taking into account variation in the hair to blood ratio and interindividual variation in toxicokinetics, CONTAM established tolarable weekly intake for MeHg of 1.3 μg/kg body weight corresponding to a hair-Hg concentration of about 1.8 μg/g (EFSA, 2012).

In addition, CONTAM has established tolerable weekly intakes (TWIs) for inorganic Hg of 4 μg/kg body weight (EFSA, 2012) based on studies involving experimental animals. As summarized in the EFSA report (2012), the human data on toxicity after oral exposure to inorganic Hg were not suitable for dose-response assessment, but they indicated that kidney effects observed in experimental animals are relevant for humans.

Overall, variations among reference levels reflect the different risk assessment assumptions, data sets and uncertainty factors employed (NRC, 2000) (Table 3).

Table 3: Variation among different intake levels estimated to be safe set by different national and international organizations during the last 20 years.

Exposure	Intake estimated to be safe	Organization	Reference
THg	4.3 µg Hg per kg body weight per week	JECFA, 1987	WHO, 1987
MeHg	0.1 µg MeHg per kg body weight per day	EPA, 1997	EPA, 1997a EPA, 1997b
MeHg	1.6 µg MeHg per kg body weight per week	JECFA, 2004	WHO, 2004; WHO, 2007
MeHg	1.3 µg MeHg per kg body weight per week	CONTAM, 2012	EFSA, 2012
Elemental Hg	0.3 µg/m^3 air	US EPA, 1995a	EPA, 1995
Inorganic Hg	0.3 µg MeHg per kg body weight per day	US EPA, 1995b	EPA, 1995
Inorganic Hg	4 µg/kg body kg body weight per week	JECFA, 2004	WHO, 2004; WHO, 2007
Inorganic Hg	4 µg/kg body kg body weight per week	CONTAM, 2012	EFSA, 2012

Due to the importance of fish consumption in relation to the MeHg exposure of the general population, a Slovenian law established the maximum allowed Hg levels in fish; according to this, the maximum allowed level of Hg in anglerfish, Atlantic catfish, bonito, eel, emperor, grenadier, halibut, marlin, megrim, mullet, pike, plain bonito, poor cod, Portuguese dogfish, rays, redfish, sail fish, scabbard fish, seabream, shark, snake mackerel, sturgeon, swordfish and tuna is 1.0 mg/kg. For other fishery products and muscle of other fish, the maximum allowed level of Hg is 0.50 mg/kg (UL L 364, 20.12. 2006, p. 5).

1.3 Exposure assessment

Exposure assessment can be defined as measurement of the amount or concentration of a chemical(s) coming into contact with the body at potential sites of entry (skin, lung, GI tract) (James et al., 2000). The aim is to measure how much of a contaminant can be absorbed by an exposed target organism, in what form, at what rate and how much of the absorbed amount is actually available to produce a biological effect. Although the same general concepts apply to other organisms, the majority of applications of exposure assessment are concerned with human health (Zartarian, 2005). Studies of exposure contaminants can be designed to identify a specific population at risk, define norms for the general population, or examine the long-term effect of the reduction or increase of exposure to a contaminant by monitoring selected groups or the general population (Lioy, 1990).

In our study exposure was assessed through food consumption data, Hg levels in foodstuffs, and through different biomarkers of exposure (Miklavčič et al., 2011a; Miklavčič et al., 2011b; Miklavčič et al., 2013a; Mikalavčič et al., 2013). Different approaches to exposure assessment are discussed below in detail.

Mathematically, exposure is defined as:

$$E = \int_{t_1}^{t_2} C(t)dt$$

Equation 1

where E is exposure, C (t) is the concentration of the contaminant, which varies as a function of time and dt is an increment of time from t_1 to t_2 (Lioy, 1990). The EPA exposure guidelines for risk assessment define exposure as a potential dose, because the units are those of mass (e.g. µg/kg day or mg/kg day) (Greim and Snyder, 2008).

However, the amount deposited on or absorbed by an organ interface is not estimated by equation 1, but is implicitly assumed to be 100% absorbed. If these estimates are included, the equation is as follows:

$$\int D_I(t)dt = \int f(x)g(ab)C(t)dt$$

Equation 2

where D is the integrated dose (mass or mass/body weight) at a target tissue or cell D(t) is a time-varying function of dose, f(x) is the contact rate, g(ab) is a variable dependent on the target organ or system, and the bioavailability that affects the extent of absorption (ab). To calculate an internal dose, the variables used for g(ab) require values for deposition in the lung, absorption through the epidermal layers of the skin, or other routes of exposure (Lioy, 1990). When applying this equitation to different Hg species, one should take into consideration that the absorption is different for different Hg species, depending on the exposure pathway (see chapter 1.2.2).

There are two basic methods for quantifying exposure: exposure measurement and exposure modelling. Exposure measurement results in the most accurate and realistic exposure data that can occur via multiple pathways for an extended period of time. However, these accurate measurements are rarely possible. Models are used in those cases where monitoring data are unavailable or inadequate for exposure assessment (James et al., 2000; Greim and Snyder, 2008). They simulate the behaviour of chemicals and predict their concentration in the environment and organisms. Various exposure models exist, including atmospheric models, surface-water models, groundwater models and food chain models. All of these models are limited by uncertainty in the data input or the capability of a generic model to predict a specific exposure scenario. However, they can in principle make predictions for an unlimited number of exposure scenarios and predict past and future exposure (James et al. 2000).

If a potential chemical hazard exists, exposure assessment is part of the risk assessment process (Greim and Snyder, 2008). Risk assessment is an ever-evolving process whereby scientific information on the hazardous properties of chemicals and the extent of exposure results in a statement as the probability that exposed populations will be harmed (James et al., 2000). The probability of harm can be expressed either qualitatively or quantitatively, depending on the nature of the scientific information available and the purposel aim of the

risk assessment. Risk assessment is not research per se, but rather a process of collating existing data (James et al., 2000). In 1983, the National Research Council described risk assessment as a four step analytical process consisting of hazard identification, dose-response assessment, exposure assessment and risk characterization (Figure 4).

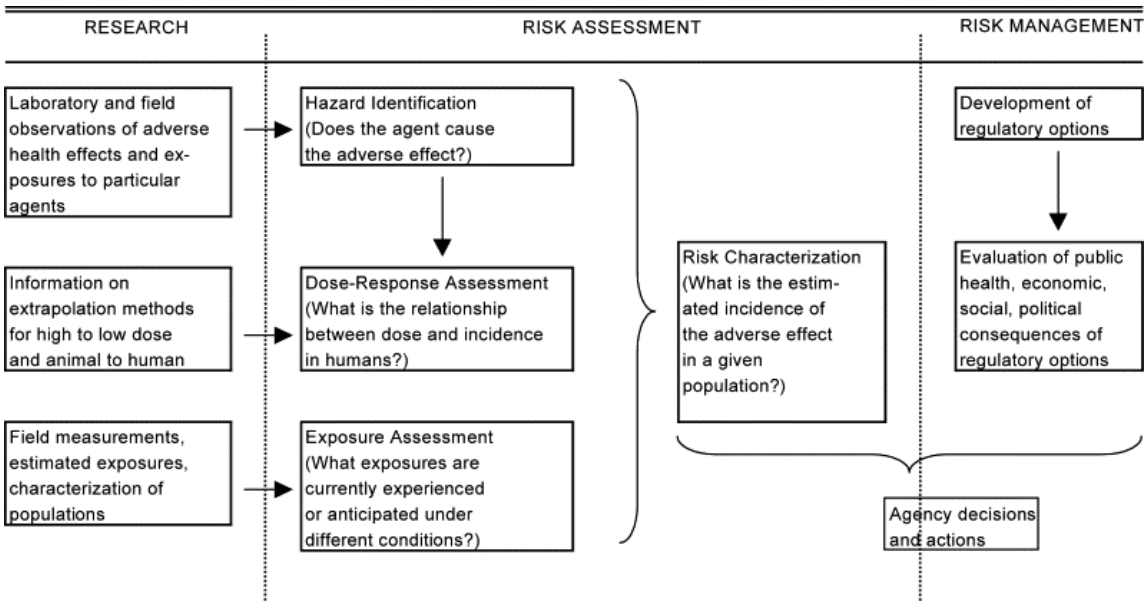


Figure 4: Elements of risk assessment and risk management. Risk assessment provides a mean to organize and interpret research data in order to inform decisions regarding human and environmental health (James et al., 2000).

1.3.1 Biomarkers

A biomarker is a substance used as an indicator of a biological state. It is a characteristic that can be objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological response to a therapeutic intervention. A biomarker of effect is a measurable biochemical, physiological or other alteration within the organism that, depending on magnitude, can be recognized as an established or potential health impairment or disease, while a biomarker of exposure is an exogenous substance, the metabolites or the product of interactions between a xenobiotic agent and some target molecule or cell that is measured in a compartment within an organism (NRC, 2000).
Biomarkers could provide, in some cases, a more accurate method for assessing exposure (Figure 5) (Schulte and Waters, 1999). They have the ability to identify if an exposure has occurred, the route of exposure, the pathway of exposure, or the resulting effects of the exposure (Schulte and Waters, 1999).

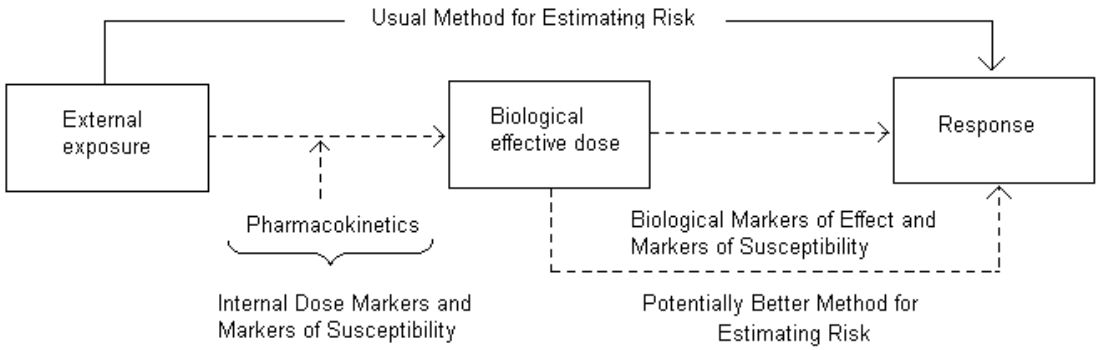


Figure 5: Rationale for using biomarkers to assess risk (Schulte and Waters, 1999).

When dealing with exposure and effect assessment, three types of biomarkers exist: biomarkers of exposure, biomarkers of susceptibility, and biomarkers of effect (Bird, 2008).

Biomarkers of exposure are the actual chemicals, or chemical metabolites, that are measured in the body or after excretion from the body to determine different characteristics of a person's exposure (NRC, 1995; Bird, 2008).

Biomarkers of Hg exposure

Because this work was mainly focused on biomarkers of Hg exposure, the kinds of biomarkers appropriate for human biomonitoring of a) occupational exposure, b) environmental exposure and c) prenatal exposure are discussed below.

As indicators of exposure to MeHg of dietary origin, generally the concentrations of total and organic Hg in blood and hair are used (NRC, 2000). THg in hair reflect past exposure to MeHg for up to a year or more, depending on the length of the hair. Thus the Hg content in the part of a hair strand closest to the scalp reflects the recent blood Hg levels (WHO, 1990). When assessing Hg exposure through THg levels in hair, possible external contamination of hair should be taken into account and potential leaching of Hg from the hair from hair treatments (Barregård, 2005; Grandjean et al., 2002). Due to the simplicity of hair sample collection, storage and THg hair analyses, THg in hair samples is the, preferred biomarker for population monitoring purposes. Hg concentrations in hair of a populations consuming no fish are normally below 0.5 µg/g; in a population with low to moderate fish consumption hair THg varies from below 1 to 2 µg/g, while people with frequent consumption (once or more per day) may have THg levels in hair exceeding 10 µg/g (UNEP and WHO, 2008). The presence of Hg in blood indicates recent or current exposure to Hg (UNEP and WHO, 2008; Horvat et al., 2011a). In the general population, MeHg from fish and Hg vapour from dental amalgam fillings influence the Hg concentrations present in blood. The exceptions are occupationally exposed people for whom Hg vapour is the major contributor to blood Hg concentration (Barregård, 1993; Langworth, 1992). Especially in blood samples, speciation analyses are important for accurate assessment of specific exposure (Horvat et al., 2011a; Vahter et al., 2000).

However, sample collection and MeHg determination in blood sample is not as practical, simple and fast as is sample collection and analytical determination of THg in hair.
The THg concentration in cord blood is the preferred biomarker for prenatal exposure, since it shows a better association with Hg-related neurobehavioural deficit in the child compared to Hg determined in maternal hair (Barregård, 2005; Grandjean et al., 2002). Moreover, inorganic Hg compounds are prevented from being transferred to the foetus and accumulate in the placenta (Ask et al., 2002). Hg levels in cord blood are in general higher than in maternal blood Hg (Horvat et al., 1988 a, b; Vahter et al., 2000; Stern et al., 2003; Murata et al., 2007), which is a consequence of higher haematocrit levels (Greener and Kochen, 1983).
Urinary Hg concentration is another commonly used biomarker that reflects exposure by inhalation of elemental Hg (Mason et al., 2001). Urine is the preferred matrix because collection of urine is non-invasive and elemental Hg is mostly excreted through urine. In the non-occupationally exposed population, the number of amalgam fillings was found to be a predictor for urinary Hg (Vahter et al., 2000; Akesson, 1991). Due to the slow extraction of Hg from kidneys through urine, urinary Hg reflects not only recent exposure to Hg, but also exposure that occurred sometime in the past (Langworth et al., 1992; Barregård, 1993). Although urinary Hg is used as a biomarker of exposure to elemental Hg, fish consumption can affect urinary Hg excretion (Soleo, 2003). This is probably not just the consequence of inorganic Hg present in fish, but also the result of *in vivo* demethylation of MeHg (Barregård, 2005).
Other biological media that are not so commonly used for biomonitoring Hg exposure purposes are cord tissue, breast milk and toenails. A strong correlation between THg levels in cord blood and cord tissue was observed, while the correlation between THg levels in maternal hair and cord tissue was weaker (Grandjean et al., 2005). In that study of Grandjean et al., 2005 cord tissue was almost as good a predictor of MeHg-associated neuropsychological deficits at 7 years of age as was Hg determined in cord blood. Hg levels determined in breast milk are used to evaluate past maternal exposure and to examine potential exposures for breast-feeding infants. Unlike placental, where MeHg moves more easily across the placenta barrier than inorganic Hg, inorganic Hg is more readily eliminated in breast milk than MeHg (Sundberg and Oskarsson, 1992). Infant exposure to MeHg or inorganic Hg via breast-feeding is low compared to the late foetal exposure (Björnberg et al., 2005). In an autopsy study a significant correlation between THg in toenails and MeHg in both blood and the occipital lobe was found (Björkman et al., 2007). However, there was no significant association between THg in toenails and inorganic Hg. The authors concluded that THg in toenails may be an indicator of MeHg stored in the body and may be a useful complement to Hg in hair for MeHg exposure assessment.
Our study was mostly focused on biomarkers of MeHg exposure because of the importance of MeHg exposure to pregnant women. However, we assumed that at the Hg contaminated site other species of Hg also contributed to the total Hg exposure significantly. Therefore, in this case we considered not only MeHg exposure through food consumption but also elemental Hg exposure through air inhalation and inorganic Hg species exposure through food consumption.

Biomarkers of effect are the quantifiable changes that an individual displays, indicating exposure to a compound and may indicate a resulting health effect (NRC, 1995; Bird, 2008). For example, different studies have reported the effects of long term low-level Hg exposure, mostly testing the association with early neurodevelopmental disorders using tests such as the Denver Developmental test II., the Bayley Scales of Infant Development (BSID-II), or the McCarthy Scales of Children's Ability (MSCA) (Barbone et al., 2004;

Barbone et al., 2003; Jedrychowski et al., 2006; Steuerwald et al., 2000; Debes et al., 2006; Freire et al., 2010). Another exsmple are the changes in the composition of proteins excreted in urine monitored by electrophoretic analyses that can very sensitively indicate early biological effects of occupational exposure to elemental Hg (Kobal et al., 2000). Biomarkers of inorganic Hg exposure are positively associated with kidney markers, especially with N-acetyl-β-glucosamidase (NAG) and also Alpha-1-microglobulin (A1M) and albumin (Jarosińska et al., 2008).

Biomarkers of susceptibility are indicators of the natural characteristics of an organism that make it more susceptible to the effects of exposure to a chemical. They can help define what sensitivities are more susceptible as well as critical times when exposures can be most harmful (NRC, 1995; Bird, 2008). For example vulnerability to prenatal Hg exposure might be enhanced or attenuated by differences in genetic susceptibility, diet, or exposure to other contaminants (NRC, 2000). Due to the ability of certain substances to protect against Hg toxicity, individuals with a dietary insufficiency of zinc, glutathione, antioxidants or Se, or those who are malnourished may be more sensitive to the toxic effects of Hg. Moreover, individuals with diseases of the liver, kidneys, nervous system and lungs are at higher risk of suffering from the toxic effects of Hg (UNEP and WHO, 2008; NRC, 2000). The sensitivity of an individual could be further influenced by polymorphism of the enzymatic activities involved in the metabolism of Hg (Gil and Pla, 2001). As was described in Chapter 1.2.1, glutathione S-transferase is involved in the metabolism of Hg, enhancing its excretion via the bile or urine. Glutathione S-transferase genes are a gene family involved in the detoxification of electrophilic compounds by conjugation with glutathione. In higher organisms at least five glutathione S-transferases gene classes have been well studied. It was shown that glutathione deficiency is associated with sensitivity to both mercury chloride and MeHg (Klautau-Guimarães et al., 2005 Gundacker et al., 2007; 2009).

For assessing risk the “ideal” biomarker does not exist. A biomarker of exposure may be appropriate in assessing exposure at a specific time. However, the health outcomes and consequent risk, may be associated at different time from those being assessed. Moreover, a biomarker of exposure could be insufficient to assess effects and therefore a biomarker of effect may be needed. Furthermore, one should be concerned about differential susceptibility in the population and thus a biomarker of susceptibility is needed (Ryan et al., 2007)

In addition, harmonised biomonitoring of Hg among different countries is crucial and should be coordinated in the future. A good example is the DEMOCHOPHES (LIFE09 ENV/BE/000410) project based on harmonised protocols developed in the EU FP6 COPHES project (Joas et al., 2011).

1.4 Mercury exposure assessment through fish consumption

Fish consumption and exposure to MeHg has been studied extensively (EFSA, 2012), and represents the most common MeHg exposure route (UNEP and WHO, 2008; EFSA, 2012). As was described previously, the major toxic effect of MeHg is on the central nervous system and the developing foetus is the most vulnerable target (NRC, 2000; WHO, 2011). Therfore, MeHg exposure assessment through fish consumption is especially important for pregnant women and their fetuses considered in our study. In this subchapter Hg assessment through fish consumption is discussed in detail, because of the importance of this MeHg exposure route for the most vulnerable population.

Assessing exposure and then the effects of Hg through fish consumption is complex because fish can also contain other chemical contaminants such us As, lead (Pb) and cadmium (Cd), polychlorinated biphenyls (PCBs)**,** organochlorine pesticides, aromatic hydrohcarbons, dioxin-like polychlorinated biphenyls, polychlorinated dibenzo-p-dioxins, dibenzofurans, polychlorinated diphenyl, polybrominated diphenyl ethers and polychlorinated naphthalenes. Moreover, fish are also an important source of protein with essential amino acids, macroelements (calcium, phosphorus, fluorine and iodine in marine fish), microelements (Se, zinc), fat-soluble vitamins and unsaturated fatty acids. They contain high levels of n-3 fatty acids such a docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA) and docosapentaenoic acid (DPA), which play an important role in the prevention of the development of cardiac and circulatory disorders, and they reduce mortality in patients with coronary diseases (Kris-Etherton, Harris, & Appel, 2002). Contrary to the negative effect of Hg on the developing nervous system, n-3 fatty acids are considered especially important to prenatal development. Rapid brain growth occurs primarily during the third trimester in humans (Rice et al., 2000), when DHA accumulates rapidly in the central nervous system (Clandinin et al., 1980).

Little is known about the potential protective effects of nutrients in fish against MeHg neurotoxicity in humans (Choi et al., 2008). Davidson et al. (2008) tested the hypothesis that the intake of selected nutrients in fish or measures of maternal nutritional status may represent important confounders when estimating the effect of prenatal MeHg exposure on child development. The study took place in the Republic of Seychelles, where fish consumption is high. The primary analysis examined the associations between MeHg, maternal nutritional measures and children's scores on the BSID-II (Bayley Scales of Infant Development-II) and showed an adverse association between MeHg and the mean Psychomotor Developmental Index scores at 30 month. However, their results contrast with those found in studies carried out in the Faroe Islands (Grandjean et al., 1997; Grandjean, 1998). There were several differences between these studies and the populations in general, but the most important reason for the different results seem to be that the diet consumed by Faroe Islandes included whale, while the Seychelles Islanders diet did not. The whale is known to contain higher levels of PCBs, methylmercury and possible other toxins than other fish (Raymond and Nicholas, 2004). Even though it is possible statistically to separate the neurodevelopmental effects of MeHg and PCBs, knowledge of the mechanisms and interactions of PCBs and MeHg is still missing (Mergler et al., 2007).

1.4.1 Fish consumption

Methods for assessing fish consumption

Different methods exist to assess fish consumption. Dietary intake measurements are discussed below in order to elucidate the issues in methods for assessing different foodstuffs intake and to better understand the information given by different methods.

Field methods

Field methods used to measure dietary intake are field methods in the sense that information is obtained either at the home of the individual concerned or from an individual who is not subject to laboratory restrictions (Rutishauser, 2005). It is possible to classify them in different ways. Rutishauser (2005) classified them into those that record intake as it occurs (records) and those that recall intake after it has occurred (recalls). Records can be descriptive or quantitative. Quantitative description can be recorded in household measures, by photographic means or by actual weighing of all the foods consumed over the period of study. The information on food intake can be converted to nutrient intake by means of food composition tables, by analysis of samples of the foods consumed or by laboratory analysis of a duplicate diet. Recalls can be quantitative, semi-quantitative and in terms of frequency. Quantitative amounts consumed can be described in household measures, by reference to photographs, three-dimensional food models and in terms of small, medium or large portions. Recalls can relate to specified (yesterday, previous year or longer) or indefinite periods of time. If the information on food intake is converted to nutrient intake, it may be presented in terms of categories (high, medium and low) rather than as absolute estimates of intake (Rutishauser, 2005).

Assessment of food consumption by food frequency questionnaire

A food-frequency questionnaire is a list of foods with a selection of options for reporting how often each food is consumed. Respondents indicate the most appropriate frequency option for each of the foods on the list by marking the appropriate column in the questionnaire. Typical options include daily, 3-4 times per week, 1-2 times per month, less than once per month and never. Depending on the focus of the questionnaire, the food list may contain only a few items or it may contain up to 200 items. A questionnaire designed to capture a high proportion of dietary intake will necessitate a much longer list than a questionnaire designed to capture a certain proportion of just one nutrient (Rutishauser, 2005). Some of them attempt to quantify the frequency information by obtaining additional data on portion size (large, medium, small or portion size in terms of a standard portion, or described by reference to a picture atlas of food portions) (Nelson et al., 1996; Rutishauser, 2005). They are almost always designed for self-completion so that they be optically scanned to save time on data entry and checking procedures (Rutishauser, 2005).
Food-frequency questionnaires are very useful when information on a large number of individuals is required, because they provide a relatively inexpensive and standardized way of collecting data. However, the data that can be obtained about food are of semi-quantitative nature with large random errors. Therefore, they only provide a guide and not a direct measure of the amounts of energy and nutrients that are ingested by an individual (Rutishauser, 2005).

In our study, which was part of the PHIME project, the participating women filled out a "short" and "long" questionnaire (see the appendix). These were designed at the University of Udine, Italy, translated from English into the local language and tested in each country. Questionnaires provide sociodemographic and health status information on the women and their family, information on the pregnancy and delivery, a detailed residential and occupational history of the women, a record of their smoking, drinking and general dietary habits, and a detailed food frequency assessment investigating their consumption of 138 food items adapted from a validated frequency questionnaire (Franceschi et al., 1993; Franceschi et al., 1995; Decarli et al., 1996). A section investigating the consumption of over 22 fish species commonly caught or marketed was also included in the long frequency questionnaire. Food intake categories in the questionnaire were converted to continuous estimates of daily intake:

1) never: 0 per day
2) less than once per month: 0.02 portions/day
3) 1-3 times per month: 0.07 portions/day
4) once time per week: 0.14 portions/day
5) 2-4 times per week: 0.43 portions/day
6) 5-6 times per week: 0.79 portions/day
7) once per day: 1 portion/day
8) 2-3 times per day: 2.5 portions/day
9) more than 3 times per day: 4.5 portions/day

The estimated portions of fish were then converted to grams. One portion of fresh or frozen fish was estimated to be around 150 g, while one portion of canned fish was estimated to be around 80 g (Valent et al., 2013; Miklavčič et al., 2013a; Miklavčič et al., 2011b). The questionnaires that were used are appended in the Annex.
To estimate the MeHg exposure of Slovenian pregnant women from frequencies of fish consumption and MeHg levels in the most frequently consumed fish, equation 3 was used to calculate the probable daily intake (PDI) (Miklavčič et al., 2011b).

$$PDI_{intake}\ (\mu g/kg\ body\ weight/\ day) = fish\ intake(g/day) \cdot [MeHg]_{most\ frequently\ eaten\ fish\ species} /\ body\ weight$$

Equation *3*

MeHg levels measured in the most frequently consumed species of fish from the Slovenian market from Miklavčič et al., 2011a were used. From previously obtained data and from the frequency of the most frequently consumed fish, we obtained an average estimate of the MeHg concentration of the most frequently consumed fish species ($[MeHg]_{most\ frequently\ eaten\ fish\ species}$) (Miklavčič et al., 2011b).
In addition, THg and MeHg concentrations were determined in the fish most frequently consumed by the women involved in our study, obtained from different supermarkets where this women most often bought fresh fish. The results have not been published yet and are listed in the Appendix. The PDI intake were calculated with levels included in Table 4.
In order to compare exposure assessment through fish consumption with exposure assessment through biomarkers of exposure the model used by EPA, 1997 was applied and the PDI was calculated according Equation 4, which is explained in details in NRC (2000).

$$PDI_{biomarkers} = \frac{C \cdot b \cdot V}{A \cdot f \cdot bw} \quad 4$$

$PDI_{biomarker}$: probable daily intake calculated according the biomarkers
C: concentrations in blood or hair. A ratio of 250:1 was used to convert hair concentration to blood concentration
b:elimination constant (0.014 days^{-1})
V: volume of blood in the body (5L)
A: absorption factor expressed as the unitless decimal fraction of 0.95
f: fraction of daily intake taken up by blood (0.05)
bw:body-weight

Table 4 shows a comparison of PDIs calculated *via* different methods is shown.

Table 4: PDIs assessed by different methods.

	Method	Calculated value µg/day kg bw
PDI_1	Food frequency questionnaire (Miklavčič et al., 2011b), MeHg levels in most frequently consumed fish (Miklavčič et al., 2011a) *(Equation 3)*	0.023
PDI_2	Biomarkers of exposure (Miklavčič et al., 2011b) *(Equation 4)*	0.029
PDI_3	Food frequency questionnaire (Miklavčič et al., 2011b), Hg levels in most frequently consumed fish (Miklavčič et al., 2011a and additional data appended) *(Equation 3)*	0.019
PDI_4	Food frequency questionnaire (Miklavčič et al., 2011b), Hg levels in most frequently consumed fish (only additional data appended - without tuna fish) *(Equation 3)*	0.0054

The assessed PDIs calculated by different methods are roughly comparable. The variations among these calculated levels are probably due to uncertainties in the applied methods that probably mostly relate to the accuracy of the data contained in the questionnaire and the uncertainties contained in the applied model (EPA, 1997; NRC, 2000). However, when we included only THg levels determined in fish from supermarkets that were probably from fish farms and excluded the levels of Hg in tuna, the PDI intake was around 4 times lower, indicating that MeHg levels in tuna fish comprised the majority of the PDI of the pregnant women involved in the study.

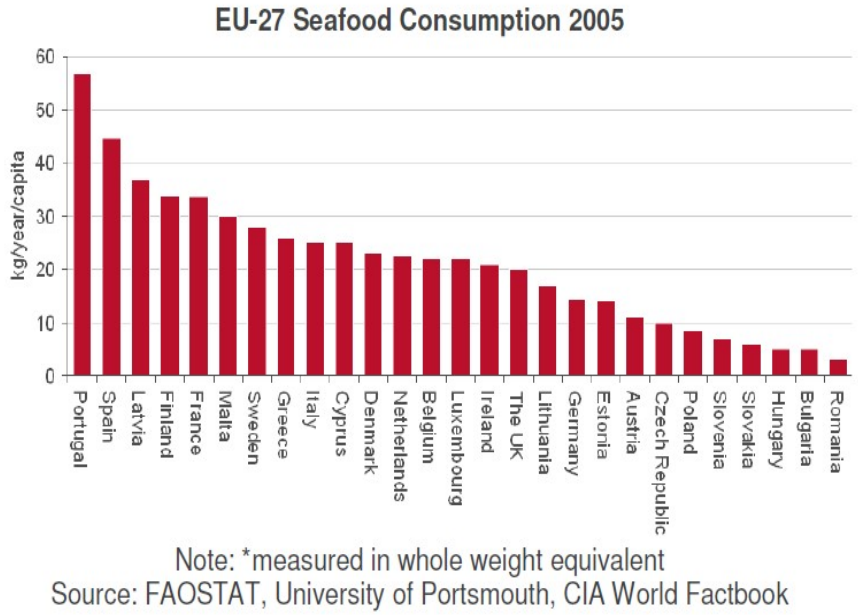
Laboratory methods of assessing dietary intake

In a laboratory study of dietary intake, the individuals whose food intake is being assessed have access only to foods that have been prepared under known conditions and for which the energy or nutrient composition has been determined. These individuals may live in a metabolic facility for the period of time or may live at home and take their meals in the metabolic laboratory. In both cases the amount of food provided to each individual is carefully measured. This approach enables precise estimation of the amounts of energy and nutrients that are ingested by an individual over a fixed period of time, but do not include the amounts of nutrients available for metabolism. Therefore, additional measurements are needed to determine the proportion of the ingested nutrients that is available for metabolism. With these methods, it is possible to measure accurately and

precisely, what people eat under certain conditions, but not what they would choose to eat, if they were not in a laboratory situation (Rutishauser, 2005).
The foods that are consumed may be sampled for analyses in a different way. A duplicate portion of all foods consumed during the period of the dietary record can be collected. Another approach is to collect only samples of the food consumed for analyses. Food samples can be reconstructed from the record of the foods that have been consumed during the period of the dietary record (Rutishauser, 2005).

Fish consumption in Slovenia

Fish consumption in Slovenia is one of the lowest in the European Union (Figures 6 and 7). Seafood consumption in Portugal was the highest in 2005 at 56.9 kg/year followed by Spain (45 kg/year). The average seafood consumption in the EU-27 is 22 kg/year. That is approximately 3 times more than in Slovenia, where the average fish consumption is only 7 kg/year (EU Seafood Industry Report, 2008). The fish contribution to total protein supply was also one of the lowest compared to other countries (Figure 7). Total fish catches in 2010 in Slovenia amounted to 1000 tonnes live weight and the same amount for aquaculture fish (Eurostat, 2010). Therefore, the annual fish production in Slovenia represents approximately only 1/7 of total fish consumption. Overall, actual data about different kinds of species consumed in Slovenia are scarce. The main species caught in the EU are herring, mackerel, blue whiting and sprat. The combined catches of Denmark and Spain represent one third of total EU catches. In Denmark the main species were sand eels, sprat and herring, while in Spain the main species were tuna, crustaceans and hake. Spain, France, the UK and Italy are the major contributors to farmed seafood and the species most widely produced are blue mussel, rainbow trout and salmon (EU Seafood Industry report, 2008). Nevertheless, from these numbers it is difficult to determine, which species are the most frequently consumed in Europe. It must be pointed out that the European Union is the world`s largest seafood market, and if EU-intra trade is included, it represented 45% of the world's total seafood imports in 2006 (EU Seafood Industry report, 2008).



Note: *measured in whole weight equivalent
Source: FAOSTAT, University of Portsmouth, CIA World Factbook

Figure 6: Annual average *per* capita fish consumption in European states (EU Seafood Industry report, 2008).

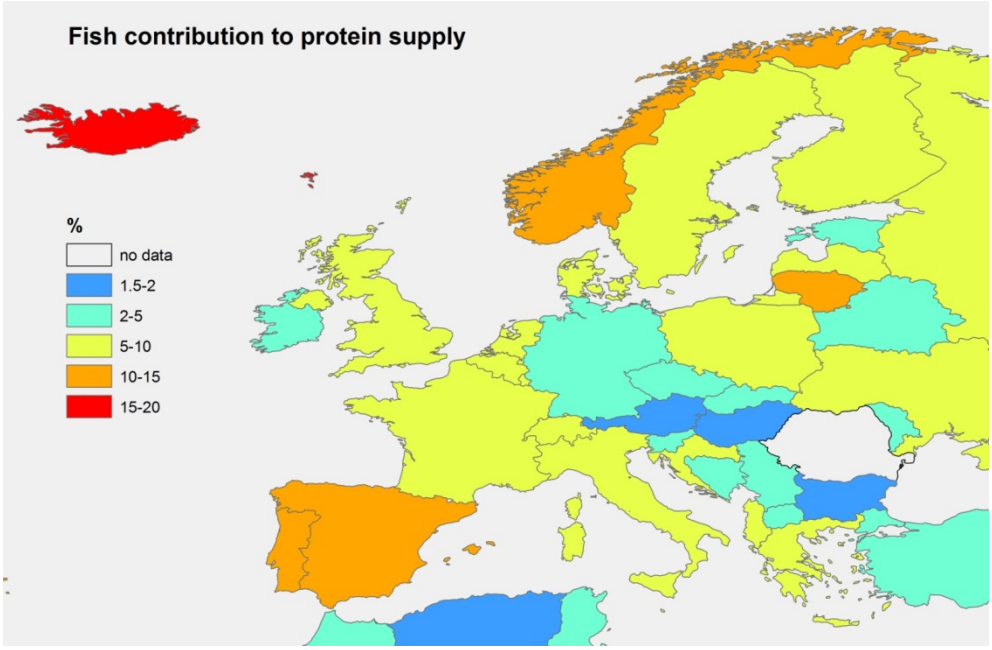


Figure 7: The relation between fish protein and total protein supply in Europe (based on data from FAO, 2007).

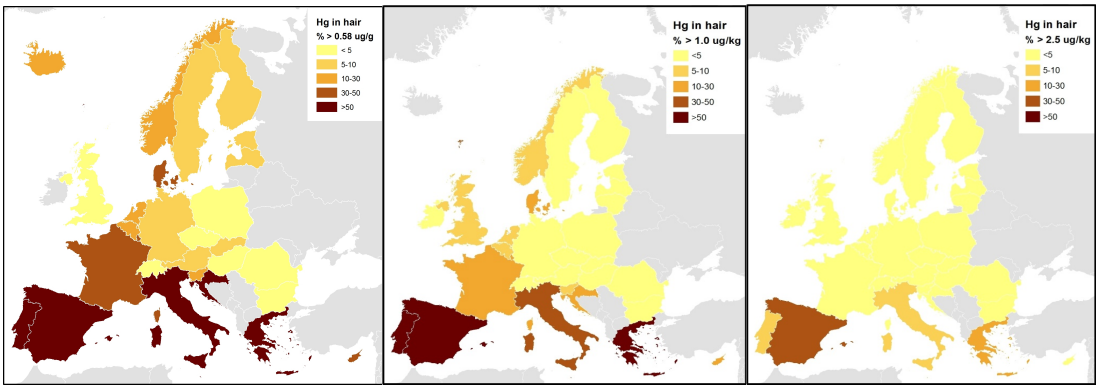


Figure 8: % of population exceeding 0.58 µg/g, 1.0 µg/g and 2.5 µg/g THg levels in hair (based on data from Bellanger et al., 2013)

Figure 8 shows that the exposure levels to MeHg in Europe (from a cross-sectional survey of European population exposure to environmental chemicals DEMOCHOPHES with a common European protocol involving 17 countries) do not coincide with the relation between fish protein and total protein supply in Europe (Figure 7). This indicates that MeHg exposure assessment made only through the frequency of fish supply assessed on a country level might be insufficient, and that actual Hg levels in fish should be included, especially when assessing MeHg exposure on the country level and not in a particular region of the country. This issue is further discussed in the next Chapter.

1.4.2 Mercury in fish

Because Hg is biomagnified in the aquatic food web, fish higher on the trophic level tend to have higher levels of Hg. Other kinds of food represent a minor source of exposure containing a negligible quantity of MeHg. The exception is foodstuffs grown on Hg contaminated sites (EFSA, 2004; Horvat et al., 2003; Falnoga et al., 2003).
Although the highest THg and MeHg levels are found in fish higher in the food chain (EPA, 1997a, 1997b, 2003a; UNEP, 2002), levels of Hg in fish are variable. The SCOOP data used by EFSA (2004) consisting of 14 912 samples resulted in a mean concentration of 109 μg/kg THg in fish with a high standard deviation (845 μg/kg). Importantly, due to the variability of Hg in fish, MeHg concentrations in fish together with the frequency of fish consumption should be included in exposure assessment (Mergler et al., 2007). As an example of the variability of Hg in fish within and between species, the levels of THg and MeHg in fresh fish and fish products found on different markets are shown in the Table 5.
The levels of Hg determined in fish can vary according to the fishing area. From the literature it is known that fish species from the Mediterranean Sea show higher levels of Hg in their tissues compared to the same species from the Atlantic Ocean (Renzoni et. al., 1997). Moreover, fish from aquaculture have lower Hg levels compared to wild fisheries (Kristan et al., 2012; Kelly et al., 2008). In addition, fish bought at the same food market can originate from fishing areas in all parts of the world (Kelly et al., 2008). The EU is the largest seafood market and if EU-intra trade is included, it represented 45% of the world's total seafood imports in 2006. Norway is the main supplier of seafood products to the EU accounting for 17.5% of total EU imports. Other suppliers are China, Iceland, Morocco, Argentina, Thailand, Vietnam, Ecuador, and Chile (EU Seafood Industry report, 2008). However, some populations such as the Faroe Islands (Weihe et al., 2005) and the population of the Greek Islands (Gibičar et al., 2006; Miklavčič et. al, 2013a), typically consume mostly local fish.
According to above listed facts, Hg exposure assessment from fish consumption can be very complex. Overall, when assessing the exposure through fish consumption, it is very important not only to take into consideration the amount of fish consumed, but also the type or species of fish consumed and the origin of the fish, since Hg in fish can vary greatly according these factors.

Table 5: THg in analyzed fresh or/and canned fish samples bought on the Italian, Polish, U.S. and Portugese market (Storelli et al., 2010; Cabañero et al., 2005; Usydus et al., 2008; Sunderland, 2007; EFSA, 2012).

Samples	**No**	**Market**	**Origin**	**THg (mg / kg wet weight)** Mean±SD (range)	**Refernce**
FRESH TUNA *Thunnus thynnus*	20	Italy	Tyrrhenian Sea	0.61 (0.07-1.76)	Storelli et al., 2010
FRESH TUNA *Thunnus* spp.		Portugal		0.31±0.01	Cabañero et al., 2005
FRESH SALMON	69	U.S.	imports	0.04±0.01	Sunderland, 2007
FRESH SALMON	11	U.S	Atlantic	0.013±0.17	EPA, 2003b
FRESH SALMON	289	U.S.	Pacific	0.04 ±0.01	EPA, 2003b
FRESH POLLOCK	12	U.S.	imports	0.03±0.002	Sunderland, 2007
FRESH POLLOCK	115	U.S.	Atlantic	0.02±0.01	EPA, 2003b
FRESH POLLOCK	37	U.S.	Pacific	0.06±0.03	FDA 2006a
FRESH ATLANTIC COD	5		Greater North Sea	0.053	EFSA, 2012
FRESH PLAICE	17		Greater North Sea	0.045	EFSA, 2012
GHOSTSHARK	10		South Adriatic Sea	3.14 (1-30-5.16)	EFSA, 2012
FRESH PILCHARD	300		Central and Southern Adriatic Sea	0.130 (<LOD-0.400)	EFSA, 2012
CANNED TUNA	8-10	Poland		0.067	Usydus et al., 2008
CANNED TUNA	45	Italy		0.41 (0.04-1.79)	Storrelli et al., 2010
CANNED MACKEREL	8-10	Poland		0.062	Usydus et al., 2008
CANNED SARDINE	8-10	Poland		0.024	Usydus et al., 2008
CANNED HERRING	8-10	Poland		0.032	Usydus et al., 2008
CANNED SPRAT	8-10	Poland		0.020	Usydus et al., 2008

The absorption and bio-availability of Hg in fish may be affected by Se in fish (ATDR, 1999). Hg and Se might bind to form Hg selenides with extremely low solubility, which are thought to be metabolically inert (Raymond and Ralston, 2004)
George et al. (2008) used L_{III}-edge X-ray absorption spectroscopy to characterize the

chemical forms of Hg and Se in swordfish samples and to compare them with the chemical forms after digestion with simulated gastric fluid. They confirmed that MeHg in fish is mostly coordinated by a single thiolate donor, which resembles cysteine. They did not find a chemical species that resembles mercury selenide. The Hg species was unchanged by treatment with simulated gastric fluid. This indicates that the more toxic methylmercury chloride will not be produced in the stomach. These results are in conflict with those of Rubenstein and Evens (1978). They thought that because of the high chloride ion content and low pH in the stomach, Hg could be converted to methylmercury chloride which could then be easily absorbed in the stomach (*5*).

$$CH_3HgS(Cys) + H^+ + Cl^- \rightarrow CH_3HgCl + (Cys)SH \quad 5$$

Cabañero et al. (2007) found that, depending on the species of fish, a low percentage of Hg in the fish is bioaccessible (less than 20%) in both simulated stomach and intestinal digestion (*in vitro*). It was concluded that the low MeHg recovery could be attributed to the low ability of enzymes *in vitro* method to release the Hg existing in the samples, perhaps due to the fact that Hg is complexed by Se, rather than a lack of bioaccessibility of methylmercury itself. In fact, they obtained a recovery of 89% from *in vitro* enzymolysis of samples spiked with MeHg. These results are consistent with Berntssen et al. (2004) findings. They showed higher foecal excretion and lower tissue accumulation of Hg in rats fed on contaminated fish than in those fed with methylmercury chloride spiked to fish.

1.4.3 The relation between mercury, selenium and arsenic in fish

Fish and other seafood represents an important source of Hg, Se and As intake (UNEP, 2002; Hughes et al., 2009; Reilly, 1996). Moreover, it is also known that As and Hg interact with essential Se. The glutathione-driven formation of compounds with these elements in blood links the metabolism of Hg^{2+} and arsenate with Se (Gailer, 2007).

Selenium

Selenium in the environment

Selenium is widely distributed in the geosphere. It can occur at relatively high concentrations in certain sedimentary rocks, such as limestones and coal deposits, where it may be associated with pyrite. It is particularly concentrated in the soils of some dry regions. The element occurs in alkaline soils in the form of selenates, which are readily available to plants. In acidic soils it occurs as selenides and to some extent as elemental Se, which are less available to plants (Reilly, 1996).
Higher plants are primary distributors of Se from soil to foods which provide human nutritional needs for this element. However, they themselves appear not to require the element for their own metabolism. Low Se soils do not inhibit plant growth and crop yields are not affected by a deficiency of the element (Reilly, 1996). However, lower plants, such as algae, have been shown to require Se for growth (Lindström, 1948).
Plants vary greatly in their ability to absorb Se from the soil. Plants that are normally rich in sulphur, such as members of the *Liliaceae family* (onions and garlic) and members of the *Cruciferae family* (including cabbage and broccoli) can be expected to be rich in Se

(Brown and Shrift, 1982).
Foods normally contain only organoselenium compounds. Inorganic compounds of the element, such as sodium selenite, only enter the diet as supplements or contaminants. Since the level of Se in soil is the primary determinant of the Se content of plant foods and animal fodder, and soil concentrations of the element can vary remarkably between countries and even between regions within countries, there is considerable variation in the levels of Se found in the different foods (Reilly, 1996; Smrkolj et al., 2005). Se levels and the variation of Se levels in different food groups are shown in Table 6.

Table 6: Se levels in different food groups (Reilly, 1993; Smrkolj et al., 2005; Smrkolj and Stibilj, 2004).

Food group	**Se (µg/g fresh weight) (Reilly, 1993)**	**Se in foodstuffs from Slovenia (µg/g fresh weight) (Smrkolj et al., 2005; Smrkolj and Stibilj, 2004)**
Cereals, cereal products	0.01-0.55	0.011; 0.009
Meat, fish, eggs	0.01-0.36	0.033-0.586
Milk, dairy products	<0.001-0.17	0.012-0.030
Vegetables, fruit	<0.001-0.022	0.0003-0.070

Selenium health impacts and recommended intake

Selenium was recognized as a nutritionally essential trace element in 1950. Until then it had only been known for its toxic effects (Reilly, 1996). Se is required for the activity of 25-35 enzymes with important functions (Rayman, 2000). Although it is normally present in all the cells of all higher animals, the functions of these enzymes are especially important in the brain (Chen and Berry, 2003; Schweizer et al., 2004) and endocrine organs (Kohrle et al., 2005). These enzymes and other Se-containing molecular species appear to be important in the prevention of cancer (Clark et al., 1996; Schrauzer, 2000) and in supporting a healthy immune system (Beck et al., 2003).
Se is toxic if is taken in excess. Exceeding the tolerable upper intake level of 800 µg per day can lead to selenosis (15 µg per kg body weight) (WHO, 1996). Symptoms of selenosis include a garlic odour on the breath, gastrointestinal disorders, hair loss, sloughing of nails, fatigue, irritability, and neurological damage. Extreme cases of selenosis can result in cirrhosis of the liver, pulmonary edema and death. Elemental Se and most metallic selenides have relatively low toxicities because of their low bioavailability. Selenates and selenites are very toxic and they have an oxidant mode of action similar to that of arsenic trioxide (Reilly, 1996).
Minimum Se requirements for humans recommended by the World Health Organization (WHO) (1996) are 30 and 40 µg per day for adult men and women. These calculations are based on the intake necessary to reach two-thirds of the maximal GSHPx (plasma glutathione peroxidase) activity. Duffield et al. (1999) calculated an upper minimal requirement of 39 µg of Se per day. In Slovenia the recommended dose of Se is 30 to 70 µg per day for both adult men and women (Referenčne vrednosti, 2004). In Slovenia analysis of 20 military total daily diet samples gave an average Se daily intake of 87 µg (Smrkolj et al., 2005) indicating an adequate Se daily intake compared to the recommended intake (Referenčne vrednosti, 2004). In addition, the levels of Se in whole blood of Slovenian residents (87±13 ng/g) were comparable with literature data for the general population (Mazej et al., 2003).

Selenium in fish

Fish and other seafood are important sources of Se in the human diet (Reilly, 1996; Smrkolj et al., 2005).
Although Se speciation is important in defining the bioavailability, mobility and toxicity of Se, and the Se contents in seafood are higher than in terrestrial foodstuffs, little is known about the chemical forms of organoselenium species in seafood (Yoshida et al., 2011). George et al. (2008) found in swordfish muscle a mixture of organic forms that resemble selenomethionine and an aliphatic selenyl sulfide such as Cys-S-Se-Cys. They found that the local chemical forms of Se do not change upon digestion of the fish with simulated gastric fluid. Cabañero et al. (2007) also found no modification of Se species during digestion, using chromatographic separation and inductively coupled plasma mass spectrometry (ICP-MS) for the quantification of Se species. These authors showed that the total amount of selenomethionine varied depending on the type of fish. In swordfish, 90% of the total Se was found to be selenomethionine, while tuna and sardine had 45% and 27% respectively (Cabañero et al., 2007). In fish muscle Kristan et al., 2013 found from 47% to 55% of soluble Se and the only species indentified was selenomethionine, which represented around 80 % of soluble Se. Moreno et al. (2001) in oyster tissue identified the trimethylselenium ion (9.8±0.8%) and selenomethionine (46±6%). According to the type of fish, between 50% and 83% of the Se was bioaccessible in simulated gastrointestinal digestion. Sardine had the highest Se bioaccessibility and the lowest Hg bioaccessibility compared to swordfish and tuna (Cabañero et al., 2007).

Arsenic

Arsenic in the environment

As is a widespread pollutant in many regions of the world, being the 20th most abundant element in the earth`s crust and a component of more than 245 minerals (Flora et al, 2005; Mandal and Suzuki, 2002). However, only a few of these are commonly encountered in significant amounts. As compounds are mobile in the environment. Weathering of rocks converts arsenic sulfides to arsenic trioxide, which enters the As cycle as dust or by dissolution in rain, rivers, or groundwater (Mandal and Suzuki, 2002). Therefore, groundwater contamination by As represents a serious problem to humans and animals in many areas (Mandel and Suzuki, 2002; Hopenhayn, 2006).

Arsenic in foodstuffs

The levels of As vary in different foods and are dependent on different factors such as the type of soil, water and geochemical activity (ASTDR, 2007). The provisional tolerable weekly intake (PTWI) for inorganic As from drinking water and food is assumed to be 15 µg/kg bw. Although foods of marine origin such as fish and shellfish have the highest levels of As, ranging from 0.3 to 2.7 mg/kg f.w. (Tao and Bolger, 1999; Perelló et al., 2008), the element is in organic forms such as arsenobetaine, arsenocholine or dimethylarsenic acid (Hughes et al., 2009; Šlejkovec et al., 2004) that are considered relatively nontoxic (Sakurai et al., 1997; Hughes et al., 2009). Arsenobetaine is rapidly excreted by humans unchanged (Vahter, 1994) and is assumed to have negligible toxicity due to its high LD50 in mice (> 10 000 mg/kg) (Kaise and Fukuit, 1992). In contrast to

organic As, it was demonstrated that inorganic As is associated with increased risk for a wide range of diseases such as cancer, diabetes, vascular disease, hypertension, neurological disorders, reproductive problems, and the well-known skin damage (Hopenhyn, 2006; NRC, 1999; IARC, 2002). Inorganic As is dominant in drinking water (Hopenhayn, 2006; van Eltern et al., 2002) and to a lesser extent in foods such as rice, flour, grape juice and spinach (Schoof et al., 1999). Some bottled Slovene mineral waters had As(V) concentrations around the permitted level of 50 μg/l for As in mineral waters (van Elteren et al., 2002).
Except for As in drinking water for the general population, diet is the major source of As (Abernathy et al., 2003).

Biomarkers of arsenic exposure

The biomarkers of As exposure normally used are its levels in urine, blood, hair or nails. The most common biomarker of inorganic As exposure is total As in urine. However, when using this biomarker caution is necessary, because consumption of seafood containing high levels of organic As can be a confounder of inorganic As exposure. As in blood is an appropriate biomarker for recent high-dose exposure. This biomarker is not reliable for inorganic As exposure, because it is cleared so rapidly, particularly at low levels of inorganic As exposure (ASTDR, 2007; Hughes, 2002; Horvat et al., 2011b).

The interactions between selenium - mercury and selenium - arsenic

Fish and other seafood are important sources of Se and a significant source of Hg. The simultaneous presence of Se in sufficient quantities in certain seafood seems to be able to counteract the toxic effects of Hg (Raymond and Ralston, 2004; Dyrssen and Wedborg, 1991). It is well recognized that Hg and Se bind to form mercury selenides with extremely low solubility, ranging from 10^{-58} to 10^{-65}, which is thus thought to be metabolically inert (Nuttall, 1987). The affinity constant of selenocysteine Se and Hg is high (~10^{-22}), and the free selenides that form during each cycle of selenocysteine synthesis have an exceptionally high affinity constant for Hg: 10^{-45} (Dyrssen and Wedborg, 1991). Following the first report on the protective effect of selenite against Hg toxicity by Parizek and Ostadalova in 1967, numerous studies have shown Se supplementation counteracts the negative impacts of exposure to Hg in all investigated species of mammals, birds and fish (Beijer and Jernelov, 1978; Lourdes et al., 1991; Whanger, 1992; Suzuki at al., 1998). Therefore, it is reasonable to assume that not only does Se have an effect on the bioavailability of Hg, but that Hg may also have an effect on the bioavailability of Se. Therefore, selenium's protective effect against Hg toxicity may simply reflect the importance of maintaining sufficient free Se to support normal selenium-dependent enzyme synthesis and activity (Suzuki et al., 1991; Raymond and Ralston, 2004). Overall, Se may offer protective effects towards Hg induced toxicity through several mechanisms such as sequestration of Hg (Magos et al., 1979; Ralston et al., 2008), antioxidative effect (Kaur et al., 2009; Ralston and Raymond, 2010), glutathione synthesis and increased glutathione peroxidase activity (Burk, 2002), high selenoprotein levels (Ralston et al., 2007) and increased demethylation (Khan and Wang, 2010).
Because Se and sulphur complex with Hg and both elements tend to be associated with sulphur in proteins, it is expected that Se and Hg tend to be bioaccumulated together in the tissues of marine organisms (Ganther et al., 1972). Se levels were found to be higher

in fish with elevated Hg levels (Kristan et al., 2012). At lower levels of Hg in fish an excess of Se in relation to Hg was observed, but no significant correlation was found between the two elements (Plessi et al., 2001; Miklavčič et al., 2011a). Moreover, different studies indicated Se and Hg co-accumulation in humans and other mammals (Falnoga and Tušek-Žnidarič; 2007). The levels of Hg and Se are strongly correlated in marine mammals (Ganther et al., 1972; Koeman et al., 1972). In certain human organs following exposure to high levels of inorganic Hg the coaccumulastion and retention of Hg and Se was established (Falnoga et al., 2000; Kosta et al., 1975).

As in the case of Hg and Se, antagonistic effects or mutual detoxification between As and Se have also been confirmed in many animal species including humans (Levander, 1977, Zeng., 2001; Zeng et al., 2005). The interaction between Se and As may occur directly and indirectly. This depends on the chemical forms and the dose of each. Increased biliary excretion of Se may be the principal mechanism by which As interacts with Se. However, the existence of an interaction between As and Se through biliary excretion at low level Se and As intakes still remains to be determined (Zeng et al., 2005).

1.5 Mercury exposure assessment in the Idrija mercury mine area

The town of Idrija in Slovenia is well known for its Hg mine. Liquid Hg was discovered in 1490. From 1575 to 1994 over 12 million tons of ore was excavated and a total of 153,000 tons of commercial Hg were extracted. In 1970`s information about the hazards of Hg and mass poisoning in Minamata (1956) and Iraq (1965) become widely known, and hence the price of Hg on the world market fell. Consequently in 1977 mining in Idrija was temporally suspended. The mine re-opened in 1983 and worked with minimal capacity until 1994 (Miklavčič, 1999). Approximately 500 years of Hg mining in Idrija has caused severe pollution in the environment of the town and its surroundings. During past years different studies were measuring Hg concentrations in sediments, soil, air, vegetation, water and animals (Horvat et al. 1999, 2002; Gosar and Šajn, 2003; Jereb et al., 2004; Kotnik et al., 2005; Groenlund et al., 2005; Kocman et al., 2011a, 2011b, Gnamuš, 2000) and elevated levels of Hg were found.
Due to the potential mixed exposure to MeHg, elemental Hg and inorganic Hg species by the most susceptible population such as pregnant women in this Hg contaminated area, samples of local foodstuff were collected and speciation analyses of Hg were made. Potential exposure was assessed through foodstuff consumption (Miklavčič et al., 2013b). To better understand the issues ivolved in exposure assessment at this contaminated site, work previously performed on Hg in this environment is discussed in the following subchapter.

1.5.1 Mercury in the environment of the Idrija mercury mine area

The concentrations of THg in soil samples vary between 8.4 and 415 mg/kg and are up to 40-fold higher than the maximum permissible (10 mg Hg / kg) set by Slovenian legislation (Kocman et al., 2004). It must be pointed out that these high levels in soil are in part due to the natural geological occurrence of Hg and anthropogenic activities. MeHg in soil was found in much lower concentrations, e.g. up to 32 µg/kg (Tomiyasu et al., 2012). The highest levels of MeHg were found in soil samples with the lowest levels of THg, and MeHg concentrations were correlated with the total organic content (Tomiyasu et al., 2012). Likewise, Hg in soil, Hg in the river Idrijca and in sedimants remained elevated (Hines et al., 2006, Žižek et al., 2007, 2011; Kocman et al., 2011b). Horvat et al. (2002) found increased THg levels downstream of the mine from a few ng/L to up to 500 ng/L. MeHg comprised round 1.5 % of THg above the town of Idrija, 0.2 to 0.7 % below Idrija and 2-3 % in river impoundments. Hg in sediments and flood plain soils increased by several-fold downstream, with MeHg comprising from 0.01 to 0.1% of THg (Horvat et al., 2002).
Hg is transfered to the river system due to erosion and surface runoff from contaminated surfaces located in and around the town of Idrija. Importantly, evidence of transformation of Hg exists, because of the increased relative contribution of dissolved Hg species downstream from the mine and as well as MeHg levels. Therefore, released Hg becomes more available. It was also reported that MeHg levels are spatially and seasonally variable, with higher values at the most contaminated sites during summer (Žižek et al., 2007) and at impounded areas (Hines et al., 2006).
After closure of the Hg mine in 1994, the levels of Hg in air have diminished significantly (Kotnik et al., 2004) from approximately 20 µg/m^3 (Kosta et al., 1974) to up to 5 µg/ m^3

in the contaminated former distillation plant during a hot summer day (Kocman et al., 2011a).

1.5.2 Mercury in fish

MeHg present in the river Idrijca bioaccumulates and is biomagnified across trophic levels from water, algae, periphyton to macroinvertebrates and fish (Žižek et al., 2007). Levels in fish from the Idrija Hg mine area are elevated (Horvat et al., 2004; Cvetković et al. 2010) compared to fish from non-contaminated areas (Kristan et al., 2012). These levels vary from 70 ng/g to 3700 ng/g wet fish muscle of THg and from 92 ng/g to 1172 ng/g of MeHg according to location and fish species (Horvat et al., 2004). However, it might be assumed that the population from this area rarely eat local fish from Idrijca river, because they are well informed about the threat from Hg and people buy fish mostly from the fish market.

1.5.3 Mercury in deer

Levels of Hg in deer are elevated compared to the levels in deer from non-contaminated areas (Table 7). General background levels amount from 1.5 ng/g to 5.0 ng/g w.w. in deer muscle (Gnamuš et al., 2000) and on average between 30 and 450 ng/g w.w. in deer kidney (Pokorny and Ribarič-Lasnik, 2002). Thus on average levels in deer levels in deer found in the Idrija Hg mine area are about 30-40 fold higher than background levels (Gnamuš et al., 2000). In terms of Hg exposure assessment from deer consumption by the population in Idrija, it must be considered that deer in Idrija are eaten rarely mostly by the hunter's family (personal communication). Based on PTWI for MeHg (1.3 µg/kg b.w.) and based on the maximum level of MeHg determined in deer from this area (72 ng/g) (Gnamuš et al., 2000), it can be estimated that a man (60 kg) can eat approximately one portion (150 g) of deer meat per day.

Table 7: Mean (X), median (Med), minimum (Min) and maximum (Max) levels of THg and MeHg in deer muscle from the Idrija Hg mine area (Gnamuš et al., 2000).

	n		**X ng/g w.w**	**Min ng/g w.w**	**Max ng/g w.w.**
Deer 2009 (our not published data)	2	THg	Roe deer: 72 Roe calf: 15		
		MeHg	Roe deer: 58 Roe calf: 15		
Deer (1990-1998)	9	THg	80	28	143
		MeHg	32	5.0	65.0
Deer (1990-1998) controls from area near Ljubljana	6	THg	2.8	1.5	5.0
		MeHg	1.0	0.4	1.5

1.5.4 Mercury in vegetables

T-Hg concentrations vary in the area of Idrija town considerably (Jereb et al., 2004; Falnoga et al., 2003). The highest levels were found in parsley leaves, followed by parsley roots, chicory and cabbage (Table 8). These elevated levels should not be overlooked especially for the vegetables such as chicory that in season could be eaten daily (Falnoga et al., 2001). MeHg in vegetables was low, varying from 0.02 to 2.3% of THg concentrations (Kobal et al., 2005). However, it must be pointed out that MeHg was determined in only a few samples. Therefore, in our study the MeHg was determined in all vegetables with the highest THg levels.

Table 8: Mean (X), median (Med), minimum (Min) and maximum (Max) levels of THg in vegetables collected from different gardens in the Idrija mine area in 2001 (Falnoga et al., 2003).

	n	X (ng/g d.w.)	Med (ng/g d.w.)	Min (ng/g d.w.)	Max (ng/g d.w.)
onion, pumpkin, tomato, carrot	15	189	63	<LOD	737
parsley leaves	8	3950	2300	242	13900
parsley roots	6	2480	1900	214	7680
cabbage	4	1061	301	241	3400
chicory	6	1960	1280	374	6430

1.5.5 Health studies performed in the Idrija mercury mine area

In 1572 Theophrastus von Hohenhain (known as Paracelsus) first described the serious condition of miners from the Idrija Hg mine area: “All the people who live there are deformed and paralysed, asthmatic and benumbed, without any hope of getting well” (Lesky, 1956; Kobal and Kobal Grum, 2010). The first physician to be employed in the Idrija Mercury Mine in 1754 was Joannes Antonius Scopoli, whose descriptions of mercurialism are still relevant today. His most important observations were on the interaction between elemental Hg and alcohol, the appearance of lung impairment, and depressive mood in mercurialism (Kobal and Kobal Grum, 2010). In 1965 a Health Safety Programme was applied in the area of occupational health of the miners, based on extensive research activities, and comprising technical protective measures, monitoring of Hg levels in the work places, evaluation of individual intensity of exposure, biological monitoring, personal protection, rotation of work places, reduction of length of exposure and targetted medical examinations. The results of this programme were successful since the number of Hg intoxicated workers dropped considerably. Although the areas worked where native Hg was present increased, there were no intoxicated workers after 1975 (Kobal and Dizdarevič; 1997).

Many studies have been made on the impact of long-term past exposure to elemental Hg in this area (Kobal et al.; 2001; Byrne et al. 1995; Boffetta et al.; 1998; Boffetta et al.; 2001; Falnoga et al.; 2000; Falnoga et al.; 2002; Kobal et al.; 2004; Grum et al.; 2006; Kobal et al.; 2008) indicating glomerular and tubular damage to exposed miners (Kobal et al.; 2001), the possibility of increased mortality among Hg miners due to ischaemic heart disease (Kobal et al. 2004; Boffetta et al.; 2001), no association with cancer risk, with the

possible exception of liver cancer (Boffetta et al.; 1998) and depression associated with negative self-concept in miners moderately exposed to elemental Hg and high alcohol consumption (Grum et al.; 2006). In addition, the potential effects of remote (past) long-term intermittent occupational elemental Hg vapour exposure on erythrocyte glutathione levels and some antioxidative enzyme activities in ex-Hg miners were studied (Kobal et al.; 2008). This study found a significant increase of glutathione levels, glutathione reductase and catalase activity in erythrocytes, indicating a possible response to the Hg burden accumulated during remote occupational exposure. Another study concluded that lifelong exposure to moderately elevated Hg levels of exposure does not have a significant influence on plasma Se (Falnoga et al. 2002). In human autopsy samples from Hg mine workers Se coaccumulation with Hg was confirmed in samples of thyroid, pituitary, kidney cortex and nucleus dentatus (Falnoga et al.; 2000).

2. Hypothesis and aims

Due to the important issue of MeHg exposure of the most susceptible general population, the study was mainly focused on MeHg exposure of Slovenian pregnant women. This exposure levels were then compared with Italian, Croatian and Greek population involved in the same project. It was expected that in central Slovenia exposure to MeHg is low, mainly due to the low frequency of fish consumption. Moreover, the highest exposure levels to MeHg would be found in populations living near the sea (such as the populations from Italy, Croatia and Greece) consuming more fish compared to inland populations. Although fish are the main exposure pathway of MeHg for the general population, they are also an important source of essential nutrients such as n-3 fatty acids and Se. Therefore, together with Hg levels Se and PUFAs were determined in fish samples available on the Slovenian market. In addition, the levels of PCBs in fish available on the Slovenian market could represent an issue worth investigating. In our study was also assumed, that particularly at low levels of exposure to MeHg from fish consumption, speciation analyses could be very important, because inorganic Hg from sources other than fish could contribute significantly. It was expected that not only Hg, but also As and Se concentrations determined in biological samples are mainly associated with fish consumption. In addition, exposure assessment of pregnant women to MeHg and other Hg species from the contaminated Idrija Hg site was included. The Hg exposure through consumption of home grown vegetables and mushrooms from the contaminated area in Idrija was considered. Elevated levels of Hg in vegetables and mushrooms from the Idrija Hg mine area have been found in the past. It is also known that vegetables from the contaminated area of the Idrija Hg mine contain mainly inorganic mercury, which has a very low bioavailability. However, in this thesis was assumed that frequent consumption of home grown vegetables from the contaminated site eaten daily could contribute significantly to Hg exposure.

Thus the aims of the thesis were as follows:

- to assess the Hg exposure of the most susceptible Slovenian population through fish consumption data, measurement Hg levels in fish from the Slovenian market and biomarkers of exposure
- to compare the exposure levels found in Slovenia with exposure levels in Mediterranean countries
- to estimate which of the biomarkers of prenatal low-level Hg exposure is the most appropriate for use in large-scale epidemiological studies

- to assess exposure of the study population to As and Se
- to assess the Hg exposure of the population living in the contaminated area (Idrija Hg mine area) through consumption of local food items

3. Publications

3.1 Mercury, selenium, PCBs and fatty acids in fresh and canned fish available on the Slovenian market

In this section, the article (Miklavčič et al., 2011a) entitled "Mercury, selenium, PCBs and fatty acids in fresh and canned fish available on the Slovenian market" by Ana Miklavčič, Prof. Dr. Vekoslava Stibilj, Prof. Dr. Ester Heath, Dr. Tomaž Polak, Janja Snoj Tratnik, Janez Klavž, Dr. Darja Mazej and Prof. Dr. Milena Horvat is presented. The article was published in Food Chemistry.

The purpose of this article was to ascertain the safety of fish consumption in Slovenia and to contribute to general understanding of the risks and benefits of consuming fish and fish products. In this article the levels of THg, MeHg and the levels of PCBs, as well as the quantity of some components that are essential for a balanced human diet (Se, n-3 and n-6 fatty acids) were determined in fresh and canned fish available on the Slovenian market. Because it is believed that human exposure to MeHg occurs mainly from eating fish, the Hg exposure of pregnant women, mostly from Ljubljana and its surroundings, was assessed from determination of THg in hair and from the frequency of fish consumption (Miklavčič et al., 2011a).

The research presented in this article was supervised by Prof. Dr. Milena Horvat, Prof. Dr. Vekoslava Stibilj, Prof. Dr. Ester Heath and Dr. Tomaž Polak. My contribution to this work was to process the Hg data, perform the statistical analyses and draft the manuscript. In addition, I carried out the analyses of THg and MeHg. Part of this work was presented at the scientific conference: 9th International Conference on Mercury as a Global Pollutant, June 7-12, 2009.

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Mercury, selenium, PCBs and fatty acids in fresh and canned fish available on the Slovenian market

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ABSTRACT

In order to contribute to the general understanding of the risks and benefits of consuming fish and fish products, total mercury (THg), monomethylmercury (MeHg), polychlorinated biphenyls (PCBs), selenium (Se), *n*-3 and *n*-6 fatty acids were determined in fresh and canned fish on the Slovenian market. Furthermore, the mercury exposure of pregnant women in Slovenia was assessed by determining total mercury (THg) in hair, and through fresh and canned fish consumption obtained by a food frequency questionnaire. Based on the frequency of fish consumption and levels of MeHg, THg and PCBs determined in the present study, it can be concluded that fish available on the Slovenian market do not represent a health risk. It was also confirmed that fish are important sources of selenium and *n*-3 fatty acids.

1. Introduction

Fish are beneficial to human health because of their high nutritional value. They are rich in protein with essential amino acids, macroelements (calcium, phosphorus, fluorine and iodine in marine fish), microelements (selenium, zinc), fat-soluble vitamins and unsaturated fatty acids. The fundamental difference between fish and other animals is in their high levels of *n*-3 fatty acids such as docosahexaenoic acid (22:6, *n*-3, DHA), eicosapentaenoic acid (20:5, *n*-3, EPA) and docosapentaenoic acid (22:5, *n*-3, DPA). They play an important role in the prevention of development of some diseases, especially in the development of cardiac and circulatory disorders and they reduce mortality in patients with coronary diseases (Kris-Etherton, Harris, & Appel, 2002). On the other hand, fish can also contain chemical contaminants such as toxic metals (mercury, arsenic, lead and cadmium), polychlorinated biphenyls (PCBs), organochlorine pesticides, aromatic hydrocarbons, dioxin-like polychlorinated biphenyls, polychlorinated dibenzo-*p*-dioxins, dibenzofurans, polychlorinated diphenyl ethers, polybrominated diphenyl ethers and polychlorinated naphthalenes.

Due to their contents of *n*-3 fatty acids, fish are considered especially important to prenatal development of the human nervous system. DHA accumulates rapidly in the central nervous system during the last trimester of pregnancy and the first 18 months of human postnatal life. Infants can receive DHA from their mother or can synthesise DHA from essential fatty acid precursors. Fish are also a significant source of the monomethylmercury cation CH_3Hg^+ (termed as MeHg in this manuscript) and the foetal brain is more sensitive than the mature organ to MeHg. The threshold level for neurological effects in adults was estimated at about 50 mg/kg of mercury in hair (Swedish Expert Group, 1971). This level may be compared with an estimated threshold as low as 10 mg/kg for prenatal effects (Cox et al., 1989). Furthermore, MeHg consumed in diet might represent a risk of prenatal damage (Clarkson, 2002).

Fish are also an important source of selenium (Se). Humans and animals require selenium for the functioning of a number of selenium-dependent enzymes. It is a trace element that is essential in small amounts, but can be toxic in larger amounts. It is also well known that mercury and selenium bind to form mercury selenides with extremely low solubility, which are thought to be metabolically inert. Numerous studies have shown selenium supplementation counteracts the negative impacts of exposure to mercury in all investigated species of mammals, birds and fish (Beijer & Jernelov, 1987; Culvin-Aralar & Furness, 1991). However, little is known about the potential protective effects of dietary selenium against MeHg neurotoxicity in humans (National Research Council, 2000).

Davidson et al. (2008) tested the hypothesis that the intake of selected nutrients in fish or measures of maternal nutritional status may represent important confounders when estimating the effect of prenatal MeHg exposure on child development. The study took place in the Republic of Seychelles, where fish consumption

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is high. The primary analysis examined the associations between MeHg, maternal nutritional measures and the scores of children on the BSID-II (Bayley Scales of Infant Development-II) and showed an adverse association between MeHg and the mean Psychomotor Developmental Index (PDI) scores at 30 months. However, their results contrast with those found in studies carried out in the Faroe Islands (Grandjean, Weihe, White, & Debes, 1998; Grandjean et al., 1997). There were several differences between these studies and the populations in general but the most important reason for different results seems to be the diet consumed by Faroe Islanders that included whale, while the Seychelles Islanders diet did not. The whale is known to contain higher levels of PCBs, MeHg and possibly other toxins compared to fish (Raymond & Ralston, 2004).

In order to ascertain the safety of fish consumption in Slovenia and to contribute to the general understanding of the risks and benefits of consuming fish and fish products, the aim of the current study was to determine the levels of total mercury (THg), MeHg and the levels of PCBs, as well as the quantity of some components that are essential for a balanced human diet (Se, *n*-3 and *n*-6 fatty acids) in fresh and canned fish available on the Slovenian market. Since it is believed that human exposure to MeHg occurs mainly from eating fish, the mercury exposure of Slovenian pregnant women was assessed from determination of THg in hair and from the frequency of fish consumption.

2. Materials and methods

2.1. Collection and storage of samples

Various species of fresh and canned fish of different geographical origin available on the Slovenian market were collected randomly in the capital city of Ljubljana. Fresh fish were bought from the fish market, while canned fish were bought in various supermarkets. The samples were collected in March, April and May of 2005. The edible part of fresh fish was homogenised with the food mixer (Kenwood). A sample was obtained from at least two fish from the same species. The content of fish in tins was first separated from the oil and then the fish content from two or three tins with the same lot code was homogenised with a food mixer. Homogenised samples were stored in a freezer at −18 °C. THg, MeHg and Se were analysed in all 52 fresh and 76 canned fish samples. Samples for PCB determination were freeze dried for 72 h at 52 °C and 0.05 mbar. The analyses of fatty acid composition and PCBs were performed on 20 and 51 fish samples, respectively (Table 1). Canned fish that were selected for PCBs and fatty acids determination were preserved in own juice without addition of various oils.

Hair samples were collected from 469 pregnant women living in Slovenia in 2008 in the last month of their pregnancy or in the first month after childbirth. Hair was cut with ceramic scissors close to the root in the occipital region of the scalp. Each hair sample was stored in a plastic bag and then analysed without any cleaning or special treatment. The participating women filled out food frequency questionnaires from which the frequency of fresh fish and canned fish was assessed. One portion of fresh fish was estimated around 150 g, while one portion of canned fish was estimated around 80 g. The pregnant women filled in the food frequency questionnaire on the same occasion that the hair samples were collected.

2.2. Study methods

2.2.1. Determination of THg in fish

An amount of 0.030–0.200 g of fish tissue was placed in a 50 ml volumetric flask, to which 1 ml of distilled water, 2 ml of a mixture of 65% HNO_3 (Merck, Germany, p.a.) – $HClO_4$ (Merck, Germany, suprapur) (1:1, v/v) and 5 ml of 96% H_2SO_4 (Merck, Germany, suprapur) were added. The open flasks were heated at 200 °C on a hotplate for 20 min. After cooling, the digested samples were filled up to 50 ml with distilled water. A semi-automated Mercury Analyser based on cold vapour atomic absorption spectrometry was used for determination of total mercury in the digested samples. Akagi (1997) described the procedure in detail.

The accuracy of the results was checked by analysing the standard reference material Tuna Fish Homogenate IAEA 350. Levels of THg determined in the reference material (4.64 ± 0.12 mg/kg) were in good agreement with the certified level (4.64 ± 0.26 mg/kg). All the samples were prepared and measured in duplicate and the deviation between them was less then 10%. The limit of detection calculated as three times the standard deviation of the blank sample was 0.1 ng/g and the limit of quantification (LOQ) was estimated at 1 ng/g.

2.2.2. Determination of THg in hair

About 0.020 g of human hair was weighed in a sample boat. THg in hair was determined by thermal combustion at 650 °C, amalgamation and atomic absorption spectrometry using the Direct Mercury Analyzer (Milestone, USA). The procedure has been described in detail elsewhere (EPA Method 7473, 1998).

The reference material NIES 13 (human hair) was used to check the accuracy of the results and the found value (4.51 ± 0.05 mg/kg) was in good agreement with the certified value (4.42 ± 0.20 mg/kg). Although the sample mass was low, the deviation between duplicates was less than 10%.

The limit of detection (LOD) of the method calculated as three times the standard deviations of the blank sample was 0.2 ng/g of hair sample and the LOQ was 2 ng/g hair sample.

2.2.3. Determination of MeHg

About 0.030–0.200 g of each sample was weighed into a micro diffusion cell containing cysteine-impregnated paper and after addition of 0.1 ml of 1% NaCl (Merck, Germany, p.a.) solution, 0.1 ml of a saturated solution of $K_4[Fe(CN)_6]$ (Merck, Germany, p.a.) and 0.5 ml of 8 M prepared from 96% H_2SO_4 (Merck, Germany, suprapur) the mixture was left in an oven at a temperature of 70 °C overnight, where volatilisation of MeHg from the samples onto the cysteine-impregnated paper took place. 0.3 ml of a solution of 4 M KBr (Merck, Germany, p.a.) and 0.3 ml of 4 M H_2SO_4 (Merck, Germany, suprapur) saturated with $CuSO_4$ (Merck, Germany, p.a.) was added to the cysteine-impregnated paper. MeHg was extracted from the cysteine-impregnated paper into 0.5 ml of toluene. Two microlitres of the toluene was injected into a packed gas chromatography column and MeHg was detected by gas chromatography–electron capture detection (GC–ECD). The procedure has been described in detail elsewhere (Horvat, Byrne, & May, 1990; Horvat, May, Stoeppler, & Byrne, 1988).

The accuracy of the results was checked by analysis of the certified reference material TORT 2 Dogfish Muscle and the found values (0.150 ± 0.005 mg/kg) were in good agreement with the certified value (0.152 ± 0.013 mg/kg). All the samples were prepared and measured in duplicate and the deviation between duplicates was less than 10%.

The LOD of the method calculated on the basis of three standard deviations of the blank was 0.2 ng/g, while the LOQ was estimated at 2 ng/g of fish sample.

2.2.4. Determination of Se

Approximately 0.4–0.5 g of sample was weighed in a Teflon tube, where mineralisation of the sample was performed using a digestion mixture of 0.5 ml of 96% H_2SO_4 (Merck, suprapur) and 1.5 ml of 65% HNO_3 (Merck, Germany, suprapur) by heating the tube in an aluminium block, kept overnight at 80 °C and then kept at 130 °C for 60 min. After cooling the solution to room temperature, 0.150 ml V_2O_2 (Merck, p.a.) in 96% H_2SO_4 (Merck, Germany,

Table 1
THg, MeHg,[a] MeHg, Se, PCBs, *n*-3 fatty acids and the ratio of *n*-6/*n*-3 fatty acids in analysed fresh and canned fish samples bought on the Slovenian market.

Samples	No	Origin	THg (mg/kg wet weight) Mean ± SD (range)	MeHg (mg/kg wet weight) Mean ± SD (range)	MeHg (%) Mean ± SD (range)	Se (mg/kg wet weight) Mean ± SD (range)	PCBs (ng/g dry weight) Mean ± SD (range)	*n*-3 fatty acids (mg/100 g wet weight) Mean ± SD (range)	*n*-6/*n*-3 Mean ± SD (range)
Fresh trout *Onchorhynchus mykiss*	1	not known	0.045	0.042	93	0.164	/	/	/
Fresh tuna *Thunnus thynnus*	3	Indonesia; FAO 41	0.596 ± 0.486 (0.162–1.110)	0.559 ± 0.508 (0.027–1.120)	93 ± 11 (81–101)	0.747 ± 0.270 (0.461–0.997)	<LOD *n* = 3	218 *n* = 1	0.18 *n* = 1
Fresh salmon *Salmo salar*	3	Norway, Holland; FAO 27	0.036 ± 0.004 (0.033–0.040)	0.027 ± 0.012 (0.015–0.039)	74 ± 27 (45–98)	0.180 ± 0.002 (0.178–0.182)	13.6 ± 1.9 (11.9–15.7) *n* = 3	1360 *n* = 1	0.36 *n* = 1
Fresh european flounder *Platichthys flesus*	2	Holland, Danmark; FAO 27	0.014; 0.005	0.010; 0.002	71; 40	0.166; 0.110	<LOD *n* = 2	112 *n* = 1	3.6 *n* = 1
Fresh cod *Gadus morhua*	4	Holland, Danmark, Croatia; FAO 27	0.069 ± 0.050 (0.031–0.139)	0.066 ± 0.059 (0.020–0.149)	87 ± 23 (54–107)	0.305 ± 0.100 (0.234–0.453)	/	315 *n* = 1	0.10 *n* = 1
Fresh mediterranean squid *Loligo vulgaris*	1	France; FAO 27	0.047	0.031	66	0.421	/	474 *n* = 1	0.05 *n* = 1
Fresh conger *Conger conger*	1	Croatia; FAO 37.1, 37.2, 37.3	0.864	0.731	85	0.417	15.3 *n* = 1	384 *n* = 1	0.41 *n* = 1
Fresh octopus *Octopus vulgaris*	1	Philippines	0.012	0.011	92	0.112	/	153 *n* = 1	0.13 *n* = 1
Fresh turbot *Psetta maxima*	1	Spain	0.042	0.036	86	0.264	1.8 *n* = 1	237 *n* = 1	0.17 *n* = 1
Fresh angler *Lophius piscatorius*	3	Croatia FAO 37.1, 37.2, 37.3	0.291 ± 0.336 (0.071–0.678)	0.287 ± 0.361 (0.045–0.702)	86 ± 21 (63–104)	0.414 ± 0.169 (0.220–0.529)	2.3 ± 0.8 (1.8–3.3) *n* = 3	131 *n* = 1	0.11 *n* = 1
Fresh scorpaena *Scorpaena scrofa*	2	Morocco FAO 34	0.134; 0.371	0.134; 0.265	100; 71	0.343; 0.396	<LOD; 0.6 *n* = 2	/	/
Fresh goatfish *Mullus Barbatus*	2	Croatia FAO region not known	0.210; 0.108	0.221; 0.080	105; 74	0.377; 0.508	21.1; 0.4 *n* = 2	/	/
Fresh common pandora *Pagellus erythrinus*	2	Croatia FAO 37.1, 37.2, 37.3	0.070; 0.936	0.076; 0.719	109; 77	0.307; 0.370	31.6;21.9 *n* = 2	388 *n* = 1	0.20 *n* = 1
Fresh grey mullet *Mugil chepalus*	2	Croatia FAO 37.1, 37.2, 37.3	0.069; 0.031	0.076; 0.023	110; 74	0.089; 0.209	33.9;30.5 *n* = 2	/	/
Fresh atlantic herring *Clupea harengus*	2	Danmark FAO 27	0.040; 0.038	0.040; 0.026	100; 68	0.404; 0.362	17.0;17.3 *n* = 2	/	/
Fresh trout *Salmo trutta*	2	Slovenia FAO 34	0.025; 0.037	0.025; 0.025	100; 68	0.169; 0.176	21.1 *n* = 1	1420 *n* = 1	0.42 *n* = 1
Fresh nile perch *Lates niloticus*	2	Tanzania FAO 51 and 57	0.134; 0.045	0.118; 0.046	88; 102	0.173; 0.085	<LOD *n* = 2	158 *n* = 1	0.31 *n* = 1
Fresh atlantic chub mackerel *Scomber scomber*	2	Slovenia FAO 37.1, 37.2, 37.3	0.056; 0.035	0.054; 0.019	96; 54	0.325; 0.350	8.4; 17.4 *n* = 2	/	/
Fresh sea bass *Dicentrachus labrax*	2	Croatia FAO 37.1, 37.2, 37.3	0.137; 0.066	0.092; 0.045	67; 68	0.161; 0.182	23.7 *n* = 1	/	/
Fresh dover sole *Solea vulgaris*	1	Danmark FAO 27	0.024	0.025	104	0.171	0.6 *n* = 1	104 *n* = 1	3.35 *n* = 1
Fresh common deniex *Dentex dentex*	2	Morocco FAO region not known	0.077; 0.053	0.064; 0.032	83; 60	0.089; 0.202	<LOD *n* = 2	150 *n* = 1	0.25 *n* = 1
Fresh gilt head bream *Sparus aurata*	4	Turkey, Croatia, not known FAO 37.1, 37.2, 37.3	0.138 ± 0.026 (0.103–0.159)	0.109 ± 0.023 (0.079–0.134)	82 ± 25 (50–102)	0.185 ± 0.021 (0.167–0.207)	23.0 ± 4.8 (17.7–27.2) *n* = 3	/	/
Fresh sparidae *Lithognathus mormyrus*	2	Croatia FAO 37.1, 37.2, 37.3	0.238; 0.078	0.246; 0.040	103; 51	0.363, 0.339	16.7 *n* = 1	568 *n* = 1	0.20 *n* = 1
Fresh john dory *Zeus faber*	1	Morocco FAO 34	0.066	0.068	103	0.578	/	202 *n* = 1	0.07 *n* = 1

(continued on next page)

Table 1 (*continued*)

Samples	No	Origin	THg (mg/kg wet weight) Mean ± SD (range)	MeHg (mg/kg wet weight) Mean ± SD (range)	MeHg (%) Mean ± SD (range)	Se (mg/kg wet weight) Mean ± SD (range)	PCBs (ng/g dry weight) Mean ± SD (range)	*n*-3 fatty acids (mg/100 g wet weight) Mean ± SD (range)	*n*-6/*n*-3 Mean ± SD (range)
Fresh pilchard *Clupea pilchardus*	2	Slovenia FAO 37.1, 37.2, 37.3	0.070; 0.143	0.077; 0.066	110; 46	0.557; 0.544	32.7; 39.2 $n = 2$	/	/
Fresh swordfish *Xiphias gladius*	1	Croatia FAO 37.1, 37.2, 37.3	1.160	1.080	93	0.452	4.2 $n = 1$	/	/
Fresh european hake *Merluccius merluccius*	1	Croatia; FAO region not known	0.052	0.056	108	0.253	3.8 ± 1.0 (2.7–4.6) $n = 3$	/	/
Canned tuna in vegetable oil	9	Spain, Thailand, Croatia*, Thailand*	0.125 ± 0.114 (0.017–0.384)	0.093 ± 0.099 (0.007–0.323)	68 ± 17 (41–88)	0.747 ± 0.137 (0.501–0.995)	/	/	/
Canned sardine in vegetable oil	8	France*, Croatia*, Thailand*, (0.004–0.144)	0.094 ± 0.041	0.070 ± 0.039 (0.002–0.109)	71 ± 22 (42–109)	0.138 ± 0.285 (0.242–1.180)	/	/	/
Canned anchovy in vegetable oil	1	Spain*	0.022	0.016	73	0.251	/	/	/
Canned tuna in olive oil	15	Italy*, Spain*, Thailand	0.243 ± 0.189 (0.022–0.800)	0.212 ± 0.158 (0.014–0.654)	85 ± 11 (64–105)	0.689 ± 0.191 (0.462–1.080)	/	/	/
Canned mackerel in olive oil	1	Portugal*	0.044	0.018	41	0.536	/	/	/
Canned mackerel in seed oil	1	Croatia*	0.063	0.059	94	0.449	/	/	/
Canned tuna in sunflower oil	3	Cote d'Ivoire	0.129 ± 0.044 (0.103–0.180)	0.112 ± 0.034 (0.092–0.151)	87 ± 3 (84–89)	0.453 ± 0.071 (0.386–0.528)	/	/	/
Canned mackerel	1	Slovenia*	0.046	0.027	59	0.399	8.5 $n = 1$	/	/
Canned tuna in own juice	8	France*, Italy*, Thailand, Thailand*, Cote d'Ivoire	0.118 ± 0.093 (0.024–0.238)	0.093 ± 0.089 (0.016–0.259)	74 ± 17 (57–109)	0.548 ± 0.204 (0.289–0.889)	0.9 ± 0.4 (0.4–1.3) $n = 5$	293 ± 60 (217–360) $n = 4$	0.18 ± 0.04 (0.14–0.24) $n = 4$
Canned mackerel with white wine aroma	1	France*	0.049	0.024	49	0.357	/	/	/
Canned tuna with vegetables	17	France*, Italy*, Spain*, Thailand*, Slovenia*, Spain*, Cote d'Ivoire, Thailand	0.132 ± 0.195 (0.021–0.858)	0.122 ± 0.197 (0.010–0.862)	90 ± 16 (45–109)	0.501 ± 0.180 (0.302–0.901)	<LOD $n = 1$	/	/
Canned sardine with vegetables	3	Croatia*, Thailand*	0.062 ± 0.051 (0.003–0.093)	0.035 ± 0.028 (0.030–0.055)	71 ± 26 (53–100)	0.653 ± 0.247 (0.376–0.850)	/	/	/
Canned cod	1	Croatia*	0.111	0.046	41	0.316	/	/	/
Canned salmon with vegetables	1	Thailand	0.027	0.022	81	0.232	1.0 $n = 1$	/	/
Canned sardine in seed oil	1	Croatia*	0.075	0.048	64	0.580	/	/	/
Canned salmon in own juice	1	United States of America	0.029	0.020	69	0.284	/	/	/
Canned herring in tomato sauce	1	Austria*	0.051	0.026	51	0.308	/	/	/
Canned mackerel with vegetables	3	Slovenia*	0.029 ± 0.011 (0.018–0.039)	0.020 ± 0.011 (0.010–0.031)	70 ± 29 (51–103)	0.232 ± 0.025 (0.206–0.255)	12.6 $n = 1$	/	/

* The country of origin of the fish is not known, instead the country of the producer is given; ** < LOD below the limit of detection; *** / the samples were not included in the data analyses; **** N_0 number of analysed samples for THg, MeHg and Se determination; SD standard deviation.

suprapur) was added and the tube reheated at 115 °C for approximately 20 min until the solution became blue in colour. To reduce Se^{6+} to Se^{4+}, 2.5 ml of concentrated 30% HCl (Merck, Germany, suprapur) was added to the solution and heated for 10 min at 100 °C. Samples were diluted with MilliQ water up to 40 ml. Hydride generation atomic fluorescence spectrometry was used for selenium detection. The procedure has been described in detail elsewhere (Smrkolj, Pograjc, Hlastan-Ribič, & Stibilj, 2005).

The accuracy of Se determination was checked by analysing the certified reference materials DORM 1 Dogfish Muscle and TORT 2 *Lobster Hepatopancreas* and found levels (DORM 1:1.56 ± 0.05 mg/kg; TORT 2:5.77 ± 0.21 mg/kg) were in good agreement with the certified values (DORM 1:1.62 ± 0.12 mg/kg; TORT 2:5.63 ± 0.67 mg/kg).

The LOQ for the HG–AFS method was estimated at 32 ng/g of fish sample.

2.2.5. Determination of PCBs

PCBs in the selected samples were first extracted by the Soxhlet method in a mixture of acetone (Mallinckrodt Baker Germany, Griesheim, Germany) – 96% *n*-hexane (Mallinckrodt Baker Germany, Griesheim, Germany) (1:1, v/v) for 16 h. The extract was concentrated with the nitrogen gas to 1 ml and dissolved in 50 ml of *n*-hexane (Mallinckrodt Baker Germany, Griesheim, Germany). Lipids that could interfere with GC–ECD determination of PCBs were removed by 96% H_2SO_4 p.a. (Carlo Erba, Milano, Italy) while any residual water was removed with Na_2SO_4 (Merck, p.a.). A PR 60/100 florisil column mesh (Supelco Park Bellefonte, United States) was used for purification of the PCB extract. Seven "indicator polychlorinated biphenyls" (congeners 28, 52, 101, 118, 138, 153 and 180) were identified and quantified by GC–ECD in conjunction with a DB–XLB capillary column. The temperature programme started at 100 °C (1 min), and increased at 10 °C/min to 300 °C, final temperature was held for 7 min. The procedure has been described in detail elsewhere (EPA 1668B, 2008; EPA Method 8082A, 1996).

The certified reference material Fish Homogenate IAEA-406 was used to determine the yield of the PCB extraction. The analytical recovery of the PCBs analysed was 47 ± 32% for congener 28 (2,4,4′-trichlorobiphenyl), 103 ± 12% for congener 52 (2,2′,5,5′-tetrachlorobiphenyl), 104 ± 12% for congener 101 (2,2′,4,5,5′-pentachlorobiphenyl), 99% ± 13% for congener 118 (2,3′,4,4′,5-pentachlorobiphenyl), 102% ± 14% for congener 138 (2,2′,3,4,4′,5′-hexachlorobiphenyl), 86 ± 15% for congener 153 (2,2′,4,4′,5,5′-hexachlorobiphenyl) and 104% ± 10% for congener 180 (2,2′,3,4,4′,5,5′-heptachlorobiphenyl). Low recovery of congener 28 is most likely a consequence of its relatively high volatility comparing to other tested PCBs. This is in agreement with the literature (Berdié & Grimalt, 1998).

The recoveries for congener 28 and congener 153 were included in the calculation of the results.

The LOD for quantification of PCBs calculated as three times the standard deviation of the blank sample was 0.01 ng/g dry weight for congeners 28, 101, 138 and 153, 0.02 ng/g dry weight for congener 118, 0.04 ng/g dry weight for congeners 52 and 180, while the LOQ was estimated at 0.11 ng/g dry weight for congener 138, 0.16 ng/g dry weight for congeners 28, 101 and 153, 0.17 ng/g dry weight for congeners 118 and 180, 0.21 ng/g dry weight for congener 52.

2.2.6. Determination of fatty acids

For identification and quantification of α-linoleic acid, stearidonic acid, EPA, eicosatetraenoic acid, DHA and DPA (*n*-3 fatty acid) the *in situ* transesterification method was used with gas chromatography–flame ionisation detection (GC–FID). The fatty acids were quantified with an internal standard (nonadecanoic acid 19:0, Sigma Aldrich, Germany, 19:0, N5252) added to the weighed samples (round 0.0700 g with precision 0.0005 g). After adding 3 ml 0.5 M NaOH (Merck, Germany, 1.06498) in methanol (Merck, Germany) and 0.3 ml methylene chloride (CH_2Cl_2; Merck, Germany, 1.06044), *in situ* transesterification was performed by heating lipid-containing foods at 90 °C for 40 min. Samples were cooled and 3 ml of 14% BF_3 (Sigma Aldrich, Germany, B1252) in methanol was added. Heating (90 °C) was continued for 10 min. After cooling, the fatty acid methyl esters (FAMEs) were extracted into hexane (Merck, Germany, 104371). Analyses of FAMEs were performed using a gas chromatograph with an HP-88 capillary column and a flame ionisation detector. The temperature programme started at 150 °C (10 min), increased by 2 °C/min to 180 °C and then increased again by 3 °C/min to 240 °C (20 min). Response factors for each FAME were calculated using an available FAME standard mixture (NuCheck 85 Prep. Inc. in NuCheck 68D Prep Inc). Fatty acids were calculated considering the stoichiometric factors (the ratio between the methyl ester and fatty acid molar masses) and response factors. The procedure was described in detail elsewhere (Fidler, Salobir, & Stibilj, 2001).

The accuracy of fatty acid determinations was checked by analysing the octadecatrienoic acid in the certified reference material BCR 163 Beef–Pork Fat Blend and the values found (0.92 ± 0.11 g/100 g) were in good agreement with the certified values (0.86 ± 0.14 g/100 g).

The repeatability of the method for quantification of *n*-3 acids varied according to each *n*-3 fatty acid, but was lower than 15%.

The LOD of the method was 0.1 μg/g of fish sample, while the LOQ was estimated at 1.0 μg/g of fish sample.

2.2.7. Statistical analysis

All data obtained were analysed using SAS/STAT software (SAS Institute Inc., 2001). The correlation between frequency of fish consumption and total mercury levels in hair was calculated by Spearman's rank correlation coefficient.

3. Results and discussion

3.1. Mercury in fish

The results for THg and MeHg levels in fresh and canned fish are shown in Tables 1 and 2. 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. In none of the tested canned fish samples did the concentration of mercury exceed the permissible limits. Only in four samples of fresh fish was the limit value for THg exceeded. These data suggest that canned fish are more controlled than fresh fish. However, the mean content of mercury in both canned fish (0.130 mg/kg) and fresh fish (0.174 mg/kg) was low compared to maximum allowed levels (Table 2). Although the mean level of THg in fresh fish was higher than in canned fish, the difference was not statistically significant.

The data for THg and MeHg were compared according to the trophic level of the fish (Figs. 1 and 2). The highest levels of THg and MeHg were found in organisms at the top of the food chain such as tuna, cod, conger, frog-fish, goatfish, swordfish, John Dory and European hake. Nevertheless, the levels of THg and MeHg were also variable within a species (Table 1). The MeHg comprised over 40% of mercury in fish (Tables 1 and 2). It is generally assumed that about 80–100% of total mercury in fish muscle is methyl mercury. However, studies where MeHg was also determined in fish lower in the food chain showed that the percentage of MeHg may be quite variable and comprised over 54% (Agah, Leermarkers, Elskens, Fatemi, & Baeyens, 2007; Magalhaes et al., 2007; Storelli, Storelli, Giacominelli-Stufler, & Marcotrigiano, 2005).

Table 2
Mean (X), median (Me), minimum (Min) and maximum (Max) levels for THg in hair and for THg, MeHg, PCBs and *n*-3 fatty acid in fresh and canned fish.

	N[b]	Sample	X	Me	Min	Max
THg (mg/kg wet weight)	469	Hair	0.385	0.306	0.015	2.080
THg (mg/kg wet weight)	128	Fish	0.150	0.080	0.003	1.110
MeHg (mg/kg wet weight)	128	Fish	0.127	0.062	0.002	1.120
MeHg (%)	128	Fish	80	83	40	110
Se (mg/kg wet weight)	128	Fish	0.471	0.421	0.085	1.180
PCBs (ng/g dry weight)	51	Fish	10	4	<LOD[a]	39.2
n-3 fatty acids (mg/100 g wet weight)	20	Fish	377	259	104	1420
n-6 fatty acids/*n*-3 fatty acids	20	Fish	0.53	0.19	0.05	3.6

[a] Below the limit of detection.
[b] Number of analysed samples.

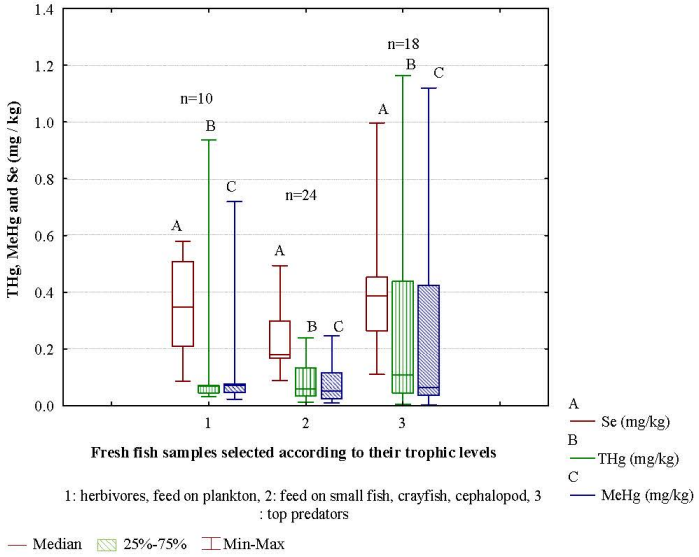


Fig. 1. Mercury and selenium levels in fresh fish selected according to their trophic level.

The EPA reference dose estimated in 1997 is 0.1 μg MeHg per kg body weight per day (EPA, 1997), while the Joint FAO/WHO Expert Committee on Food Additives (JECFA) has established a provisional tolerable weekly intake (PTWI) for MeHg at 1.6 μg/kg body weight (WHO, 2004). Variations among reference levels reflect different risk assessment assumptions, data sets and uncertainty factors employed (National Research Council, 2000). As the levels of MeHg are highest in organisms at the top of the food chain, the number of fish meals including swordfish and tuna should be limited. The JECFA PTWI implies that, according to the 95th percentile level of MeHg in fish on top of the food chain measured in this study, a 70 kg man can eat a portion (150 g) of fish on the top of food chain approximately once per week or approximately three portions (150 g) per week of fish lower in the food chain. Sensitive population groups such as pregnant women and children should consume balanced food including fish, but it is very important which species of fish and how often fish is consumed.

3.2. Selenium in fish

Se levels in fish ranged from 0.085 mg/kg to 1.180 mg/kg (Table 2). The values determined are in the same range as those reported in the literature (Cabañero, Madrid, & Cámara, 2004; Klapec et al., 2003; Plessi, Bertelli, & Monzani, 2001; Sirichakwal, Puwastien, Polngam, & Kongkachuichai, 2004; Wyatt, Meléndez, Acuña, & Rascon, 1996; Yoshida, Abe, Fukunaga, & Kikuchi, 2002). Recommended values for an adequate selenium intake for adults that are valid in Slovenia range from 30 to 70 μg/day (German Nutrition Society, 2002). Based on our results (mean value for selenium in fish), the daily requirement for selenium in Slovenia is met by approximately 60–150 g canned or fresh fish per day. Therefore according to our study the frequency of fish consumption in Slovenia (Figs. 5 and 6) is too low to cover the adequate selenium intake.

Selenium levels in fish vary greatly according to species and fishing area (Wyatt et al., 1996). We did not find a significant correlation between trophic level and selenium content.

3.3. PCBs in fish

Total PCB levels of indicator PCB congeners (congeners 28, 52, 101, 118, 138, 153 and 180) (Table 2) ranged from below the limit of detection to 0.039 mg/kg of dry weight of edible part of fish. Although it is well known that PCBs bioaccumulate in marine

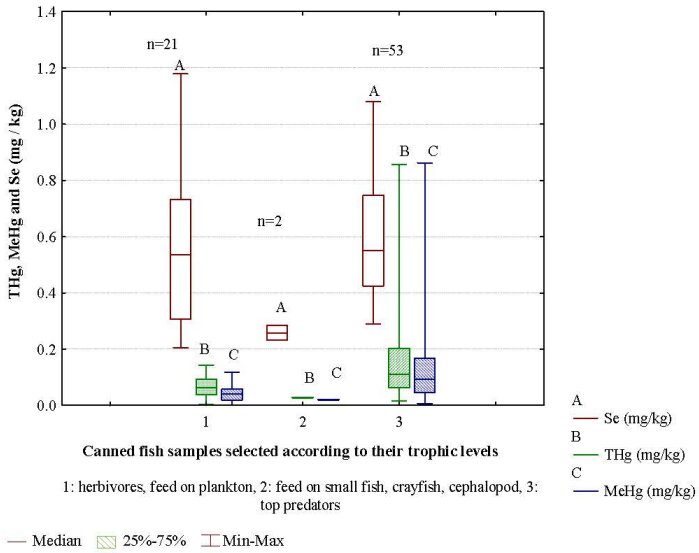


Fig. 2. Mercury and selenium levels in canned fish selected according to their trophic level.

species, we did not find higher levels of PCBs in fish on the top of food chain. The highest PCBs levels were measured in fish from fishing areas in the Mediterranean (Croatia, Slovenia and Turkey) (Fig. 3). Due to a relatively small number of results, further investigations are necessary to confirm these observations. In comparison to our results, Stefanelli et al. (2004) determined higher levels of PCBs (15 congeners) in edible fish from the Italian Adriatic Sea, while Yang, Matsuda, Kawano, and Wakimoto (2006) found lower levels of total PCBs in edible fish from China. However, comparison between results is difficult, because of the variability of PCB congeners that have been analysed in the different surveys and other differences concerning the expression of the results (wet weight, dry weight or lipid weight).

Legislation regarding PCB content in different foodstuffs on European level is not unified, while US Food and Drug Administration (FDA) reports safety level for fish consumption of 2 μg/g of total PCBs in the edible part of fish, wet weight, what we estimate to be cca 10 μg/g of dry weight. Also, FDA reports total PCB content, while we report seven main PCB congeners that usually encompass the majority of PCBs present in samples. Taking all these proximities into account, one can conclude that determined values in fish from the Slovene market are still far below the limit values of FDA and fish are therefore safe to consume.

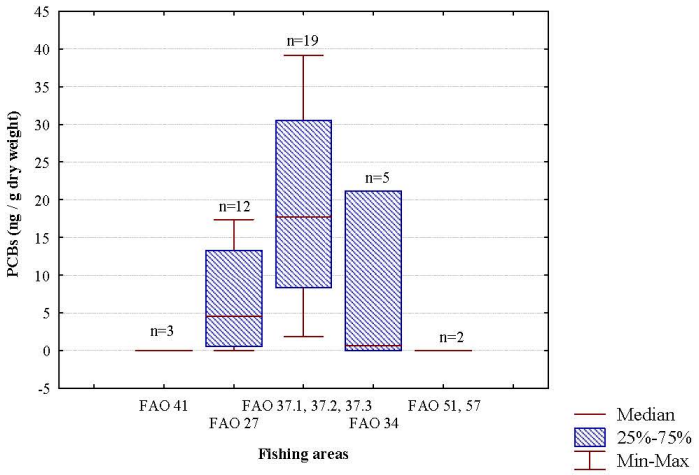
3.4. *n*-3 fatty acids in fish

The results for median, mean, maximum and minimum levels for *n*-3 fatty acids and the ratio of *n*-6 and *n*-3 fatty acids are shown in Table 2. According to data from the EFSA (European Food Safety Authority) (2005), the recommended intake for *n*-3 fatty acids is 0.5 g/person/day. Approximately 200 g of the fresh and canned fish samples analysed meets the daily requirement. However, the ratio between *n*-3 and *n*-6 is very important for estimation of recommended dose intake. The present reference values for intake of linoleic acid (*n*-6) and α-linolenic acid (*n*-3) valid in Slovenia are in the ratio of 5:1 (German Nutrition Society., 2002). The mean ratio between *n*-6/*n*-3 was low (0.5:1). Since the ratio of these acids in other food is higher than recommended, our study confirmed that fish consumption is beneficial, because it helps to ensure an appropriate ratio of *n*-6 and *n*-3 fatty acids in the whole diet.

Levels of *n*-3 fatty acids in fresh and canned fish selected according to the trophic levels are shown in Fig. 4. The highest levels of *n*-3 fatty acids were found in organisms that feed on small fish, crayfish and cephalopods. This is in accordance with the literature. According to Mahaffey (2004), several species of salmon, mackerel and herring were high in *n*-3 fatty acids, but relatively low in MeHg, while fish species that were on the top of food chain such as swordfish, tilefish and shark were not particularly rich in *n*-3 fatty acids.

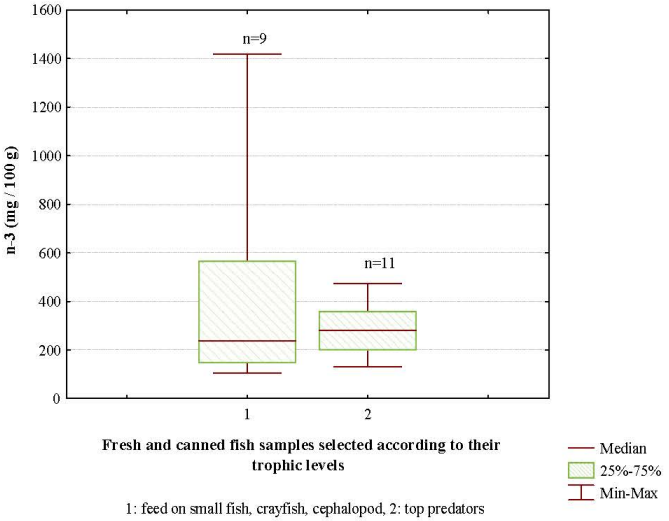
3.5. Assessment of mercury exposure of pregnant women from fresh and canned fish consumption

The frequency questionnaire showed that pregnant women in Slovenia consume on average 3 portions (150 g) of fresh fish per month and 2 tins (80 g) of canned fish per month. Mercury determination in hair was used as a biomarker in assessment of mercury exposure of pregnant women because it can provide a simple, integrated and noninvasive method for estimating long-term average exposure. Hair mercury analyses have been found to be highly suitable, although somewhat imprecise (Grandjean, Jœrgensen, & Weihe, 2002). In particular this applies to hair levels in pregnant women in their late pregnancy period as MeHg can be transported to the growing foetus (Björnberg et al., 2003). In our study hair was collected during late pregnancy or in the first month after childbirth. It is therefore suspected that mercury levels in hair are lower than in the population of nonpregnant women of childbearing age. The result for total mercury in hair is shown in Table 2. In general, the levels are far below the estimated MeHg threshold level (50 mg/kg) for neurological effects and below the estimated MeHg threshold level for prenatal effects (10 mg/kg), which is in



FAO 41: Indonesia; FAO 27: Denmark, Norway, Holland, France; FAO 37.1, 37 .2, 37.3: Croatia, Slovenia, Turkey; FAO 34: Morocco; FAO 51 and 57: Tanzania

Fig. 3. PCBs levels in fresh fish selected according to the fishing areas.



1: feed on small fish, crayfish, cephalopod, 2: top predators

Fig. 4. Levels of *n*-3 fatty acids in canned and fresh fish selected according to their trophic level.

agreement with the study, where the same population in Slovenia was included (Horvat et al., 1988).

As expected, the concentration levels of total mercury in hair were low due to low consumption of fish and relatively low levels of mercury in fish. Spearman's rank correlation between the frequency of fish consumption and THg in hair was low (Figs. 5 and 6). At low levels of exposure, as documented in the present study, a number of factors can influence the strength of the correlation between the levels in hair and the intake of MeHg. One of the important factors is the accuracy of the data contained in the questionnaires. In our study, the food frequency questionnaire provided only approximate information on fish consumption and not a precise measure of fish ingested by an individual. Moreover, the amount and frequency of specific types of fish consumed was not

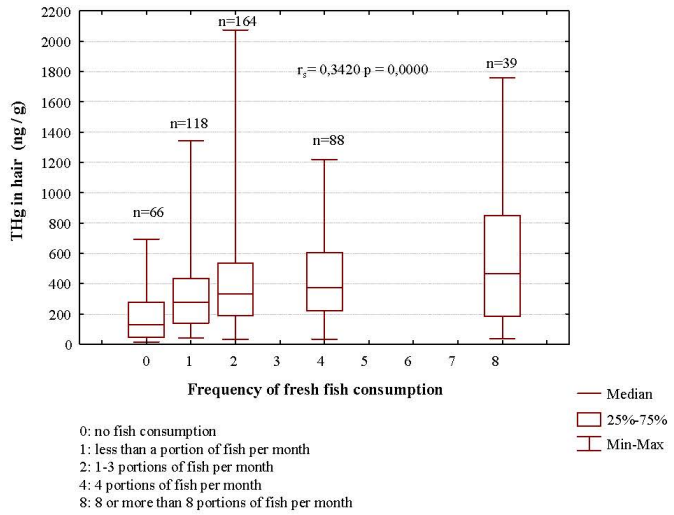


Fig. 5. Spearman's rank correlation between frequency of fresh fish consumption and total mercury levels in hair.

specified. Grundacker et al. (2007) showed that mercury content in hair is influenced not only by fish consumption but by the epistatic effects of glutathione-S-transferase deletion polymorphisms as well.

Therefore, inter-individual differences may affect the outcome, particularly at low levels of exposure. In addition, total mercury in hair was measured as a proxy for MeHg exposure through fish consumption. At low levels of exposure, the presence of inorganic mercury in hair may contribute significantly. It has been shown previously (Barbone et al., 2004; Lee et al., 2000) that at this level of exposure the percentage of mercury present in hair as MeHg is variable with a mean value of 59% (Lee et al., 2000). This is in contradiction to the generally accepted fact that MeHg represents the overwhelming majority of mercury in hair. At a low level of exposure, a better correlation can only be expected if data for MeHg in fish are correlated with MeHg in hair.

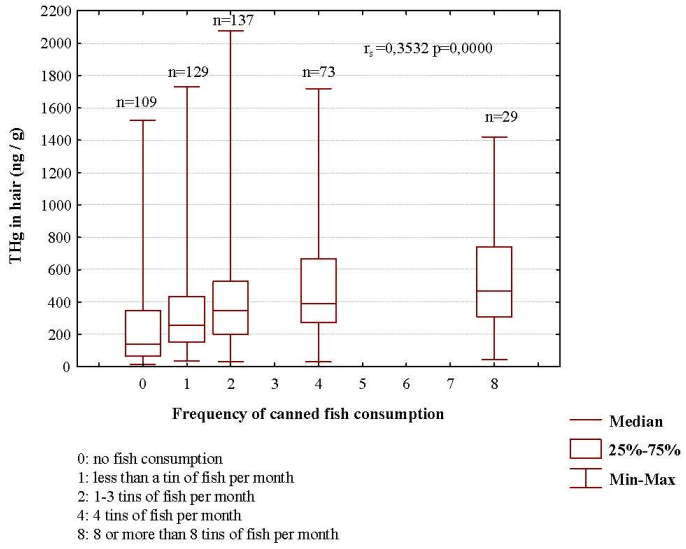


Fig. 6. Spearman's rank correlation between frequency of canned fish consumption and total mercury levels in hair.

4. Conclusions

Based on the present study and the current JECFA PTWIs for MeHg, it can be concluded that the levels of MeHg in fish available on the Slovenian market consumed by Slovenian pregnant women do not represent a health risk for pregnant woman and for susceptible developing fetuses and that fish represent an important source of selenium and *n*-3 fatty acids. Furthermore, the frequency of fish consumption of Slovenian pregnant women is relatively low. Considering the PCB tolerance limit recommended by the FDA, we conclude that edible fish from the Slovenian market pose a minimal health risk for humans. Our study also confirmed that fish lower in the food chain contain relatively low levels of MeHg and higher levels of *n*-3 fatty acids than fish on the top of the food chain. Regular monitoring of mercury levels in fish, particularly fresh fish, should be performed and the results communicated to the public, together with trustworthy advice for various population groups on the safety of fish consumption.

In terms of science based-questions related to the safety of fish consumption for the most susceptible groups of the population, more studies are needed to better understand the bioavailability of mercury and the role of genetic factors influencing the use of hair mercury as a biomarker of MeHg exposure through fish consumption (Grundacker et al., 2007). This would also contribute to further advancement of studies to identify inter-individual variance in human detoxification capacity for mercury.

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References

Agah, H., Leermarkers, M., Elskens, M., Fatemi, S. M. F., & Baeyens, W. (2007). Total mercury and methyl mercury concentrations in fish from the Persian Gulf and the Caspian Sea. *Water Air Soil Pollution, 181*, 95–105.

Akagi, H. (1997). Analytical methods for evaluating human exposure to mercury due to gold mining. In: *Proceedings of the international workshop on health and environmental effects of mercury due to mining operations* (pp. 131-141). Manila: National Institute for Minamata Disease.

Barbone, F., Valent, F., Pisa, F., Daris, F., Fajon, V., Gibicar, D., et al. (2004). Prenatal low-level mercury exposure and child development in an Italian coastal area. *Seychelles Medical and Dental Journal, 7*(1), 149–156.

Beijer, K., & Jernelov, A. (1987). Ecological aspects of mercury–selenium interactions in the marine environment. *Environmental Health Perspectives, 25*, 43–45.

Berdié, L., & Grimalt, J. O. (1998). Assessment of the sample handling procedures in a labor-saving method for the analysis of organochlorine compounds in a large number of fish samples. *Journal of Chromatography A, 823*, 373–380.

Björnberg, K. A., Vahter, M., Petersson-Grawé, K., Glynn, A., Cnattingius, S., Darnerund, P. O., et al. (2003). Methyl mercury and inorganic mercury in Swedish pregnant women and in cord blood: Influence of Fish Consumption. *Environmental Health Perspective, 111*(4), 637–641.

Cabañero, A. I., Madrid, Y., & Cámara, C. (2004). Selenium and mercury bioaccessibility in fish samples: An in vitro digestion method. *Analytica Chimica Acta, 526*, 51–61.

Clarkson, T. W. (2002). The three modern faces of mercury. *Environmental Health Perspectives, 110*, 11–22.

Cox, C., Clarkson, T. W., Marsh, D. O., Almin-Zaki, L., Al-Tikriti, S., & Myers, G. G. (1989). Dose-response analysis of infants prenatally exposed to methyl mercury: An application of a single compartment model to single strand hair analysis. *Environment Research, 49*(2), 318–332.

Culvin-Aralar, L. A., & Furness, R. W. (1991). Mercury and selenium interaction: A review. *Ecotoxicology and Environmental Safety, 21*, 348–364.

Davidson, P. W., Strain, J. J., Myers, G. J., Thurston, S. W., Bonham, M. P., Shamlaye, C. F., et al. (2008). Neurodevelopmental effects of maternal nutritional status and exposure to methylmercury from eating fish during pregnancy. *Neurotoxicology, 29*(5), 767–775.

EFSA (European Food Safety Authority) (2005). Opinion of the scientific panel on contaminants in food chain on a request from the European Parliament related to the safety assessment of wild and farmed fish. *The EFSA Journal*. 236, 1-118.

EPA Method 1668B (2008). Chlorinated biphenyl congeners in water, soil, sediment, biosolids and tissue by HRGC/HRMC.

EPA 1997a (1997). Mercury study report to congress. Vol. I.: Executive summary. EPA-452/R-97-003. U.S. Environmental Protection Agency, Office of Air Quality Planning and Standards, and Office of Research and Development.

EPA Method 7473 (1998). Mercury in solids and solutions by thermal decomposition amalgamation and atomic absorption spectrophotometry.

EPA Method 8082A (1996). Polychlorinated biphenyls (PCBs) by gas chromatography.

Fidler, N., Salobir, K., & Stibilj, V. (2001). Fatty acid composition of human colostrum in Slovenian women living in urban and rural areas. *Biology of the Neonate, 79*, 15–20.

German Nutrition Society (2002). *Reference values for nutrient intake*. Bonn: Umschau Braus GmbH.

Grandjean, P., Jergensen, P.J., & Weihe, P., (2002). Validity of mercury exposure biomarkers. In Wilson, S.H., & Suk, W.A. (Eds.), *Biomarkers of environmentally associated disease* (pp. 235–234).Boca Raton: CRM Press/Lewis.

Grandjean, P., Weihe, P., White, R. F., & Debes, F. (1998). Cognitive performance of children prenatally exposed to »safe« levels of methylmercury. *Environmental research, 77*, 165–172.

Grandjean, P., Weihe, P., White, R. F., Debes, F., Araki, S., Yokayama, K., et al. (1997). Cognitive deficit in 7-year-old children with prenatal exposure to methylmercury. *Neurotoxicology and Teratology, 19*(6), 417–428.

Grundacker, C., Komarnicki, G., Jagiello, P., Gencikova, A., Dahmen, N., Wittmann, K. J., et al. (2007). Glutathione-S-transferase polymorphism, metallothionein expression, and mercury levels among students in Austria. *Science of the Total Environment, 385*, 37–47.

Horvat, M., Byrne, A. R., & May, K. (1990). A modified method for the determination of methylmercury by gas chromatography. *Talanta, 37*(2), 207–212.

Horvat, M., May, K., Stoeppler, M., & Byrne, A. R. (1988). Comparative studies of methylmercury determination in biological and environmental samples. *Applied Organometallic Chemistry, 2*, 850–860.

Klapec, T., Mandić, M. L., Grgić, J., Primorac, L., Perl, A., & Krstanović, V. (2003). Selenium in selected foods grown or purchased in eastern Croatia. *Food Chemistry, 85*, 445–452.

Kris-Etherton, P. M., Harris, W. S., & Appel, L. J. (2002). For the nutrition committee. AHA scientific statement. Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. *Circulation, 106*, 2747–2757.

Lee, W.-C., Lee, M.-J., Lee, S.-M., Kim, J.-S., Bae, C.-S., & Park, T.-K. (2000). An observation on the mercury contents of scalp hair in the urban residents of South Korea. *Environmental Toxicology and Pharmacology, 8*, 275–278.

Magalhaes, M. C., Costa, V., Mebezes, G. M., Pinho, M. R., Santos, R. S., & Monteiro, L. R. (2007). Intra- and inter-specific variability in total and methylmercury bioaccumulation by eight marine fish species from the Azores. *Marine Pollution Bulletin, 54*, 1654–1662.

Mahaffey, K. R. (2004). Fish and shellfish as dietary sources of methylmercury and the *n*-3 fatty acids, eicosahexaenoic acid and docosahexaenoic acid: Risks and benefits. *Environmental Research, 95*, 414–428.

National Research Council (2000). *Toxicological effects of methylmercury*. Washington: National Academy Press.

Plessi, M., Bertelli, D., & Monzani, A. (2001). Mercury and selenium content in selected seafood. *Journal of Food Composition & Analysis, 11*, 47–53.

Raymond, L., & Ralston, V. C. N. (2004). Mercury: Selenium interactions and health implications. *Seychelles Medical and Dental Journal, 7*(1), 72–75.

Sirichakwal, P. P., Puwastien, P., Polngam, J., & Kongkachuichai, R. (2004). Selenium content of Thai foods. *Journal of Food Composition and Analysis, 18*(1), 47–59.

Smrkolj, P., Pograjc, L., Hlastan-Ribič, C., & Stibilj, V. (2005). Selenium content in selected Slovenian foodstuffs and estimated daily intakes of selenium. *Food Chemistry, 90*, 691–697.

Stefanelli, P., Di Muccio, A., Ferrara, F., Barbini, D. A., Generali, T., Pelosi, P., et al. (2004). Estimation of intake of organochlorine pesticides and chlorobiphenyls through edible fishes from the Italian Adriatic Sea during 1997. *Food Control, 15*, 27–38.

Storelli, M. M., Storelli, A., Giacominelli-Stufler, R., & Marcotrigiano, G. O. (2005). Mercury speciation in the muscle of two commercially important fish, hake (*Merluccius merluccius*) and striped mullet (*Mullus Barbatus*) from the Mediterranean sea: Estimated weekly intake. *Food Chemistry, 89*, 295–300.

Swedish Expert Group (1971). Methylmercury in fish. A toxicological epidemiological evalouation of risks. *Nord Hyg Tidskr, 4*, 319-364.

WHO (2004). Techical Report Series 922. Sixty-first report of the Joint FAO/WHO Expert Commitee on Food Additives (JEFCA), 133.

Wyatt, C. J., Meléndez, J. M., Acuña, N., & Rascon, A. (1996). Selenium (Se) in foods in northern Mexico, their contribution to the daily Se intake and the relationship of Se plasma levels and glutathion peroxidase activity. *Nutrition Research, 16*(6), 949–960.

Yang, N., Matsuda, M., Kawano, M., & Wakimoto, T. (2006). PCBs and organochlorine pesticides (OCPs) in edible fish and shellfish from China. *Chemospehere, 63*, 1342–1352.

Yoshida, M., Abe, M., Fukunaga, K., & Kikuchi, K. (2002). Bioavailability of selenium in the defatted dark muscle of tuna. *Food Additives and Contamination, 19*(10), 990–995.

3.2 Biomarkers of low-level mercury exposure through fish consumption in pregnant and lactating Slovenian women

In this section, the article (Miklavčič et al., 2011b) entitled "Biomarkers of low-level mercury exposure through fish consumption in pregnant and lactating Slovenian women" by Ana Miklavčič, Dr. Petra Cuderman, Dr. Darja Mazej, Janja Snoj Tratnik, Mladen Krsnik, Petra Planinšek, Prof. Dr. Joško Osredkar and Prof. Dr. Milena Horvat is presented. The paper was published in Environmental Research (Elsevier).

In this article MeHg exposure of the most susceptible Slovenian population was assesed using detailed fish consumption data in combination with biomarkers of exposure. Importantly, this study used the MeHg levels measured in the most frequently eaten species of fish of the studied population, and not just THg levels in fish. Moreover, the aim was to estimate which of the biomarkers of prenatal low-level Hg exposure is the most appropriate for use in large-scale epidemiological studies. The Hg speciation data obtained in this study represent valuable information for future large epidemiological studies that will deal with Hg risk assessment and are important in the study of the toxicokinetics of Hg (Miklavčič et al., 2011b).

This study was part of a research project within the EU 6th Framework Programme Public Health Impact of Long-term Low-level Mixed Element Exposure in Susceptible Population Strata (PHIME)and all the coauthors of this article collaborated in this project. My contribution to this work was first to perform the analyses of THg in hair and cord blood samples, and second to carry out the calculations and statistical analyses and interpret the data. As first author my task was to draft the manuscript.
Part of the research included in this article was presented at the scientific conferences: 15th International Conference on Heavy Metals in the Environment, September 19-23, 2010 and that on Environmental Health 6-9 February 2011.

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Biomarkers of low-level mercury exposure through fish consumption in pregnant and lactating Slovenian women ☆

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ABSTRACT

In order to assess the mercury exposure of pregnant and lactating women in Slovenia, levels of total mercury (THg) and methylmercury (MeHg) were determined in hair, cord blood and breast milk. In addition, the frequency of fish consumption was estimated, because fish is generally the main pathway for human exposure to MeHg. Hair samples were collected from 574 women participating in this study, while cord blood and breast milk samples were collected from 446 and 284 women, respectively. As expected, the levels of THg in hair (median (Med)=297 ng/g, 10th percentile (P10)=73 ng/g, 90th percentile (P90)=781 ng/g), cord blood (Med=1.5 ng/g, P10=0.5 ng/g, P90=4.2 ng/g) and breast milk (Med=0.2 ng/g, P10=0.06 ng/g, P90=0.6 ng/g) were low, due to low consumption of fish (X=25 g/day). A significant linear correlation was found between levels of ln THg in hair and ln THg in cord blood (r=0.87, 95% confidence interval (CI): 0.84–0.89), between levels of ln THg in hair and ln MeHg in cord blood (r=0.94, 95% CI: 0.90–0.96) and between ln THg levels in cord blood and ln THg levels in breast milk (r=0.36, 95% CI: 0.25–0.47). Spearman's rank correlations between the frequency of fish consumption and THg in hair (r_s=0.35, 95% CI: 0.28–0.42), and between the frequency of fish consumption and THg in cord blood (r_s=0.43, 95% CI: 0.36–0.51) or MeHg in cord blood (r_s=0.31, 95% CI: 0.06–0.52) were weak. This could be due to the approximate information on fish consumption obtained from the questionnaires, the high variability of MeHg concentrations in fish and a relatively high proportion of inorganic mercury in the biomarkers which originates from sources other than fish. In conclusion, THg levels in cord blood, THg levels in hair and MeHg levels in cord blood are suitable biomarkers of low-level Hg exposure through fish consumption. Compared to cord blood, hair samples are easy to collect, store and analyse.

1. Introduction

MeHg contamination is a challenge to public health, because it is mainly contained in fish, which is a highly nutritious food with known benefits to human health.

The major toxic effects of MeHg are on the central nervous system. In particular, MeHg affects neurodevelopment and the developing foetus is the most vulnerable (NRC, 2000).

People are exposed to MeHg mainly through their diet, especially through the consumption of freshwater and marine fish, and consumption of other animals that consume fish (such as marine mammals). The highest levels are found in fish that are apical predators of older age such as king mackerel, pike, shark, swordfish, walleye, barracuda, large tuna, scabbard, marlin and fish-consuming mammals such as seals and toothed whales (EPA, 1997a, 1997b, 2003; UNEP, 2002; Miklavčič et al., 2011). Trimming, skinning off and cooking the mercury-contaminated fish does not reduce the mercury content of the fillet portion. However, people who consume

Abbreviations: CI, confidence interval; CRM, certificate reference material; GM, geometric mean; k, coverage factor; LOD, limit of detection; LOQ, limit of quantification; Max, maximum; Min, minimum; Med, median; MeHg, methylmercury; P10, 10th percentile; P90, 90th percentile; PDI, probable daily intake; PHIME, Public Health Impact of Long-term Low-level Mixed Element Exposure in Susceptible Population Strata; PTWI, provisional tolerable weekly intake; RM, reference material; STD, standard devotion; THg, total mercury; X, mean

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moderate amounts of a variety of fish are not at risk (UNEP, 2002). Based on the levels of MeHg in fish available on the Slovenian market and the JECFA provisional tolerable weekly intake (PTWI) for MeHg, a 70 kg man can eat a portion (150 g) of fish from the top of food chain approximately once per week or approximately three portions (150 g) of fish lower on the food chain (Miklavčič et al., 2011). Moreover, although 95% of MeHg ingested is thought to be absorbed in the gastrointestinal tract, the absorption and bioavailability of MeHg may be affected by dietary components in food such as dietary fibre found in cereal products or selenium in fish. Most MeHg is eliminated from the body by demethylation and excretion of the inorganic form (ATSDR, 1999).

Exposure can be estimated by measuring pollutant levels in various body tissues. These measurements are also known as biomarkers of exposure. THg levels in hair and blood are used as biomarkers of exposure, when it could be assumed that almost all of THg in hair or THg in blood originate from exposure to MeHg (NRC, 2000). THg concentrations in scalp hair are proportional to the simultaneous concentrations in blood but are about 250 times higher. They are also proportional to concentrations in the target tissue, the brain. Levels in cord blood are proportional to but slightly higher than levels in maternal blood (Cernichiari et al., 1995). According to Gradjean et al. (2002), mercury in cord blood shows a better association with mercury-related neurobehavioural deficit in the child compared to mercury determined in maternal hair. Hair mercury concentrations can be affected by several factors, including hair colour and variable growth rates, which limit its usefulness as an indicator of mercury concentrations in the body (Gradjean et al., 2002). MeHg is also excreted in breast milk. However, unlike placenta, where MeHg moves more easily across the placental barrier than inorganic mercury, inorganic mercury is more readily eliminated in breast milk than MeHg (Sundberg and Oskarsson, 1992).

The objective of this study was to assess the exposure to MeHg of the most susceptible Slovenian population using detailed fish consumption data in combination with biomarkers of exposure. Importantly, our study used the MeHg mercury levels measured in the most frequently eaten species of fish of the studied population and not just THg levels in fish. Moreover, our aim was to estimate which of the biomarkers of prenatal low-level mercury exposure is the most appropriate for use in large-scale epidemiological studies. The mercury speciation data obtained in this study represent valuable baseline information for future large epidemiological studies that will deal with mercury risk assessment and are important in the study the toxicokinetics of mercury.

2. Materials and methods

2.1. Collection and storage of samples

585 pregnant women involved in this study were permanent residents in the study areas (at least 2 years), aged a minimum of 18 years, with no absence from the study area for more than 6 weeks during the pregnancy, no history of drug abuse, no serious health problems, no serious complications of pregnancy and no twin gestations. Samples were mainly ($n=513$) collected in the capital of Slovenia, Ljubljana, and in the outskirts of the city, while a smaller number ($n=72$) of samples were collected in other towns of Slovenia. The recruitment started in February 2007 and took place at the Gynecology Clinic of Ljubljana and in health centres located in Izola, Koper, Piran, Idrija and Kočevje.

574 hair samples were collected from participating women in 27–32 week of their pregnancy or in the first two months after childbirth. They were cut with ceramic scissors close to the root in the occipital region of the scalp. Each hair sample was stored in a plastic bag and then analysed without any cleaning or special treatment. 446 cord blood samples were collected by the personnel of the Obstetrics and Gynecology Divisions who assisted the women during delivery. Before the analyses they were stored in a freezer below −24 °C. 284 breast milk samples were collected in the first or two months after childbirth, by trained research personnel at the participant's homes. In a very few cases the women preferred to hand in the samples to research staff at the Institute. The breast milk samples were stored in a freezer below −24 °C.

The participating women filled out a short questionnaire from which the frequency of fresh, canned and frozen fish consumption was assessed. Moreover, from a long questionnaire, data about the most frequently consumed fish and the number of amalgam fillings were obtained from the participating women. The pregnant women filled out the short questionnaire at the time of recruitment by study researchers and the long questionnaire was compiled after delivery. Fish intake categories in the questionnaires were converted to continuous estimates of monthly intake and the estimated portions of fish were converted in grams. One portion of fresh or frozen fish was estimated to be around 150 g, while one portion of canned fish was estimated to be around 80 g. Low accuracy estimates of the number of amalgam fillings were obtained from food frequency questionnaire, because of initial categorisation in four groups (less than 3 amalgam fillings, 3–5 amalgam fillings, 6–9 amalgam fillings, more than 10). A categorised numbers of amalgam fillings were converted to continuous estimetes.

2.2. Study methods

2.2.1. Determination of THg in hair and cord blood

THg in hair and THg in cord blood were determined by thermal combustion at 650 °C, amalgamation and atomic absorption spectrometry using a Direct Mercury Analyzer (Milestone Srl., Italy). The procedure has been described in detail elsewhere (EPA Method 7473, 1998). About 0.020 g of human hair or about 0.200 g of cord blood was weighed in a sample boat. NIES CRM (Certified Reference Material) No 13 (human hair) from the National Institute for Environmental Studies in Japan was used to check the accuracy of the results of THg in hair and the value found (4380 ± 100 ng/g, $n=58$) was in good agreement with the certified reference value (4420 ± 200 ng/g). The reference material (RM) Seronorm Trace Elements in Whole Blood L-1 from the SERO AS in Norway (LOT No: MR4206) was used to check the accuracy of the results for THg in cord blood and the value found (2.2 ± 0.15 ng/ml, $n=63$) was in good agreement with the reference value (2.2 ± 0.2 ng/ml). In addition, our laboratory participated in the interlaboratory comparisons organised within the project PHIME. Three intercomparisons used lyophilised samples of human blood from: (A) non-exposed persons, (B) people occupationally exposed to elemental mercury and (C) fish eaters. In the fourth intercomparison (D) fresh blood from general population was used. Assigned values (A: 7.8 ± 0.9 ng/g, B: 54 ± 8 ng/g, C: 103 ± 16 ng/g, D1: 1.39 ± 0.14 ng/g, D2: 1.80 ± 0.16 ng/g, D3: 1.09 ± 0.20 ng/g) for THg were in agreement with the obtained values (A: 6.4 ± 0.2 ng/g, B: 52 ± 3.7 ng/g, C: 106.4 ± 5.9 ng/g, D1: 1.28 ± 0.07 ng/g, D2: 1.64 ± 0.03 ng/g, D3: 0.96 ± 0.03 ng/g).

The limit of detection (LOD) of the method calculated as three times the standard deviations (STD) of the blank sample was 0.2 ng/g hair sample and 0.02 ng/g cord blood sample, while the limit of quantification (LOQ) calculated as ten times the STD of the blank sample was 0.7 ng/g hair sample and 0.07 ng/g cord blood sample.

Although the sample mass was low, the precision calculated on the basis of the STD of THg determined in the CRM for hair samples was 7% (coverage factor (k) was 2). The precision (calculated as previously described) of determination of THg in blood samples at levels higher or equal to 1 ng/g was 7% ($k=2$), while at lower levels (less than 1 ng/g) it was 14% ($k=2$). In addition, the stability of the blood samples during freezing and defrosting was checked at three different concentration ranges of THg. Defrosting lasted 2 h at room temperature and freezing and defrosting was repeated three times. In sets of blood samples where THg in hair was below 1100 ng/g the levels of THg in blood decreased by up to 20%, while in the higher concentration range of THg in blood, the decrease of concentrations was not observed.

2.2.2. Determination of THg in breast milk

An amount of 2 ml of breast milk was placed in 30 ml screw capped volumetric flask, to which 1 ml of distilled water, 2 ml of a mixture of 65% HNO_3 (Merck, Germany, p.a.)–$HClO_4$ (Merck, Germany, suprapur) (1:1, v/v) and 5 ml of 96% H_2SO_4 (Merck, Germany, suprapur) were added. The flask was heated at 220 °C on a hotplate for 20 min. After cooling, the digested samples were filled to 30 ml with distilled water. A semi-automated Mercury Analyser based on cold vapour atomic absorption spectrometry was used for determination of THg in the digested samples. Akagi (1997) described the procedure in detail. The accuracy of the results was checked by analysing the standard RM Non-Fat Milk Powder NIST 1549 (Lot No 1) from the National Institute of Standard and Technology (Gaithersburg) and the measured value (0.38 ± 0.08 ng/g, $n=33$) was in good agreement with the reference value (0.3 ± 0.2 ng/g). The precision calculated on the basis of the STD of THg determined in breast milk samples was 13% ($k=2$). This relatively low precision of THg determination in breast milk was due to the prevalent low levels of THg in breast milk.

The LOD of the method calculated on the basis of three STD of the blank was 0.045 ng/ml of breast milk sample, while the LOQ calculated as ten times the STD of the blank sample was 0.2 ng/ml breast milk sample.

In addition, the stability of the breast milk samples during freezing and defrosting was checked at three different concentrations range of THg. As described in Section 2.2.1, defrosting lasted 2 h at room temperature and freezing and defrosting were repeated three times. No decrease of concentrations was observed at any concentrations range of THg in breast milk.

2.2.3. Determination of MeHg in hair

About 0.015–0.030 g of hair sample was weighed into a Teflon tube, to which 10 ml of 6 M of HCl (Merck, Germany, suprapur) was added and the mixture was shaken overnight. MeHg in the extract was subsequently back extracted with 0.5 ml of toluene. Two microlitres of the toluene was injected into a packed gas chromatography column and MeHg was measured by gas chromatography-electron capture detection (GC-ECD). A complete description of the method is given in Horvat et al. (1988, 1990).

NIES CRM No 13 (human hair) from the National Institute for Environmental Studies in Japan was used to check the accuracy of the results of MeHg in hair and the found value (3850 ± 130 ng/g, $n=18$) was in good agreement with the certified reference value (3800 ± 400 ng/g). The precision of the method calculated on the basis of the STD of the MeHg determined in the CRM was 12% ($k=2$).

The LOD of the method calculated on the basis of three STD was 0.2 ng/g hair samples, while the LOQ calculated as ten times the STD of the blank sample was 0.7 ng/g hair sample.

2.2.4. Determination of MeHg in cord blood and breast milk

About 200 mg of cord blood or about 1 g of breast milk sample was weighed directly in a 30 ml screw capped Teflon vial to which 6 ml of a mixture of 5% H_2SO_4 (Merck, Germany, suprapur), 18% KBr (Merck, Germany, p.a.) and 1.0 ml of a 1 M solutions of $CuSO_4$ (Merck, Germany, p.a.) was added. After shaking the vials vigorously the solvent extraction and aqueous phase ethylation were performed. The ethylated MeHg as ethylmercury was purged onto a Tenax trap for 15 min with nitrogen gas. The Tenax trap was then connected to a flow of argon and MeHg was thermally desorbed (180 °C) onto an isothermal GC column. Hg species were converted to Hg^0 by pyrolysis at 600 °C and measured by a cold vapour atomic fluorescence detector (CV AFS). The procedure has been described in detail elsewhere (Horvat et al., 1993a, 1993b; Liang et al., 1994).

The accuracy of the results for MeHg in cord blood and MeHg in breast milk was checked by analysing RM lyophilised whole human blood PT-WB1 obtained from non-exposed population. MeHg in PT-WB1 was determined by the laboratories participating in the PHIME interlaboratory comparison. The assigned value (6.3 ± 0.5 ng/g) was in good agreement with the determined value (6.2 ± 0.3 ng/g, $n=17$). In addition, our laboratory participated in the previously described interlaboratory comparison for MeHg determination in lyophilised whole human blood organised within the project PHIME. The assigned value (6.3 ± 0.5 ng/g) was in agreement with the obtained value ($5.8. \pm 0.5$ ng/g). The precision of MeHg calculated on the basis of the STD of MeHg determined in the cord blood samples was 12% ($k=2$) at levels higher or equal to 1 ng/g and 14% ($k=2$) at lower levels (less than 1 ng/g). The precision calculated on the basis of the STD of MeHg determined in breast milk samples was 17% ($k=2$).

The LOD of the method for MeHg determination in cord blood calculated on the basis of three STD was 0.02 ng/g, while the LOQ calculated as ten times the STD of the blank sample was 0.07 ng/g cord blood sample. The LOD of the method for MeHg determination in breast milk calculated on the basis of three STD was 0.003 ng/g, while the LOQ calculated as ten times the STD of the blank sample was 0.01 ng/g breast milk samples.

2.2.5. Statistical analysis

All data obtained were analysed using SAS/STAT software (SAS Institute, Inc., 2001). Logarithmic transformation of THg in hair, THg and MeHg in cord blood and THg in breast milk was used to conform to the requirements of a normal distribution. The Pearson correlation was used for bivariate comparisons. Furthermore, the Pearson correlation was used for identification of possible predictors of the element concentrations. The variables tested were the consumption of specific fish species. The predictors with $p<0.05$ were further explored by multiple linear regression analyses. The correlation between frequency of fish consumption and THg and MeHg levels in hair, cord blood and hair was calculated by Spearman's rank correlation coefficient. The Mann–Whitney test was used for comparison of two different groups of %MeHg in cord blood samples. This nonparametric test was applied, because the distribution of %MeHg in cord blood at high levels (paired hair samples exceeding 1000 ng/g) and the distribution of %MeHg in cord blood at low levels (paired hair samples below 1000 ng/g) significantly differed from the normal distribution.

The level of statistical significance was set to $p<0.05$.

2.2.6. Exposure assessment

To estimate the MeHg exposure of Slovenian pregnant women, the following equitation was used to calculate the probable daily intake (PDI):

$$\mathrm{PDI_{intake}}\ (\mu g/kg\ \text{body weight/day}) = \text{fish intake (g/day)} \times [\mathrm{MeHg_{most\ frequently\ eaten\ fish\ species}}]/\text{body weight} \quad (1)$$

MeHg levels measured in the most frequently consumed species of fish from the Slovenian market from a previous study (Miklavčič et al., 2011) were used. From previously obtained data and from the frequency of the most frequently consumed fish, we obtained an average estimate of the MeHg concentration of the most frequently consumed fish species ($[\mathrm{MeHg_{most\ frequently\ eaten\ fish\ species}}]$).

3. Results and discussion

3.1. Mercury in hair, cord blood and breast milk

The results for THg levels in all samples collected are shown in Table 1.

The levels of THg in cord blood from Slovenian women (Med=1.5 ng/g, minimum ($_{\mathrm{Min}}$)=0.2 ng/g, maximum ($_{\mathrm{Max}}$)=14 ng/g) are comparable with levels of MeHg in cord blood from Swedish women (Med=1.3 μg/l, Min=0.1 μg/l, Max=5.7 μg/l) consuming approximately the same amount of fish per day (25 g/day) (Björenberg et al., 2004). However, the levels in cord blood from Slovenian women (geometric mean (GM)=1.4 ng/g) are much lower compared to levels in cord blood collected from women in the Faroe Islands during the years 1986 and 1987 (GM=22.6 μg/l, Min=0.9 μg/l, Max=351 μg/l) (Budtz-Jørgensen et al., 2004). The differences in biomarkers of mercury exposure between these countries were due to the higher fish consumption and whale meat consumption in the Faroe Islands. The questionnaire completed by Faroese adults showed a mean daily consumption of 72 g fish and 12 g pilot whale muscle (Vestergaad and Zachariassen, 1987). Thus we obtained a result for hair and breast milk comparable with Swedish pregnant women (Björenberg et al., 2004; Oskarsson et al., 1996) and much lower results for hair and breast milk compared to women from the Faroe Islands in 1986 and 1987 (Budtz-Jørgensen et al., 2004). Nevertheless, more recent study (Weihe et al., 2005) reported substantially lower whale meat consumption and consequently lower THg hair levels for pregnant Faroese women. Moreover, the levels of THg in hair are far below the estimated MeHg threshold level for prenatal effects (10 mg/kg) (Cox et al., 1989) or BMDL (the lower limit on the 95% confidence interval of the benchmark dose) from the Faroe Island study (12–15 mg/kg) and from the New Zealand study (4–6 mg/kg) (Rice, 2004). Overall, as expected the concentrations of THg in cord blood, milk and hair were low, due to the low consumption of fish and their relatively low levels of mercury (Miklavčič et al., 2011).

In addition we analysed MeHg in samples where THg levels in hair exceeded or were equal to 1000 ng/g. The concentrations of MeHg in these selected samples are shown in Table 2. At higher levels MeHg on average comprised 89% (P10=55%, P90=110%) of THg in hair, 94% (P10=87%, P90=115%) of THg in cord blood and 38% (P10=3%, P90=70%) of THg in breast milk. Therefore our results showed that at higher levels MeHg comprised almost all the THg in hair and cord blood, while in breast milk the percentage of MeHg was lower and variable. The lower percentage of MeHg in breast milk compared to the percentage of MeHg in hair or cord blood is probably due to the more ready elimination of inorganic mercury in breast milk than MeHg (Sundberg and Oskarsson, 1992). Due to a relatively small number of results, further investigations are necessary to confirm these observations.

To evaluate the influence of other sources than fish in biomarkers of exposure at low levels, MeHg mercury was additionally

Table 1
X, Med, P10, P90 levels for THg in hair, cord blood and breast milk.

Sample	*N*	*X* (ng/g)	Med (ng/g)	P10 (ng/g)	P90 (ng/g)
Cord blood	446	2.0	1.5	0.5	4.2
Hair	574	377	297	73	781
Breast milk	284	0.3	0.2	0.06	0.6

Table 2
X, Med, P10 and P90 levels and standard deviation (STD) levels for MeHg in hair, cord blood and breast milk in sets of samples where THg levels in hair exceeded or were equal to 1000 ng/g and *X*, Med, P10, P90 and STD levels for MeHg in cord blood where THg levels in hair were below 1000 ng/g.

Sample	THg in hair (ng/g)	*n*	*X*	Med	P10	P90	STD
Cord blood							
MeHg (ng/g)	≥ 1000	13	6.4	6.2	3.3	9.9	2.3
MeHg (%)	≥ 1000	13	94	93	87	115	17
MeHg (ng/g)	< 1000	44	1.7	1.3	0.3	4.0	1.5
MeHg (%)	< 1000	44	79	76	57	103	19
Hair							
MeHg (ng/g)	≥ 1000	15	1270	1350	624	1630	359
MeHg (%)	≥ 1000	15	89	93	55	110	20
Breast milk							
MeHg (ng/g)	≥ 1000	11	0.68	0.07	0.03	6.2	1.8
MeHg (%)	≥ 1000	9	38	48	3	70	28

determined in 44 samples of cord blood, where THg levels in hair were below 1000 ng/g. We observed a lower Med value for the percentage of MeHg in cord blood samples where THg levels in samples of hair were below 1000 ng/g (Med=76%, P10=57%, P90=103%) compared to the percentage of MeHg in cord blood where THg levels in samples of hair exceeded 1000 ng/g (Med=93%, P10=87%, P90=115). A statistical test (Mann–Whitney test) showed that the difference between these two groups is statistically significant ($Z=-5.06$, $p<0.001$). Overall at lower levels of exposure the percentage of MeHg in cord blood was lower and more variable (Table 2). This could be due to the proportionally higher contribution of inorganic mercury from sources other than fish present in cord blood at lower levels of exposure.

3.2. Correlations of mercury levels between biomarkers

The natural logarithm (ln) of THg levels in hair was significantly and strongly associated with ln THg levels in cord blood ($r=0.87$, 95% CI: 0.84–0.89) (Fig. 1), while the regression correlations between the levels of ln THg in breast milk and ln THg levels in cord blood ($r=0.36$, 95% CI: 0.25–0.47) and the regression correlation between ln THg levels in breast milk and ln THg levels in hair ($r=0.24$, 95% CI: 0.12–0.35) were weaker, but statistically still significant.

A stronger regression correlation between ln THg levels in hair and ln MeHg levels in cord blood (Fig. 2) was found compared to ln THg levels in hair and ln THg levels in cord blood (Figs. 1 and 2). While the literature suggests that cord blood should be the preferred sample for assessment of individual risk levels in studies of prenatal neurotoxicity risks (Gradjean et al., 2002), the strong correlation between ln THg levels in hair and ln THg levels in cord blood obtained in our study indicate that THg levels in scalp hair could also be an appropriate biomarker. Moreover because of the simplicity of hair sample collection, storage and THg hair analyses, THg in hair should be the preferred biomarker for population monitoring purposes.

Due to the strong correlations between the levels of mercury in scalp hair in cord blood, the ratios between these biomarkers can be calculated, which is very important in risk assessment studies. The ratio between the concentrations of THg in hair and concentrations of THg in cord blood was on average 190 and the range between the 5th percentile and 95th percentile was 80–330. This is in agreement with the literature (Budtz-Jørgensen et al., 2004). The ratio between the concentrations of THg in hair and concentrations of MeHg in

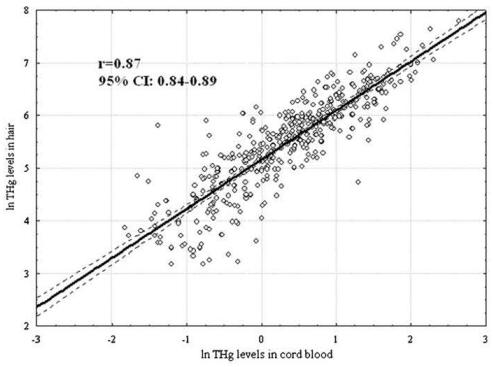

Fig. 1. Regression correlation between ln THg levels in hair and ln THg levels in cord blood.

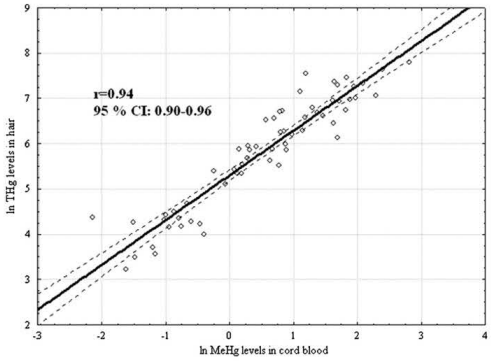

Fig. 2. Regression correlation between ln THg levels in hair and ln MeHg levels in cord blood.

cord blood was on average 220 and the range between the 5th percentile and 95th percentile was 110–390.

3.3. Fish consumption of pregnant and lactating Slovenian women and MeHg exposure assessment

The frequency questionnaires showed that pregnant women in Slovenia consume on average 2.5 portions (150 g) of fresh fish per month, 2.2 tins (80 g) of canned fish per month and 1.1 portions (150 g) of frozen fish per month (Table 3). This would correspond to a mean consumption of about 25 g/day (range: 0–185 g/day). Moreover, from the questionnaires it was estimated which species of fish are the most frequently consumed. The most frequently eaten species of fish are tuna ($X=2.3$ portions/month, Med=2 portions/month), trout or salmon ($X=1.3$ portions/month, Med=1 portion/month), cod ($X=1.2$ portions/month, Med=1 portion/month), gilt head bream ($X=0.84$ portions/month, 1 portion/month) and sea bass ($X=0.79$ portions/month, Med=1 portion/month) (Table 3). The median value for the frequency consumption of other species of fish was less than 1 portion per month. From among the women that eat tuna, round 70% of women buy canned tuna, while from among the women that

Table 3
X, Med, P10, P90 levels for frequency of fresh fish consumption, frequency of canned fish consumption, frequency of frozen fish consumption, frequency of total fish consumption, frequency of tuna consumption, frequency of trout or salmon consumption, frequency of gilt head bream consumption, frequency of sea bass consumption and frequency of the most frequently eaten species among pregnant women involved in this study.

Sample	*X*	Med	P10	P90
Frequency of fresh fish consumption (portions/month)	2.5	2.0	0.0	4.0
Frequency of canned fish consumption (tins/month)	2.2	2.0	0.0	4.0
Frequency of frozen Fish consumption (portions/month)	1.1	1.0	0.0	2.0
Frequency of total fish consumption (portions/month)	5.8	5.0	2.0	11.0
Frequency of tuna consumption (portions/month)	2.3	2.0	0.0	4.0
Frequency of trout or salmon consumption (portions/month)	1.3	1.0	0.0	2.0
Frequency of cod consumption (portions/month)	1.2	1.0	0.0	2.0
Frequency of gilt head bream consumption (portions/month)	0.84	1.0	0.0	2.0
Frequency of sea bass consumption (portions/month)	0.79	1.0	0.0	2.0
Frequency of the most frequently eaten species (portions/month)	6.3	6.0	1.0	12

Table 4
Spearman rank correlation coefficients between biomarkers of exposure and frequency of fish consumption or the sum of mean levels of MeHg in the most frequently eaten species of fish or estimated number of amalgam fillings.

	THg hair	THg cord blood	THg breast milk	MeHg cord blood
Frequency of fish consumption	**r_s=0.35, 95% CI: 0.28-0.42**	**r_s=0.43, 95% CI: 0.36–0.51**	r_s=0.08, 95% CI: −0.04–0–20	**r_s=0.31, 95% CI: 0.06–0.52**
MeHg levels in the most frequently eaten species of fish	**r_s=0.41, 95% CI: 0.32–0.50**	**r_s=0.42, 95% CI: 0.32–0.50**	**r_s=0.14, 95% CI: 0.02–0.25**	**r_s=0.42, 95% CI: 0.16–0.63**
Number of amalgam fillings	r_s= −0.07, 95% CI: −0.04–0.18	r_s=0.03, 95% CI: −0.08–0.14	r_s=0.05, 95% CI: −0.07–0.17	r_s=0.06, 95% CI: −0.26–0.36

eat cod, round 60% women buy frozen cod. Gilt head bream and sea bass are mostly bought fresh. The frequencies of fish consumption of specific types of fish are not in accordance with frequencies of total fish consumption (Table 3). The women involved in this study probably overestimated the frequency of specific types of fish consumption. It must be also taken into a consideration that they probably did not remember exactly how much fish had they eaten or exactly which type of fish they had consumed. Therefore, it must be pointed out that the questionnaire in our study provided only approximate information on fish consumption and not a precise measure of the fish ingested by an individual.

According to Eq. (1), the PDI_{intake} for Slovenian pregnant women was calculated to amount to 0.023 μg/day/kg. As expected, the PDI_{intake} for Slovenian pregnant women was low due to low consumption of fish and the relatively low levels of mercury in fish. Moreover, the calculated PDI intake is approximately 4 times lower than the EPA reference dose (EPA, 1997a) or 10 times lower than the provisional tolerable weekly intake (PTWI) for MeHg established by the Joint FAO/WHO Expert Committee on Food Additives.

THg in hair and THg in cord blood significantly increased with increasing frequency of total consumption of fish, but no correlation between THg levels in breast milk and the frequency of fish consumption was found (Table 4). However, an exceedingly weak correlation was found between THg in breast milk and calculated MeHg levels in the most frequently eaten species of fish. Although our study was mainly focused on MeHg exposure, we additionally studied the correlation of the biomarkers of exposure with the number of amalgam fillings of the women involved in this study. Amalgam fillings, in fact, with the exception of certain occupational exposures, are reported to be the main source of human exposure to mercury vapour (Clarkson, 2002). In contrast to the literature (Oskarsson et al., 1996) we did not find any relation between THg in breast milk and the number of amalgam fillings (Table 4). This is probably due to the low accuracy of the data obtained from the questionnaire, while the information acquired was a categorised number of amalgam fillings (less than 3 amalgam fillings, 3–5 amalgam fillings, 6–9 amalgam fillings, more than 10) and not the exact number of amalgam fillings.

As is shown in Table 4, a slightly stronger Spearman's rank correlation was obtained when the sum of mean levels of MeHg in the most frequently eaten species of fish was correlated with THg in hair (r_s=0.41, 95% CI: 0.32–0.50), while the correlation between THg levels in cord blood and the sum of mean levels of MeHg in the most frequently eaten species of fish remained approximately the same (r_s=0.42, 95% CI: 0.32–0.50) compared to the correlation between THg levels in cord blood and the frequency of total fish consumption (r_s=0.43, 95% CI: 0.36–0.51). A slightly stronger Spearman's rank correlation was obtained when the sum of the mean levels of MeHg in the most frequently eaten species of fish was correlated with MeHg in cord blood (r_s=0.42, 95% CI: 0.16–0.63) compared to the Spearman's rank correlation between the frequency of fish consumption and MeHg in cord blood (r_s=0.31, 95% CI: 0.06–0.52).

To elucidate which fish species contributed most to the levels of THg in cord blood and levels of THg in hair, stepwise multiple regression analyses were performed. In the model with ln hair THg as dependent variable, consumption of tuna, gilt head bream and cod remained significant (adj. R^2=0.18, $p<0.001$). The same independent variable remained significant when ln cord blood THg was chosen as the dependent variable (R^2=0.19, $p<0.001$). This could be due to higher levels of MeHg in tuna ($X_{\text{fresh tuna}}$= 559 ng/g, $X_{\text{canned tuna}}$=93 ng/g), gilt head bream (X=109 ng/g) and cod (X=66 ng/g) compared to fresh trout (X=25 ng/g) or salmon (X=27 ng/g) bought on the Slovenian market. Because of the relatively low number of results, further analyses of MeHg in the most frequently eaten types of fish available on the Slovenian market should be conducted to confirm these observations.

To summarise, our study showed that according to the correlations with the frequency of fish consumption there was not much difference between biomarkers of low-level mercury exposure such as levels of THg in hair and levels of THg or MeHg in cord blood. Moreover, it was confirmed that THg in breast milk

is not appropriate biomarker for MeHg assessment through fish consumption. The correlation between the biomarkers and the estimated sum of the mean levels of MeHg in the most frequently eaten species of fish was not considerably stronger compared to the correlation between the biomarkers and the frequency of fish consumption in general. Therefore from our results it can be concluded that the data obtained for the frequency of canned, fresh and frozen fish consumption are sufficient for large epidemiological studies and that there is no need for collection of additional data on the frequency of specific types of fish consumption, especially in a population that does not eat a lot of large fish at the top of the food chain. However, it must be pointed out that the collection of information about fish consumption should be improved, especially in informing the subjects exactly how much is a portion of fish and exactly which species of fish they exactly consume. In this way the frequency of specific types of fish consumption might provide more valuable information compared to the frequency of general fish consumption. However, in addition to the imprecision of the data on fish consumption from the questionnaire, the weak correlation between the biomarkers of exposure and the frequency of fish consumption can also be due to the low frequency of fish consumption, the high variability of MeHg between and within different species of fish (Miklavčič et al., 2011) and particularly at low-level exposure, to the presence of inorganic mercury, which originates from sources other than fish, possibly contributing significantly to measured levels of THg in cord blood or hair. Moreover, inter-individual differences may also affect the biomarkers of low-level mercury exposure (Grundacker et al., 2007).

4. Conclusion

Overall we can conclude that the concentrations of THg in cord blood, hair and breast milk in Slovenian women are low, due to the low frequency of fish consumption. At low levels of exposure a number of factors can influence the strength of the correlation between biomarkers of exposure and intake of MeHg through fish consumption. The important factors are the accuracy of the data contained in the questionnaire, the high variability of MeHg in fish, the presence of inorganic mercury from sources other than fish and inter-individual differences. Our study also confirmed that THg in hair and THg or MeHg in cord blood are suitable biomarkers of mercury exposure from fish consumption. Moreover, for large epidemiological studies THg level in hair is the most appropriate biomarker for exposure assessment, because of its simple and practical collection and storage of the samples, and due to the relatively simple, fast and precise analytical determination of THg determination in hair.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.envres.2011.07.006.

References

Akagi, H., 1997. Analytical method for evaluating human exposure to mercury due to gold mining. In: Proceedings of the International Workshop on Health and Environmental Effects of Mercury due to Mining Operations. National Institute for Minamata Disease, Manila, pp. 131–141.

ATSDR, 1999. Toxicological Profile for Mercury Atlanta, GA: Agency for Toxic Substances and Disease Registry.

Björenberg, K.A., Vahter, M., Petersson-Grawè, K., Glynn, A., Cnattingius, S., Darnerund, P.O., Atuma, S., Aune, M., Becker, W., Berglund, M., 2004. Methyl mercury and inorganic mercury in Swedish pregnant women and cord blood: influence of fish consumption. Environ. Health. Persp. 111 (4), 637–641.

Budtz-Jørgensen, E., Grandjean, P., Jørgensen, P.J., Weihe, P., Keiding, N., 2004. Association between mercury concentration in blood and hair in methylmercury exposed subjects at different ages. Environ. Res. 95, 385–393.

Cernichiari, E., Brewere, R., Myers, G.J., Marsh, D.O., Lapham, L.W., Cox, C., Shamlaye, C.F., Berlin, M., Davidson, P.W., Clarkson, T.W., 1995. Monitoring methylmercury during pregnancy: maternal hair predicts fetal brain exposure. Neurotoxicology 16 (4), 705–710.

Clarkson, T.W., 2002. The Three Modern Faces of Mercury. Environ. Health Persp. 110, 11–23.

Cox, C., Clarkson, T.W., Marsh, D.O., Amin-Zaki, L., Al-Tikriti, S., 1989. Dose response analysis of infants prenatally exposed to methylmercury poisoning: relationship between concentration in single strands of maternal hair and child effects. Environ. Res. 49, 318–332.

EPA Method 7473. Mercury in Solids and Solutions by Thermal Decomposition Amalgamation and Atomic Absorption Spectrometry, 1998.

EPA, 1997a. Mercury Study Report to Congress, Volume I: Executive Summery. EPA-452/R-97-007. Washington, DC. Office of Air Quality Planning and Office of Reaseach and Development.

EPA, 1997b. Mercury Study Report to Congress, Volume IV: Assessment of Exposure to Mercury in the United States. Washington, DC. Office of Air Quality Planning and Office of Reaseach and Development. EPA-452/R-97-007.

EPA, 2003. Fact Sheet. Update: National Listing of Fish and Wildlife Advisories. Washington, DC: Office of Woter. EPA 540-R-01-005.

Gradjean, P., Jørgensen, P.J., Weihe, P., 2002. Validity of mercury exposure biomarkers. In: Wilson, S.H., Suk, W.A. (Eds.), Biomarkers of Environmentally Associated Disease. CRC Press, Boca Raton, pp. 235–247.

Grundacker, C., Komarnicki, G., Jagiello, P., Gencikova, A., Dahmen, N., Wittmann, K.J., Gencik, M., 2007. Glutathione-S-transferase polymorphism, metallothionin expression, and mercury levels among students in Austria. Sci. Tot. Environ. 385, 37–47.

Horvat, M., Byrne, A.R., May, K., 1990. A modified method for the determination of methylmercury by gas chromatography. Talanta 37 (2), 207–212.

Horvat, M., May, K., Stoeppler, M., Byrne, A.R., 1988. Comparative studies of methylmercury in biological and environmental samples. Appl. Organomet. Chem. 2, 515–524.

Horvat, M., Liang, L., Bloom, N.S., 1993a. Comparison of distillation with other current isolation methods for the determination of methyl mercury compounds in low level environmental samples. Part 1: Sediments. Anal. Chim. Acta 282 (1), 153–168.

Horvat, M., Liang, L., Bloom, N.S., 1993b. Comparison of distillation with other current isolation methods for the determination of methyl mercury compounds in low level environmental samples. Part 2: Water. Water. Anal. Chim. Acta 282 (1), 153–168.

Liang, L., Horvat, M., Bloom, N.S., 1994. An improved method for speciation of mercury by aqueous phase etylation, room temperature precollection, GC separation and CV AFS detection. Talenta 3, 371–379.

Miklavčič, A., Stibilj, V., Heath, E., Polak, T., Snoj, Tratnik, J., Klavž, J., Mazej, D., Horvat, M., 2011. Mercury, selenium, PCBs and fatty acids in fresh and canned fish available on the Slovenian market. Food Chem. 124, 711–720.

NRC (National Research Council), 2000. Toxicological Effects of Methylmercury. National Academy Press, Washington, DC.

Oskarsson, A., Schültz, A., Skerfving, S., Hallèn, I.P., Ohlin, R., Lagerkvist, B.J., 1996. Total and inorganic mercury in breast milk in relation to fish consumption and amalgam lactating women. Arch. Environ. Heath 51 (3), 234–241.

Sundberg, J., Oskarsson, A., 1992. Placental and lactational transfer of mercury from rats exposed to methylmercury in their diet: speciation of mercury in the offspring. J. Trace Elem. Exp. Med. 5 (1), 47–56.

3.3 Mercury, arsenic and selenium exposure levels in relation to fish consumption in the Mediterranean area

In this section, the article (Miklavčič et al., 2013a) entitled "Mercury, arsenic and selenium exposure levels in relation to fish consumption in the Mediterranena area" by Ana Miklavčič, Anica Casetta, Janja Snoj Tratnik, Darja Mazej, Mladen Krsnik, Dr. Marika Mariuz, Katia Sofianou, Dr. Zdravko Spirić, Prof. Dr, Fabio Barbone and Prof. Dr. Milena Horvat is presented. The article was published in Environmental Research (Elsevier).

In this article Hg, As and Se exposure levels of the most susceptible population of Mediterranean areas, namely pregnant women, were assessed through fish consumption using detailed fish consumption data in combination with different biomarkers of exposure. The main strength of this study was the high number of women (over 2000) from whom different biomarkers of Hg, As and Se exposure and nutritional status data were collected. This is a comprehensive study that involves the same data collection approach and the same sampling methods in all four countries (Italy, Slovenia, Croatia and Greece) and in which analysis of all biological samples was performed by the same laboratory. Furthermore, in terms of simultaneous low-level exposure to a group of elements that can interact with each other, this study represents an invaluable data base for future risk assessment studies. In addition, the Hg speciation data obtained in this work are of great importance for studying the toxicokinetics of Hg. Given the cost of analyses of MeHg at low levels of exposure in epidemiological studies, this is a uniquely extensive study in which speciation of Hg was determined in a large number of biological samples (Miklavčič et al., 2013a).

This study was part of a research project PHIME within the EU 6th Framework Programme. My contribution to this work was first to evaluate all data, carry out the calculations and supervised by Prof. Dr. Barbone and Anica Cassetta, perform the statistical analyses. I interpretated the data and drafted the manuscript. In addition, I carried out the analyses of THg in more than 2000 samples. I was supervised by Prof. Dr. Milena Horvat.
Part of this work was also presented and published at scientific conferences: the 3rd International Symposium on Trace Elements & Health, 24-27, May, in 2007 and the 11th International Symposium on Neurobehavioral Methods and Effects in Occupational and Environmental Health, June 5-10, 2011.

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Mercury, arsenic and selenium exposure levels in relation to fish consumption in the Mediterranean area

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ABSTRACT

In order to assess mercury (Hg), selenium (Se) and arsenic (As) exposure in the Mediterranean area, total mercury (THg), monomethylmercury (MeHg), Se and As levels were measured in umbilical cord blood and breast milk from Italian (n=900), Slovenian (n=584), Croatian (n=234) and Greek (n=484) women. THg, MeHg, As, and Se levels were also determined in blood samples of the same mothers from Italy and Croatia. In addition, THg and MeHg were determined in the same women's hair from all the countries involved in this study and As and Se levels were determined in the mother's urine samples from Italy, Croatia and Greece. Besides recording the consumption of other food items, the frequencies of fish consumption were assessed by detailed food frequency questionnaires, since fish represents an important source of Hg, Se and As in humans. The highest levels of THg and As were found in cord blood ($Med_{(THg)}$=5.8 ng/g; $Med_{(As)}$=3.3 ng/g) and breast milk ($Med_{(THg)}$=0.6 ng/g; $Med_{(As)}$=0.8 ng/g) from Greek women, while the highest Se levels were found in cord blood (Med=113 ng/g) from Italy. Significant linear correlations were found between Hg, Se and As in blood, cord blood and breast milk. In addition, significant relations were found between the frequencies of total fish consumption and biomarkers of As, MeHg and Se exposure, with the strongest Spearman rank coefficients between frequencies of total fish consumption and THg levels in cord blood (r_s=0.442, $p<0.001$) or THg levels in hair (r_s=0.421, $p<0.001$), and between frequencies of total fish consumption and As levels in cord blood (r_s=0.350, $p<0.001$). The differences in Hg and As exposure between countries were probably due to different amounts of fish consumption and the consumption of different species of fish of different origin, while the highest Se levels in women from Italy were probably the consequence of the more frequent consumption of different non specific food items. Moreover, fish consumption, the possible common source of As, Hg and Se intake, could explain the correlations between the elements determined in cord blood, mother's blood or breast milk.

1. Introduction

An important source of Hg, Se and As exposure in man is the consumption of fish and other food sea (UNEP, 2002; Hughes et al., 2009; Reilly, 1996).

Although inorganic Hg it is also present in fish (Miklavčič et al., 2011a), MeHg is of particular concern because it can build up in fish higher in the food chain and marine mammals to levels that are many times higher than the levels in the surrounding water, also to levels exceeding what is regarded as safe (Horvat et al., 2011a). Moreover, 95% of MeHg is to be absorbed in the gastro-intestinal tract, while animal studies indicate that the absorption of inorganic Hg is approximately 10–30% (Piotrowski et al., 1992; Morcillo and Santamaria, 1995). Overall, the general population is

Abbreviations: CRM, certified reference material; k, coverage factor; LOD, limit of detection; LOQ, limit of quantification; Max, maximum; Min, minimum; Med, median; MeHg, monomethylmercury; P5, 5th percentile; P95, 95th percentile; PHIME, Public Health Impact of Long-term Low-level Mixed Element Exposure in Susceptible Population Strata; PUFAs, polyunsaturated fatty acids; RM, reference material; THg, total mercury; X, mean

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exposed to MeHg mainly through the consumption of freshwater and marine fish, and consumption of other animals that consume fish (EPA, 1997a,b, 2003; UNEP, 2002). The levels of Hg in fish from different FAO regions ranged from 0.003 to 1.110 mg/kg (Miklavčič et al., 2011a). The highest levels are found in fish of older age that are apical predators, such as king mackerel, pike, shark, swordfish, walleye, barracuda, large tuna, scabbard, marlin and fish-consuming mammals such as seals and toothed whales (EPA, 1997a,b, 2003; UNEP, 2002; Miklavčič et al., 2011a). Commonly used cooking techniques including panfrying, deep-frying, baking, boiling, and smoking do not appreciably reduce the Hg content of the fillet portion of fish (Morgan et al., 1997; Perelló et al., 2008). The major toxic effect of MeHg is on the central nervous system and the developing foetus is the most vulnerable target (NRC, 2000). Scalp hair has been widely used as a good indicator of MeHg exposure. Hg in umbilical cord blood is preferentially measured instead of Hg in maternal blood for prenatal MeHg exposure assessment (Horvat et al., 2011a).

For most people, excluding sources of As pollution and drinking water contamination, the diet is the major source of As (Abernathy et al., 2003). The levels of As vary widely in different foods and are dependent on the type of soil, water and geochemical activity (ASTDR, 2007). Foods of marine origin, such as fish and shellfish, tend to have the highest levels of As, primarily in organic form such as arsenobetaine and arsenocholine (Hughes et al., 2009). Tuna, shrimp, hake and sardine have levels of As ranging from 0.3 to 2.7 mg/kg (Tao and Bolger, 1999; Perelló et al., 2008). Compared to inorganic As, the organic forms of As in seafood are considered relatively nontoxic (Sakurai et al., 1997; Hughes et al., 2009). The prevalent species of As that occurs in marine organisms, arsenobetaine (Edmonds and Francesconi, 2003) is rapidly excreted by humans unchanged (Vahter, 1994) and is assumed to have negligible toxicity because of its high LD50 in mice (>10 000 mg/kg) (Kaise and Fukuit, 1992). In contrast to organic As, it was demonstrated that inorganic As is associated with increased risk for a wide range of diseases. To date, it has been linked to high risks of several types of cancer, as well as of diabetes, vascular disease, hypertension, neurological disorders, reproductive problems, and the well-known skin damage (Hopenhayn, 2006; NRC, 1999; IARC, 2000). Inorganic As is predominant in drinking water (Hopenhayn, 2006) or to a lesser extent in foods such as rice, flour, grape juice and spinach (Schoof et al., 1999). Different studies reported decreases or increases in total As after cooking (Devesa et al., 2001; Van Eltern and Šlejkovec, 1997; Perelló et al., 2008). The increase in As levels could be only apparent due to loss of water, while solubilisation or volatilization of As compounds during cooking may cause a decrease in the concentration of As (Devesa et al., 2001). Biomarkers of As exposure include the concentration in urine, blood, hair, or nails. The most common inorganic As biomarker of exposure is total As in urine. However, consumption of seafood containing high levels of organic As can critically confound estimation of inorganic As exposure. Analysis of blood for As is best suited for recent high-dose exposure and cannot be a reliable biomarker for inorganic As exposure because it is cleared so rapidly, particularly at low levels of inorganic As exposure (ASTDR, 2007; Hughes, 2002; Horvat et al., 2011b).

Se is an essential trace element, but be toxic in larger amounts. The range between insufficiency and toxicity is rather narrow. A Joint Food and Agriculture Organization/World Health Organization (FAO/WHO) Expert Committee on Human Vitamin and Mineral Requirements proposed a recommended nutrient intake for Se of 26 and 34 μg/day in adult women (55 kg) and men (65 kg), respectively, and 6 μg/day in infants aged 0–6 months (6 kg) (Joint FAO/WHO, 1998). However, different intuitions recommended different values. The recommended adequate intakes of selenium published by D_A_CH are 30–70 μg/day for adults, 4–15 μg/day until infants are 6 months old and 7–30 μg/day for infants aged 4 to 12 months (Reference Values for Nutrition Intake, 2002). Fish and other seafood are the most important providers of Se in the human diet (Reilly, 1996) and the levels vary greatly according to species and fishing area (Wyatt et al., 1996). Total Se levels found in fish from different FAO regions ranged from 0.085 mg/kg to 1.180 mg/kg (Miklavčič et al., 2011a). Beside fish, food items high in Se can include meat, nuts, cereals and bread. Importantly, the Se content of foods can be extremely variable, depending on the combination of geo-environmental factors and Se supplementation of fertilizers and animal feedstuffs (Barclay et al., 1995; Thomson and Robertson, 1990; NIH, 2011; Smrkolj et al., 2005). Foods normally contain only organoselenium compounds. Inorganic compounds of the element, such as sodium selenite, only enter the diet as supplements or contaminants (Reilly, 1996). Plasma, erythrocyte and whole-blood Se respond to changes in Se intake. More large, high quality, randomized controlled trials are needed for Se biomarkers to explore heterogeneity in response to Se intakes (Ashton et al., 2009). Several studies have shown Se supplementation counteracts the negative impacts of exposure to Hg in all investigated species of mammals, birds and fish (Beijer and Jernelov, 1987; Culvin-Aralar and Furness, 1991). However, little is known about the potential protective effects of dietary Se against MeHg neurotoxicity in humans (NRC, 2000). Antagonistic effects or mutual detoxification between As and Se have also been confirmed in many animal species including humans (Levander, 1977; Zeng., 2001; Zeng et al., 2005). The interaction between Se and As can occur directly and indirectly, depending on the chemical forms and dose of each. Increased biliary excretion of Se may be the principal mechanism by which As interacts with Se. The existence of an interaction between As and Se through biliary excretion at low level Se and As intakes still remains to be determined (Zeng et al., 2005).

Although fish are a source of pollutants such as toxic metals (Hg, As, lead and cadmium), polychlorinated biphenyls, organochlorine pesticides and aromatic hydrocarbons, they are also an important source of protein, microelements (Se, iodine, zinc), macroelements (calcium, phosphorus), polyunsaturated fatty acids (PUFAs) and fat-soluble vitamins. While Hg in fish negatively affects neurodevelopment (NRC, 2000), PUFAs in fish have a potential beneficial effect on neurological development (Nettleton, 1993; Helland et al., 2008). A considerable body of literature exists focused on the effects of prenatal exposure through fish consumption on neurodevelopment. However, the findings are inconsistent, particularly when assessing effects at low levels of exposure (Schoeman et al., 2009). Some of the divergent outcomes from different studies could be due to different end points and ages used in testing, use of different biomarkers, choice of covariates for statistical models, differences in the study populations and different levels of Hg and other contaminants in fish (Schoeman et al., 2009).

The objective of this study was to assess the Hg, As and Se exposure of the most susceptible population of Mediterranean areas, namely pregnant women, through fish consumption using detailed fish consumption data in combination with different biomarkers of exposure. The main strength of this study is the high number of women (over 2000) from whom different biomarkers of Hg, As and Se exposure and nutritional status data were collected. Although a small part of the data on Hg exposure in Slovenia has already been published elsewhere (Miklavčič et al., 2011b); this is a comprehensive study that involves the same data collection approach and the same sampling methods in all the four countries and in which analysis of all biological samples was performed by the same laboratory. Furthermore, in terms of simultaneous low-level exposure to a group of elements that can interact with each other, this study represents invaluable

information for future risk assessment studies. In addition, the Hg speciation data obtained in this work are of the great importance for studying the toxicokinetics of Hg. Given the cost of analyses of MeHg at low levels of exposure in epidemiological studies, this is a uniquely extensive study in which speciation of Hg was determined in a large number of biological samples.

2. Methods

2.1. Study design, collection and storage of the samples

This prospective cohort study included 4 recruitment areas: (a) the coastal Province of Trieste, Italy, (b) the city of Ljubljana, Slovenia and its surroundings (number of samples (n)=513) and Izola, Koper, Piran, Idrija and Kočevje (n=72), (c) the coastal city of Rijeka and its county, Croatia, and (d) the Greek islands of Lesvos, Chios, Samos and Leros in the Eastern Aegean. It must be pointed out that in this study three different populations were involved: (1) the central Slovenian population with a low frequency of fish consumption; (2) Italian and Croatian populations from coastal and near coastal areas; and (3) a Greek population consuming mostly fish from local areas. The age structure of pregnant women involved in this study was: 18–20 years (n=2), 20–30 years (n=118), 30–40 years (n=558), more than 40 years (n=72) in Italy; 20–30 years (n=147), 30–40 years (n=209), more than 40 years (n=12) in Slovenia; 20–30 years (n=81), 30–40 years (n=104), more than 40 years (n=3) in Croatia; 18–20 years (n=3); 20–30 years (n=157), 30–40 years (n=173), more than 40 years (n=11) in Greece. The women from Italy were slightly older compared to women from other countries (Z=14,518, $p<0.001$; Wilcoxon–Mann–Whitney test). All women were permanent residents in the study areas with no absence from the study area for more than 6 weeks during pregnancy, no history of drug abuse, no serious health problems, no serious complications of pregnancy, and no twin gestation. In Italy, the recruitment took place at the Burlo Garofolo Children's Hospital in Trieste; in Slovenia at the Maternity Hospital of the University Medical Centre of Ljubljana and in health centres located in Izola, Koper, Piran, Idrija and Kočevje; in Croatia at the University Hospital of Rijeka and in Greece at the General Regional Hospitals of Mytilini (Lesvos), Chios, Samos and Leros. At recruitment, eligible women were approached for consent following their routine morphological ultrasound scan between the 20th and 22nd weeks of gestation (Italy), at their 34–38th week of gestation visit (Croatia), or during their hospital stay for delivery (Slovenia, Croatia and Greece). For logistic reasons, the timing of data and sample collection was different among the 4 centres and some biological samples were not collected in all the countries (Table 1). However, the data collection protocol and sampling methods were the same in all 4 countries and all analysies of biological samples were performed by the same laboratory at the "Jožef Stefan" Institute in Ljubljana.

The participating women filled out a short and long questionnaire. These were designed at the University of Udine, Italy, translated from English into the local language and tested in each country. A short questionnaire was completed at the time of recruitment to verify the absence of exclusion criteria and to provide a quick assessment of demographic information, smoking status or of food frequency consumption (vegetables, milk and milk products, eggs, meat, fresh fish, frozen fish, canned fish, alcoholic beverages). The long questionnaire collected sociodemographic and health status information on the women and their family, information on the pregnancy and delivery, a detailed residential and occupational history of the women, a record of their smoking, drinking, and general dietary habits, and a detailed food frequency assessment investigating their consumption of 138 food items adapted from a validated frequency questionnaire (Franceschi et al., 1993,1995; Decarli et al., 1996). Moreover, a section investigating the consumption of over 22 fish species commonly caught or marketed was also included in the long frequency questionnaire. Food intake categories in the questionnaire were converted to continuous estimates of weekly intake; the estimated portions of fish were then converted to grams. One portion of fresh or frozen fish was estimated to be around 150 g, while one portion of canned fish was estimated to be around 80 g.

Sampling was performed according to a protocol developed by the Jožef Stefan Institute, Ljubljana, and by the University Medical Centre, Clinical Institute of Clinical Chemistry and Biochemistry, Ljubljana, using standardized sample containers. Approximately 7 ml of whole mother's venous blood or whole mixed umbilical cord blood was collected for THg, MeHg, Se and As determination. Samples of mother's blood or umbilical cord blood were immediately withdrawn into the tubes containing sodium heparinate. Collected Samples were separated into three polypropylene tubes for THg, MeHg and As, Se analysis. These samples were collected by ad-hoc research trained personnel in Italy and by hospital personnel in other countries. An approximately 50 mL portion of 24-hour breast milk for THg, MeHg, Se and As determination was collected in most cases by research trained personnel at the participant's home. In a very few cases the women preferred to hand in the samples to research staff at the study hospital. All breast milk, mother's blood, cord blood and urine samples were stored in a freezer below −24 °C. Before analyses the samples were defrosted and reconstituted. Hair samples were cut with stainless steel scissors close to the root in the occipital region of the scalp. Each hair sample was stored in a plastic bag and then analysed without any cleaning or special treatment.

2.2. Study methods

2.2.1. Determination of THg in hair and blood

THg in hair and THg in blood were determined by thermal combustion at 650 °C, amalgamation and atomic absorption spectrometry using a Direct Mercury Analyser (Milestone, USA). The procedure has been described in detail elsewhere (EPA Method 7473, 1998). About 0.020 g of human hair or about 0.200 g of blood was weighed in a sample boat. NIES CRM (Certified Reference Material) no 13 (human hair) from the National Institute for Environmental Studies in Japan was used to check the accuracy of the results of THg in hair and the value found (4380 ± 100 ng/g, n=58) was in good agreement with the certified reference value (4420 ± 200 ng/g). The reference material (RM) Seronorm Trace Elements in Whole Blood L-1 (LOT no: MR4206) was used to check the accuracy of the results for THg in blood and the value found (2.2 ± 0.18 ng/ml) was in good agreement with the reference value (2.2 ± 0.2 ng/ml). In addition, the IJS laboratory participated in the interlaboratory comparisons organised within the PHIME project. Three intercomparisons using lyophilised samples of human blood from: (A) non-exposed persons; (B) people occupationally exposed to elemental Hg; (C) fish eaters were employed. In the fourth intercomparison (D) fresh blood from the general population was used. The obtained values (A: 6.4 ± 0.2 ng/g, B: 52 ± 3.7 ng/g, C: 106.4 ± 5.9 ng/g, D1: 1.28 ± 0.07 ng/g, D2: 1.64 ± 0.03 ng/g, D3: 0.96 ± 0.03 ng/g) for THg were in agreement with the assigned values (A: 7.8 ± 0.9 ng/g, B: 54 ± 8 ng/g, C: 103 ± 16 ng/g, D1: 1.39 ± 0.14 ng/g, D2: 1.80 ± 0.16 ng/g, D3: 1.09 ± 0.20 ng/g).

The limit of detection (LOD) of the method calculated as three times the standard deviation of the blanks was 0.2 ng/g hair and 0.02 ng/g blood, while the limit of quantification (LOQ) calculated as ten times the standard deviation of the blanks was 0.7 ng/g hair and 0.07 ng/g blood.

Table 1
Protocol summary: study phases and samples, and data collected in each phase in each country.

Country	Recruitment start and end dates and number of women recruited	Recruitment, short questionnaire, mother's hair collection	Prenatal/perinatal sample collection	Long questionnaire	Postnatal sample collection
Italy	Start: 03-Apr-2007End: 03-Mar-2009N=900	20–22 weeks estimated gestational age (EGA)	Maternal blood and urine at 20–22 weeks EGA and urine at 30–32 weeks EGA	30–32 weeks EGA 1 month post-delivery	Cord blood at delivery. Breast milk at 1 month post- delivery.
Slovenia	Start: 18-Feb-2007End: 29-Jul-2009N=584	Perinatal period	No	1 month post-delivery	Cord blood at delivery. Breast milk and maternal hair at 1 month.
Croatia	Start: 04-Jun-2007End: 05-Nov-2008N=234	34–38 weeks EGA or perinatal period	Maternal blood at 34–38 weeks EGA or in perinatal period	1 month post-delivery	Cord blood at delivery. Breast milk and urine at 1 month.
Greece	Start: 08-Sep-2006End: 26-Jun-2009N=484	Perinatal period	No	3–6 months post-delivery	Cord blood and maternal hair at delivery. Breast milk and urine at 3–8 months.

Although the sample mass was low, the estimated precision of the determination of THg in hair samples was 7% (with a coverage factor (k) of 2). The estimated precision of determination of THg in blood samples at levels higher or equal to 1 ng/g was 7% ($k=2$), while at lower levels (less than 1 ng/g) it was 14% ($k=2$).

2.2.2. Determination of THg in breast milk

2 ml of breast milk was placed in a 30 ml screw capped Teflon volumetric flask, to which 1 ml of distilled water, 2 ml of a mixture of 65% HNO_3 (Merck, Germany, p.a.) $HClO_4$ (Merck, Germany, suprapur) (1:1, v/v) and 5 ml of 96% H_2SO_4 (Merck, Germany, suprapur) were added. The flask was heated at 220 °C on a hotplate for 20 min. After cooling, the digested samples were diluted to 30 ml with distilled water. A semi-automated Mercury Analyser based on cold vapour atomic absorption spectrometry was used for determination of THg in the digested samples (Akagi, 1997). The accuracy of the results was checked by analysing the standard RM Non-Fat Milk Powder NIST 1549 (Lot no. 1) from the National Institute of Standards and Technology (Gaithersburg) and the measured value (0.38 ± 0.08 ng/g) was in good agreement with the reference value (0.3 ± 0.2 ng/g). The estimated precision of the determination of THg in breast milk samples was 13% ($k=2$). This relatively low precision of THg determination in breast milk was a result of the prevalent low levels of THg in breast milk.

The LOD of the method calculated on the basis of three times the standard deviation of the blanks was 0.045 ng/ml of breast milk, while the LOQ calculated as ten times the standard deviation of the blanks was 0.2 ng/ml breast milk.

2.2.3. Determination of MeHg in hair

About 0.015–0.030 g of hair sample was weighed into a screw capped Teflon tube, to which 10 ml of 6 M HCl (Merck, Germany, suprapur) was added and the mixture shaken overnight. MeHg in the extract was subsequently back extracted into 0.5 ml of toluene. Two microlitres of the toluene was injected into a packed gas chromatography column and MeHg was measured by gas chromatography–electron capture detection (GC–ECD). A complete description of the method was given elsewhere (Horvat et al., 1988,1990).

NIES CRM no 13 (human hair) from the National Institute for Environmental Studies in Japan was used to check the accuracy of the results of MeHg in hair and the value found (3840 ± 130 ng/g) was in good agreement with the certified reference value (3800 ± 400 ng/g). The estimated precision of the method calculated on the basis of the standard deviation of the MeHg determination in the RM was 12% ($k=2$).

The LOD of the method calculated on the basis of three times the standard deviation of the blanks was 0.2 ng/g hair, while the LOQ calculated as ten times the standard deviation of the blanks was 0.7 ng/g hair.

2.2.4. Determination of MeHg in blood and breast milk

About 200 mg of blood or about 1 g of breast milk sample was weighed directly in a 30 ml screw capped Teflon vial to which 6 ml of a mixture of 5% H_2SO_4 (Merck, Germany, suprapur), 18% KBr (Merck, Germany, p.a.) and 1.0 ml of a 1 M solutions of $CuSO_4$ (Merck, Germany, p.a.) was added. After shaking the vials vigorously, solvent extraction and aqueous phase ethylation were performed. The ethylated MeHg as ethylmercury was purged onto a Tenax trap for 15 min with nitrogen gas. The Tenax trap was then connected to a flow of argon and MeHg was thermally desorbed (180 °C) onto an isothermal GC column. Hg species were converted to Hg^0 by pyrolysis at 600 °C and measured by a cold vapour atomic fluorescence detector (CV AFS). The procedure has been described in detail elsewhere (Horvat et al., 1993a,b; Liang et al., 1994).

The accuracy of the results for MeHg in mother's blood or cord blood and MeHg in breast milk was checked by analysing RM lyophilised whole human blood PT-WB1 obtained from a non-exposed population. MeHg in PT-WB1 was determined by the laboratories participating in the PHIME interlaboratory comparison. The determined value (6.1 ± 0.3 ng/g) was in good agreement with the assigned value (6.3 ± 0.5 ng/g). In addition, our laboratory participated in the previously described interlaboratory comparison for MeHg determination in lyophilised whole human blood organised within the project PHIME. The obtained value (5.8 ± 0.5 ng/g) was in agreement with the assigned value ($6.3. \pm 0.5$ ng/g). The estimated precision of MeHg determination in blood samples was 12% ($k=2$) and the estimated precision of MeHg determination in breast milk samples was 17% ($k=2$).

The LOD of the method for MeHg determination in blood calculated on the basis of three times the standard deviation of the blanks was 0.02 ng/g blood, while the LOQ calculated as ten times the standard deviation of the blanks was 0.07 ng/g blood. The LOD of the method for MeHg determination in breast milk calculated on the basis of three times the standard deviation of the blanks was 0.003 ng/g breast milk, while the LOQ calculated as ten times the standard deviation of the blanks was 0.01 ng/g breast milk.

2.2.5. Determination of As and Se in blood samples

An aliquot of 0.3 mL of blood sample was diluted ten times with an alkaline (Merck, suprapur) solution containing Triton X-100 (Sigma Aldrich, sigmaultra) and ethylenediaminetetraacetic acid disodium salt dehydrate (EDTA, Fisher Scientific, analytical reagent grade) (Bárány et al., 1997). An aliquot of an internal standard solution containing Ga, Gd, Y and Sc was added. For calibration the standard addition procedure was performed. Measurements of prepared solutions were made on an Octapole Reaction System Inductively Coupled Plasma Mass Spectrometer (7500ce, Agilent) equipped with an ASX-510 Autosampler (Cetac). Instrumental conditions were as follows: Babington nebuliser, Scott-type spray chamber, spray chamber temperature 5 °C, plasma gas flow rate 15 L/min, carrier gas flow rate 0.8 L/min, make-up gas flow rate 0.1 L/min, sample solution uptake flow rate 1 mL/min, RF power 1500 W, reaction cell gas helium 4 mL/min, isotopes monitored ^{75}As, ^{77}Se, ^{78}Se. Tuning of the instrument was made daily using a solution containing Li, Mg, Y, Ce, Tl and Co. Quantification of all isotopes was performed using one central point of the spectral peaks and three repetitions.

The reference material (RM) Seronorm Trace Elements in Whole Blood L-1 (LOT no: MR4206, Sero) was used to check the accuracy of the results for As and Se in blood and the values found (As: 2.1 ± 0.2 ng/ml, Se: 72 ± 10 ng/ml) were in good agreement with the reference values (As: 1.8 ± 0.4 ng/ml, Se: 79.8 ± 5.4 ng/ml). The limit of detection for As and Se calculated as three times the standard deviation of the blanks were 0.13 and 5 ng/g blood, respectively.

2.2.6. Determination of As and Se in breast milk samples

For determination of As and Se in milk samples two sample preparation procedures were used:

a) Microwave digestion: About 1 mL of milk sample (0.15 g of lyophilised sample) was weighed into a quartz tube. 1 mL 65% HNO_3, (Merck, Germany, suprapur) and 1 mL 30% H_2O_2, (Merck, Germany, suprapur) were added and the samples were subjected to closed vessel microwave digestion (Microwave system ETHOS 1, MILESTONE SN 130471) at a max. power of 1500 W: ramp to 130 °C for 10 min, ramp to 200 °C for 10 min, hold 20 min and cooling 20 min. Then the samples were equilibrated to room temperature. The solution was quantitatively transferred to a 10 mL polyethylene graduated tube and filled to the mark with Mili-Q water.
b) Dilution: An aliquot of 1 mL of milk sample was diluted five times with alkaline (Merck, suprapur) solution containing Triton X-100 (Sigma Aldrich, sigmaultra) and ethylenediaminetetraacetic acid disodium salt dehydrate (EDTA, Fisher Scientific, analytical reagent grade). An aliquot of the internal standard solution containing Ga, Gd, Y and Sc was added.

For calibration the standard addition procedure was utilised in both procedures.

Measurements of prepared solutions by inductively coupled plasma mass spectrometry (ICP-MS) were described in Section 2.2.5.

The accuracy of the results was checked by analysing the certified reference material Whole Milk Powder NIST 8435 from the National Institute of Standards and Technology (Gaithersburg) and the measured values (microwave: As: 2.0 ± 0.6 ng/g, Se: 121 ± 16 ng/g; dilution: As: 1.3 ± 0.6 ng/g, Se: 128 ± 15 ng/g) were in good agreement with each other and with the certified reference value for Se of 131 ± 14 ng/g and the informative value for As of 1 ng/g. An internal milk control material in liquid form was also used for accuracy checking. The values found by microwave digestion (As: 0.21 ± 0.07 ng/g, Se: 14.2 ± 2.7 ng/g) were comparable with the values obtained by dilution (As: 0.27 ± 0.11 ng/g, Se: 14.0 ± 1.7 ng/g). Comparison of the value obtained for As was also made by the independent method of radiochemical neutron activation analysis (0.28 ± 0.02 ng/g) (Byrne, 1987; Byrne and Vakselj, 1974).

The limits of detection of procedure (a) for As and Se calculated as three times the standard deviation of the blanks were 0.04 and 1.5 ng/g blood, respectively. The limits of detection of procedure (b) for As and Se were 0.04 and 0.9 ng/g, respectively.

2.2.7. Determination of As and Se in urine samples

An aliquot of 1 ml of urine sample was diluted five times with 1% nitric acid (Merck, suprapur), and internal standard solution containing Ga, Gd, Y and Sc was added. For calibration the standard addition procedure was utilised (Heitland and Köster, 2004).

Measurements of prepared solutions by ICP-MS were described in Section 2.2.5.

The reference material (RM) Clinchek®Urine Control (Lot 923, Recipe) was used to check the accuracy of the results for As and Se in urine and the values found (As: 41 ± 3 ng/ml, Se: 28 ± 3 ng/ml) were in good agreement with the reference values (As: 43.5 ± 8.7 ng/ml, Se: 28.3 ± 5.7 ng/ml). The limits of detection for As and Se calculated as three times the standard deviation of the blanks, were 0.3 ng/mL and 4 ng/mL for As and Se, respectively.

2.2.8. Statistical analyses

All data obtained were analysed using STATA11/SE software. The distribution of THg, MeHg, Se and As levels was displayed as box plots. The normality of the THg, MeHg, Se, As distributions was assessed using the Shapiro–Wilk test. Logarithmic transformation of THg, MeHg, Se and As levels in mother's blood, cord blood and breast milk was used to conform with the requirement for a normal distribution. Even though in some cases the logarithmically transformed

levels of THg, MeHg, Se or As levels were not normally distributed, the logarithmically transformed levels approached a normal distribution more closely than the non-logarithmically transformed levels. The Pearson correlation was used for bivariate comparison. Furthermore, the Pearson correlation was used for identification of possible predictors of the element concentrations. The variables tested were consumption of specific fish species. The predictors with $p<0.05$ were further explored by multiple linear regression analyses. The correlation between the frequency of fish consumption, alcohol consumption or mother's age and THg, MeHg, Se and As levels in mother's blood, cord blood and breast milk, Se and As in urine or THg and MeHg in hair was assessed by Spearman's rank correlation coefficient. The Wilcoxon–Mann–Whitney test was applied for comparison of two different groups (smokers, non-smokers; the users and non-users of vitamin supplements; differences between countries). This nonparametric test was applied because the levels of THg, MeHg, As, Se levels in mother's blood, cord blood and breast milk significantly differed from a normal distribution. The level of statistical significance was set to $p<0.05$.

In addition we tested the correlation of Se, As and Hg biomarkers of exposure with different food items. Because of significant correlations between variables of different food consumption, factor analysis was applied. After running the factor analysis, rotation of the factor loads was performed to get a clearer pattern. From the different frequencies of food items new variables were created ($n=26$) if the "eigenvalues" were more than 1, and the Spearman rank correlation was used to correlate them with THg, Se and As in cord blood, mother's blood and breast milk.

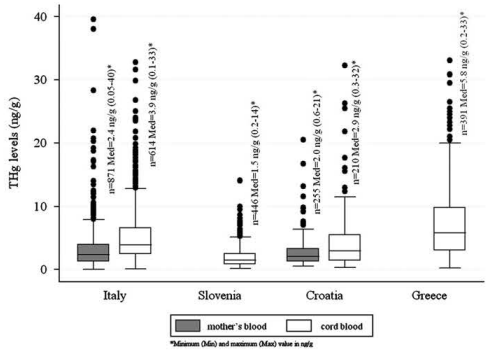

Fig. 1. THg levels in mother's blood from Italian and Croatian women, and in cord blood from Italian, Slovenian, Croatian and Greek women.

3. Results and discussion

3.1. Hg in cord blood, mother's blood and breast milk

The results for THg levels in mother's blood and cord blood from different countries are shown in Fig. 1. The levels of THg in cord blood were slightly higher than the levels in maternal blood, which is in accordance with the literature (Cernichiari et al., 1995; Vahter et al., 2000). The highest levels of THg were found in cord blood from Greek pregnant women (Med=5.8 ng/g, 5th percentile (P5)=1.2 ng/g, 95th percentile (P95)=20 ng/g), while the lowest levels of THg in cord blood were found in Slovenian pregnant women (Med=1.5 ng/g, P5=0.3 ng/g, P95=5.3 ng/g). A statistical test (Wilcoxon–Mann–Whitney test) showed that these differences were significant ($Z=-12.844$, $p<0.001$ for Italy compared to other countries; and $Z=19.303$, $p<0.001$ for Slovenia compared to other countries). The differences in Hg exposure between these countries were probably due to the different frequency of fish consumption and consumption of different species of fish (Table 2) of different origin. While pregnant women in Greece consume approximately the same amount of fish compared to Italy and Croatia (Table 2), in Greece they mainly consume fresh fish that most of the women (62%) buy from fishermen. Hence most Greek pregnant women consume fish from the local area that might have higher levels of Hg. Several species of fish from the Mediterranean Sea show higher levels of Hg in their tissues compared to the same fish species from the Atlantic Ocean (FAO, 1986). From the food frequency questionnaires it was also found that Italian women buy fish mostly from their local fish market (65%), whereas in Croatia they mostly buy fish from the local fish market (37%) and fish counter in the supermarket (25%). Therefore, they probably buy fish from different FAO regions and from aquaculture that may have lower Hg levels. Moreover, according to FAOSTAT data (2009), 39% of Italian total fishery production is aqua-cultured (FAO, 2011). Further investigations that would include Hg analysis of the most frequently eaten species of fish bought from the most common providers of fish in each country would be necessary to confirm these observations. Less likely, genetic factors that could influence the Hg levels in mother's blood or cord blood (Gundacker et al., 2007) might vary between countries.

Table 2
X (mean), Med, P5, P95 levels for frequency of different species of fish consumed in different countries*.

Sample	Italy				Slovenia				Croatia				Greece			
	X	Med	P5	P95	X	Med	P5	P95	X	Med	P5	P95	X	Med	P5	P95
Frequency of **fish broth** consumption (portions/week)	0.009	0	0	0.070	0.005	0	0	0.020	0.027	0.020	0	0.140	**0.079**	**0.070**	**0**	**0.140**
Frequency of **sea bass** consumption (portions/week)	**0.050**	**0.020**	**0**	**0.140**	0.026	0.020	0	0.070	0.033	0.02	0	0.070	0.015	0	0	0.070
Frequency of **gilt head bream** consumption (portions/week)	**0.050**	**0.020**	**0**	**0.140**	0.027	0.020	0	0.070	0.007	0	0	0.020	**0.046**	**0.020**	**0**	**0.140**
Frequency of **tuna** consumption (portions/week)	**0.095**	**0.070**	**0**	**0.430**	**0.079**	**0.07**	**0**	**0.430**	**0.072**	**0.070**	**0**	**0.140**	**0.049**	**0.020**	**0**	**0.140**
Frequency of **smooth dogfish** consumption (portions/week)	0.002	0	0	0	0.001	0	0	0	0.002	0	0	0.020	0.028	0.020	0	0.070
Frequency of **anchovy** consumption (portions/week)	0.018	0	0	0.070	0.006	0	0	0.020	**0.063**	**0.02**	**0**	**0.140**	0.027	0	0	0.070
Frequency of **sardine** consumption (portions/week)	0.013	0	0	0.070	0.025	0	0	0.140	**0.063**	**0.020**	**0**	**0.140**	0.049	0.020	0	0.140
Frequency of **Mediterranean shad** consumption (portions/week)	0.030	0.020	0	0.070	0	0	0	0	0.008	0	0	0.020	0.0024	0	0	0
Frequency of **trout/salmon** consumption (portions/week)	0.026	0.020	0	0.070	**0.041**	**0.020**	**0**	**0.140**	0.023	0	0	0.070	0.004	0	0	0.020
Frequency of **European Hake/Cod** consumption (portions/week)	0.032	0.020	0	0.014	**0.038**	**0.020**	**0**	**0.140**	0.060	0.020	0	0.140	0.053	0.020	0	0.140
Frequency of **other fish** consumption (portions/week)	0.019	0	0	0.014	0.011	0	0	0.070	0.052	0.020	0	0.140	0.011	0	0	0.070
Frequency of **fresh fish** consumption (g/week)	151	150	0	375	91	75	0	300	187	150	30	450	236	150	0	600
Frequency of **frozen fish** consumption (g/week)	73	30	0	300	47	30	0	150	77	75	0	150	25	0	0	150
Frequency of **canned fish** consumption (portion/week)	59	40	0	160	42	40	0	160	38	16	0	80	15	0	0	80
Frequency of **total fish** consumption (g/week)	**283**	**241**	75	616	178	145	0	460	**304**	**265**	91	610	**276**	**225**	0	750

*Frequencies of eel, grey mullet, common pandora, blue whiting, piked dogfish, mackerel, plaice, monkfish, shark and john dory consumption are not shown in this table, while the Med levels for all 4 countries were 0.

THg was also measured in hair samples because of its simple and practical collection and storage, and its relatively simple, fast and precise analytical determination. The Med level of THg in hair of women from Italy was 770 ng/g (n=891, P5=235 ng/g, P95=2570 ng/g); of women from Slovenia 297 ng/g (n=574, P5=44 ng/g, P95=966 ng/g); of women from Croatia 604 ng/g (n=234, P5=76 ng/g, P95=2480 ng/g) and of women from Greece 1120 ng/g (n=454, P5=242 ng/g, P95=3840 ng/g). The measured levels of THg in hair are below the estimated MeHg threshold level for prenatal effects (10 μg/g) (Cox et al., 1989) or the BMDL (the lower limit of the 95% confidence interval of the benchmark dose) from the Faroe Islands study (12–15 μg/g) and from the New Zealand study (4–6 μg/g) (Rice, 2004). However, the P95 levels were higher compared to the LOAEHC (lowest observable adverse effect hair concentration) at 0.5 μg/g determined by a more recent study (Schoeman et al., 2009) that reviewed all relevant research on humans focused on the effects of prenatal Hg exposure through fish consumption. It must be pointed out that due to inconsistency of the results focused on the effects of prenatal exposure through fish consumption, in heavy fish eating populations it is still unclear whether removing fish from the diet entirely would be more harmful a continuing to consume contaminated fish (Schoeman et al., 2009).

As shown in Fig. 2, the highest levels of THg in breast milk were found in pregnant women from Greece (Med=0.6 ng/g, P5= <LOD, P95=9.4 ng/g). The differences between THg levels from Greece and other countries was significant (Wilcoxon–Mann–Whitney test, Z= −5.953, $p<0.001$). Although in Slovenia women consume the lowest amount of fish (Table 2), the levels of THg in breast milk (Med=0.2 ng/g, P5= <LOD, P95=0.8 ng/g) were comparable to the levels in breast milk from Italy (Med=0.2 ng/g, P5=0.05 ng/g, P95=0.8 ng/g) or Croatia (Med=0.2 ng/g, P5= <LOD, P95=1.0 ng/g). This might be due to higher exposure to Hg from sources other than fish in the Slovenian population. In fact from the frequency questionnaires it was found that Slovenian women have the highest number of amalgam fillings (6.5) compared to Italy (5.6), Croatia (2.7) or Greece (2.07) and the difference is significant (Wilcoxon–Mann–Whitney test, Z=7.337 $p<0.001$). Moreover, a significant but very weak Spearman correlation was found between number of amalgam fillings and THg in breast milk from all the countries (r_s=0.1187, p=0.001) or between the number of amalgam fillings and inorganic Hg (calculated from THg and MeHg) in breast milk from all the countries (r_s=0.1813, p=0.012). However, no correlation was found between THg in breast milk and the number of amalgam fillings in Slovenian women.

In addition we analysed MeHg in samples where THg levels in mother's hair exceeded or were equal to 1000 ng/g. MeHg comprised almost all the THg in mother's blood, hair and cord blood, while in breast milk the percentage of MeHg was lower and variable (Table 3). Considerably the lowest percentages of MeHg were found in breast milk from Greece (Wilcoxon–Mann–Whitney test, Z=4.643, $p<0.001$). In the first place this may be due to different sources of exposure that in Greece should be studied in more detail. While some studies suggest that most MeHg is eliminated from the body by demethylation and excretion of the inorganic form (ATSDR, 1999), the lower percentages of MeHg in breast milk from Greece might be also due to more efficient metabolism of elimination that could be the consequence of higher MeHg exposure and different genetic factors influencing the metabolism of MeHg (Gundacker et al., 2007).

3.2. Se in cord blood, mother's blood and breast milk

The highest levels of Se in mother's blood (Med=117 ng/g, P5=85 ng/g, P95=166 ng/g) or cord blood (Med=113 ng/g, P5=75 ng/g, P95=162 ng/g) were found in pregnant women from Italy (Fig. 3). The differences between Se levels in mother's

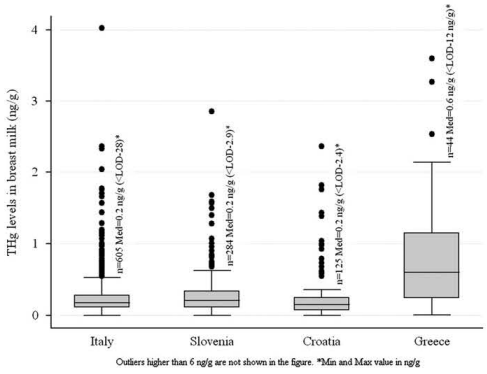

Fig. 2. THg levels in breast milk from Italian, Slovenian, Croatian and Greek women.

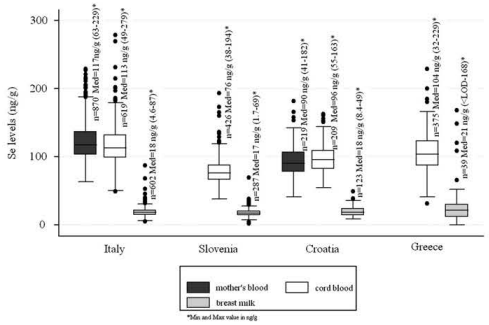

Fig. 3. Se levels in mother's blood from Italian and Croatian women, in cord blood from Italian, Slovenian, Croatian and Greek women, and in breast milk from Italian, Slovenian, Croatian and Greek women.

Table 3
The percentage of MeHg in mother's blood from Italian and Croatian women, and the percentage of MeHg in cord blood, hair and breast milk from Italian, Slovenian, Croatian and Greek women.

	Italy				Slovenia				Croatia				Greece			
	n	Med	P5	P95	*n*	Med	P5	P95	*n*	Med	P5	P95	*n*	Med	P5	P95
Mother' s blood % MeHg	223	91	52	100	–	–	–	–	47	100	54	100	–	–	–	–
Cord blood % MeHg	330	99	65	100	14	97	52	100	47	100	85	100	207	100	71	100
Hair % MeHg	323	100	59	100	28	100	55	100	49	99	82	100	247	100	60	100
Breast milk % MeHg	224	60	16	100	7	47	3	71	26	56	23	100	21	7	2	96

blood or cord blood from Italy and other countries was significant (Wilcoxon–Mann–Whitney test for Se levels in mother's blood: $Z=14.178$, $p<0.001$; Wilcoxon–Mann–Whitney test for Se levels in cord blood: $Z=17.563$, $p<0.001$). Because Se levels in food can be extremely variable (Barclay et al., 1995), the higher levels of Se in Italy are probably due to the more frequent consumption of different non specific food items in Italy that have higher Se levels. Moreover, we did not find a significant and sufficiently strong correlation ($r_s>0.2$) between the consumption frequency of different food items and Se levels in cord blood, mother's blood, breast milk and urine, or a more frequent use of vitamin supplements containing Se in Italy compared to other countries. While the highest Se levels were found in breast milk from Greece (Med=21 ng/g, P5=5.6 ng/g, P95=149 ng/g), the difference between Se levels in breast milk from Greece and Se levels from other countries was not significant (Wilcoxon–Mann–Whitney test $Z=-1.692$, $p=0.0906$). The levels of Se in blood and breast milk are in the same range as their levels in populations determined in other studies (Batáriová et al., 2005; Gundacker et al., 2006; Goullé et al., 2005; Valent et al., 2011). According to our results for breast milk and the recommended nutrient intake (RNI) for Se (6 μg/day in infants aged 0–6 months weighing approximately 6 kg) set by the Joint Food and Agriculture Organization/World Health Organization (FAO/WHO) Expert Committee on Human Vitamin and Mineral Requirements (Joint FAO/WHO, 1998), the recommended levels of Se are met for infants consuming approximately 0.8 L/day. However, the US Institute of Medicine proposed a higher AI (adequate intake) of 15 μg/day for infants aged 0–6 months which is met by only approximately 50% of the presently studied population (Institute of Medicine, 2000).

In addition, Se levels were measured in urine samples from Italy (Med=31 μg/g creatine (Cr)/Med=25 μg/L, P5=5.7 μg/L, P95=59 μg/L, $n=721$), Croatia (Med=24 μg/g Cr/Med=19 μg/L, P5= < LOD, P95=48 μg/L, $n=217$) and Greece (Med=24 μg/g Cr/Med=24 μg/L, P5=5.5 μg/L, P95=62 μg/L, $n=302$) and the results are in the same range as the levels found in the literature (Heitland and Köster, 2006). Although some studies (Ashton et al., 2009) suggested that urinary Se may be a useful marker of exposure, more studies are needed to confirm this.

3.3. As in cord blood, mother's blood and breast milk

The highest levels of As (Med=3.3 ng/g, P5=0.8 ng/g, P95=20 ng/g), and which were significantly different compared to the other countries, were found in cord blood from Greece (Wilcoxon–Mann–Whitney test $Z=-17.485$, $p<0.001$) or breast milk (Med=0.8 ng/g, P5=0.3 ng/g, P95=3.5 ng/g) from Greece (Wilcoxon–Mann–Whitney test $Z=-6.442$, $p<0.001$) (Figs. 4 and 5). The main source of As exposure in the general population could be seafood, which in general contains the greatest amounts of As (Hughes et al., 2009). Therefore, as for Hg, the highest levels in biological samples from Greece might be due to different fish consumption with higher As levels. On the other hand, higher As levels in samples from Greece might also be due to some other source of As such as the consumption of food other than fish. However, we did not find a significant and relevantly strong correlation between As levels in cord blood, mother's blood or breast milk and the frequency of different food item consumption.

The levels of As in blood or breast milk are in the same range compared to those found in blood or breast milk from the general non-exposed population in other studies (Goullé et al., 2005; Sternowsky et al., 2002). The levels found in blood are very far from the chronic toxicity levels of As in whole blood (100–500 μg/L) and far from the acute toxicity levels in whole blood (600–9300 μg/L) (Burtis and Ashwood, 1999).

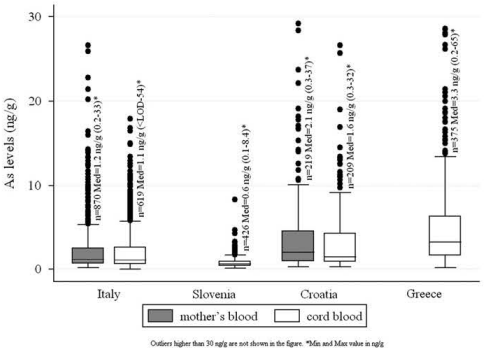

Fig. 4. As levels in mother's blood from Italian and Croatian women and in cord blood from Italian, Slovenian, Croatian and Greek women.

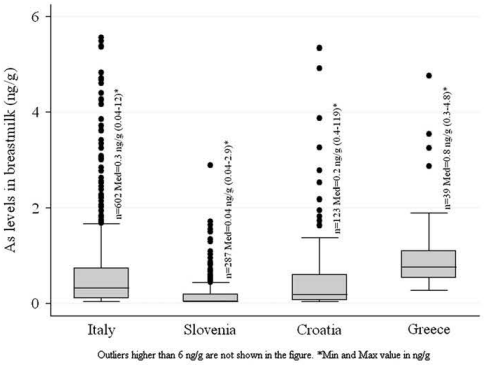

Fig. 5. As levels in breast milk from Italian, Slovenian, Croatian and Greek women.

In addition, we determined As levels in urine from the women involved in this study, since due to the fast clearance of As in blood, blood levels of As do not appear to be a reliable indicator of chronic exposure to low levels of As (ASTDR, 2007). The Med values of As in urine were 17 μg/g Cr/ 13 μg/L in samples from Italy (P5=2.3 μg/L, P95=199 μg/L, $n=721$), 24 μg/g Cr/17 μg/L in samples from Croatia (P5=2.0 μg/L, P95=267 μg/L, $n=217$) and 37 μg/g Cr/31 μg/L in samples from Greece (P5=2.8 μg/L, P95=621 μg/L, $n=312$). The Med levels of As in urine in our study are comparable to those found in urine from healthy volunteers from Germany (Heitland and Köster, 2006). The limitations of this study include the lack of As speciation analysis. It must be pointed out that the an important limitation to the use of total urinary As a biomarker of exposure is that arsenobetaine is excreted (unmetabolized) in urine after ingestion of seafood (Brown et al., 1990; Tam et al., 1982). Therefore, analytical methods based on total urinary As content probably overestimate exposure to As species that are health concern. Especially in cases where the levels of As are more than 10 times higher than the Med value, further investigations and As speciation analyses would be necessary to confirm that the As determined in this study is not harmful for human health.

3.4. Correlations between biomarkers of exposure

Interestingly, significant linear correlations were found between the natural logarithm (ln) of THg levels and ln Se levels, between ln As and between ln Se levels and ln As and ln THg levels in cord blood, as well as between ln THg levels and ln Se levels, ln As and ln Se levels, and ln As and ln THg levels in mother's blood, and further between ln THg levels and ln Se levels, ln As and ln Se levels and ln As and ln THg levels in breast milk (Table 4). At these low levels of exposure it is hardly probable that these correlations could be due to the protective role of Se against Hg and As toxicity, while in the literature there is no evidence of Se–Hg or Se–As interactions at these low levels of exposure. The more probable explanation could be that these correlations between Hg, As and Se biomarkers of exposure are due to the same source of exposure, that is, seafood consumption.

3.5. Correlations between biomarkers and frequency of fish consumption

A significant Spearman rank correlation was found between the biomarkers of MeHg, Se and As levels and the frequency of fish consumption, with the exception of non-significant Spearman rank correlations between the frequencies of total fish consumption and Se levels in breast milk or Se levels in urine samples (Table 5). It should be pointed out that the low correlations could be due to the low accuracy of the data contained in the questionnaire, since the questionnaire in our study provided only approximate information on fish consumption and not a precise measure of the fish ingested by an individual. Moreover, the correlations could be low due to variation of Se, As or Hg levels in portions of fish. However, the strongest Spearman rank coefficients found between the frequencies of total fish consumption and THg levels in cord blood (r_s=0.4416, $p<0.001$) or THg levels in hair (r_s=0.4209, $p<0.001$) and between the frequencies of total fish consumption and As levels in cord blood (r_s=0.3497, $p<0.001$).

In addition, we investigated correlations of Se, As and Hg biomarkers of exposure with different food items. Due to significant correlations between the variables of different food consumption, factor analysis was applied. However, except for the factors that included fish consumption, where the Spearman correlation was significant, but still weak ($r_s<0.5$), we did not find any significant and relevantly strong Spearman rank correlations ($r_s \leq 0.2$) between factors that included the consumption of different food items and Hg, Se and As biomarkers of exposure. The influence of other variables on THg, MeHg, As or Se biomarkers of exposure such as age, vitamin supplements, smoking status and alcoholic drink consumption was also evaluated. A statistical test (Wilcoxon–Mann–Whitney test) showed that the differences in THg, MeHg, As or Se levels in cord blood, blood or breast milk between smokers and non smokers or between women who were taking vitamin supplements and women that did not take them was not significant. The only exception was the significant and unexpected difference (p=0.0153) in Se levels in urine between women that took vitamin supplements (Med=22 μg/L, n=847) and women who did not take them (Med=26 μg/L, n=231). Furthermore, the mother's age was correlated with THg in cord blood (r_s=0.155; $p<0.005$), THg in mother's blood (r_s=0.155; $p<0.005$), As in breast milk (r_s=0.130; $p<0.005$), Se in cord blood (r_s=0.214; $p<0.005$) and Se in breast milk (r_s=0.130; $p<0.005$). The frequencies of alcohol drink consumption was correlated with THg in mother's blood (r_s=0.129; $p<0.005$), As in mother's blood (r_s=0.112; $p<0.005$), THg in cord blood (r_s=0.176; $p<0.005$), As in cord blood (r_s=0.111; $p<0.005$) and THg levels in hair (r_s=0.141; $p<0.005$). Nevertheless it must be pointed out that these correlations are very weak.

As shown in Table 2, the species of fish that are the most frequently consumed in each country are different. The most frequently consumed fish are tuna, gilt head bream and sea bass

Table 5
Spearman rank correlation between THg, MeHg, Se and As levels in cord blood, mother's blood and breast milk from Mediterranean women (Italy, Slovenia, Croatia and Greece) and frequency of total fish consumption.

Biomarkers of exposure			
THg	**MeHg**	**Se**	**As**
Frequency of total fish consumption (g per week)			
Cord blood			
r_s=0.4416	r_s=0.2429	r_s=0.2689	r_s=0.3497
$p<0.001$	$p<0.001$	$p<0.001$	$p<0.001$
n=1240	n=286	n=1223	n=1223
Mother's blood			
r_s=0.3291	r_s=0.1953	r_s=0.0847	r_s=0.2775
$p<0.001$	$p<0.001$	p=0.005	$p<0.001$
n=1090	n=382	n=1083	n=1083
Breast milk			
r_s=0.0977	r_s=0.1377	not significant	r_s=0.2984
p=0.002	p=0.027	–	$p<0.001$
n=1005	n=259	–	n=1003
Urine			
–	–	Not significant	r_s=0.1820
–	–		$p<0.001$ n=914
Hair			
r_s=0.4209	r_s=0.1270	–	–
$p<0.001$	p=0.01	–	–
n=1681	n=399	–	–

Table 4
Linear correlations between levels of ln THg, ln MeHg, ln Se and ln As in cord blood, mother's blood and breast milk from Italy, Slovenia, Croatia and Greece.

	Cord blood			**Mother's blood**			**Breast milk**		
	ln MeHg levels	**ln Se levels**	**ln As levels**	**ln MeHg levels**	**ln Se levels**	**ln As levels**	**ln MeHg levels**	**ln Se levels**	**ln As levels**
ln THg levels	r=0.9480	r=0.4414	r=0.6027	r=0.9157	r=0.2266	r=0.5302	r=0.4668	r=0.2993	r=0.2857
	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.001$
	n=491	n=1616	n=1615	n=382	n=1086	n=1086	n=278	n=1030	n=1031
ln MeHg levels	–	r=0.2107	r=0.4761	–	r=0.2732	r=0.4150	–	Not significant	r=0.2800
	–	$p<0.005$	$p<0.001$	–	$p<0.005$	$p<0.001$	–	–	$p<0.001$
	–	n=480	n=480	–	n=374	n=374	–	–	n=266
ln Se levels	–	–	r=0.3259	–	–	r=0.1325	–	–	r=0.2421
	–	–	$p<0.001$	–	–	$p<0.001$	–	–	$p<0.001$
	–	–	n=1628	–	–	n=1086	–	–	n=1050

in Italy, tuna, trout or salmon and European hake or cod in Slovenia, tuna, anchovy and sardine in Croatia and fish broth, gilt head bream and tuna in Greece. The THg and As compounds present in fish tissues differ depending on their trophic position (diet) (Miklavčič et al., 2011a; Kirby and Maher, 2002). To elucidate which species contributed the most to the levels of THg or As in cord blood in each country, stepwise multiple regression analyses were performed. THg or As in cord blood was chosen since the Spearman correlation for these biomarkers and total fish consumption was the strongest. Moreover, for the model only variables of specific fish consumption in each country for which the Med values were higher than 0 portions per day (Table 2) were tested. In the model with ln THg cord blood from Italy as the dependent variable, consumption of tuna, sea bass and other fish remained significant ($R^2_{adj}=0.2284$, $p<0.001$), whereas for ln As in cord blood from Italy as the dependent variable, consumption of Mediterranean shad and gilt head bream remained significant ($R^2_{adj}=0.1143$, $p<0.001$). In the model with ln THg cord blood from Slovenia as the dependent variable, consumption of sea bass, tuna and hake remained significant ($R^2_{adj}=0.1096$, $p<0.001$), whereas for ln As in cord blood from Slovenia as the dependent variable, consumption of sea bass and hake remained significant ($R^2_{adj}=0.0384$, $p<0.001$). In the model with ln THg cord blood from Croatia as the dependent variable, consumption of fish broth and sea bass remain significant ($R^2_{adj}=0.1861$, $p<0.001$), whereas for ln As in cord blood from Croatia as the dependent variable, consumption of fish broth and anchovy remained significant ($R^2_{adj}=0.1653$, $p<0.001$). In the model with ln THg cord blood from Greece as the dependent variable, consumption of smooth dog fish and fish broth remained significant ($R^2_{adj}=0.0285$, $p=0.0039$), whereas for ln As in cord blood from Greece as the dependent variable, none of the variables of fish consumption remained significant. The weak correlation of THg with consumption of different species of fish in Greece and the lack of a correlation between As and consumption of different species of fish might be due to non specific variation of As or THg between the species eaten in Greece, depending on other factors or the low accuracy of the data collected through the questionnaires. Overall, because of consumption of different species of fish in each country, the species of fish contributing the most to the Hg or As levels in cord blood for each population were different.

4. Conclusions

In conclusion the highest levels of Hg and As were found in cord blood and breast milk from Greek women, while the highest Se levels were found in cord blood from Italy. The differences in As and Hg exposure between the countries were probably due to the different amounts of fish consumption and the consumption of different species of fish of different origin. Since speciation analyses of As were not included in this study, it is still not clear whether As levels came mainly from fish or in part also from some sources other than fish, which could also influence the differences in As levels between countries. Because Se levels in different food items also depend on the origin of food, the highest levels of Se in Italy are probably the consequence of more frequent consumption of different non-specific food items in Italy that have higher Se levels. The percentages of MeHg in biological samples did not differ between the four countries, with the exception of the percentages of MeHg in breast milk. The lower percentages of MeHg in breast milk from Greece could be due to different source of Hg exposure or might be a consequence of a more efficient metabolism of MeHg elimination in women from Greece. While the levels of Hg or As found are far below the threshold levels for acute or chronic toxicity, it is still unknown if such low long-term exposure levels could affect the most susceptible population. Interestingly, significant linear correlations were found between ln THg levels and ln Se levels, ln As and ln Se levels, and ln As and ln THg levels in cord blood, mother's blood and breast milk. In addition, significant correlations were found between the frequency of fish consumption and different biomarkers of exposure. Fish consumption, the possible common source of As, Hg and Se exposure, could explain the correlations between the elements determined in cord blood, mother's blood or breast milk.

Role of funding source

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The research protocol was approved by the Ethics Committee of the Republic of Slovenia (KME 98/05/06). Evidence of this ethical approval is additionally appended.

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References

Abernathy, C.O., Thomas, D.J., Calderon, R.L., 2003. Health effects and risk assessment of arsenic. J. Nutr. 133, 1536S–1538S.

Akagi, H., 1997. Analytical method for evaluating human exposure to mercury due to gold mining. In: Proceedings of the international workshop on health and environmental effects of mercury due to mining operations. National Institute for Minamata Disease, Manila, pp. 131–141.

Ashton, K., Hooper, L., Harvey, L.J., Hurst, R., Casgrain, A., Fairweather-Tait, S.J., 2009. Methods of assessment of selenium status in humans: a systematic review. Am. J. Clin. Nutr. 89, 2025S–2039S.

ATSDR, 1999. Toxicological Profile for Mercury, Atlanta, GA: Agency for Toxic Substances and Disease Registry.

ASTDR, 2007. Toxicological Profile for Arsenic, GA: Agency for Toxic Substances and Disease Registry. ⟨http://www.atsdr.cdc.gov/toxprofiles/tp2.pdf⟩.

Bárány, E., Bergdal, I.A., Schutz, A., Skerfving, S., Oskarsson, A., 1997. Inductively coupled plasma mass spectrometry for direct multi-element analysis of diluted human blood and serum. JAAS 12, 1005–1009.

Barclay, M.N.I., MacPherson, A., Dixon, J., 1995. Selenium Content of a Range UK Foods. J. Food Comp. Anal. 8, 307–318.

Batáriová, A., Černá, M., Spěváčková, V., Čejchanová, M., Beneš, B., Šmíd, J., 2005. Whole blood selenium content in healthy adults in the Czech Republic. Sci. Total Environ. 338, 183–188.

Beijer, K., Jernelov, A., 1987. Ecological aspects of mercury–selenium interactions in the marine environment. Environ. Health Perspect. 25, 43–45.

Brown, R.M., Newton, D., Pickford, C.J., Sherlock, J.C., 1990. Human metabolism of arsenobetaine ingested with fish. Hum. Exp. Toxicol. 9, 41–46.

3.4 Mercury in food items from the Idrija mercury mine area

In this section, the article (Miklavčič et al., 2013b) entitled "Mercury in food items in the Idrija mercury mine area" by Ana Miklavčič, Dr. Darja Mazej, Dr. Radojko Jaćimović, Tatjana Dizdarević and Prof. Dr. Milena Horvat, in press in the Special Issue "Hg in the contaminated sites" of Environmental Research (Elsevier), is presented.

In this article the potential exposure to Hg species of the population living in the contaminated Idrija area was assessed through analyses of local food items consumed. 27 different kinds of vegetables were collected in 10 individual domestic gardens selected randomly in Idrija town and its surroundings. Besides checking the present situation in these samples, levels of THg and MeHg were also determined in mushrooms and fish from the Idrija Hg mine area, since in the past elevated levels of Hg were found in these kinds of foodstuffs collected in this area. In addition, Cd and Se concentrations were determined in selected vegetable samples in order to assess exposure levels to these elements. Determination of Se was included in the analyses due to its detoxifying role as an essential component of antioxidative enzymes and in forming insoluble complexes with metals and metalloids, either in food, especially of animal origin, or after ingestion in humans. Moreover, the results presented in this study are important in checking the present situation regarding the contents of Hg in various selected local vegetables, mushrooms and fish samples, and in following any changes resulting from closure of the mine and environmental remediation measures (Miklavčič et al., 2013b).

In this study I prepared all the samples and carried out the analyses of THg and MeHg, performed the calculations, statistical analyses and interpreted the data. As first author I drafted the manuscript. Part of this work was presented and published at the 6th SETAC World Congress, 20-24 May, 2012.

Mercury in food items from the Idrija mercury mine area

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Abbreviations: CRM, certified reference material; IHg, inorganic mercury; LOD, limit of detection; LOQ, limit of quantification; Max, maximum; Min, minimum; Med, median; MeHg, monomethylmercury; PDI, probable daily intake; PTWI, provisional tolerable weekly intake; RM, reference material; STD, standard deviation; THg, total mercury; X, mean

Abstract

As a consequence of 500 years of mining and smelting activities (1490-1995), and of its natural geological occurrence, the soil in the Idrija region is highly contaminated with Hg. In order to assess the present situation regarding the Hg contents in local food samples, concentrations of total mercury (THg) and monomethyl mercury (MeHg) were determined in selected vegetables, mushrooms and fish from the Idrija Hg mine area. Hg levels in the foodstuffs analysed were not very high but were elevated compared to the levels in food from non-contaminated areas. The study showed that THg accumulates in mushrooms (X=5680 ng/g dry weight, Min=346 ng/g dry weight, Max=17100 dry weight) and chicory (X=1950 ng/g dry weight, Min=86 ng/g dry weight, Max=17100 ng/g dry weight). In addition, Se and Cd concentrations were determined by ICP-MS in those vegetable and mushroom species in which the highest Hg levels were found. The levels of Cd and Se were below the threshold levels. Based on previously available data, we can conclude that the levels of Hg in food have not diminished significantly during the past 15 years after closure of the Hg mine. Special attention should be given to vegetables such as chicory representing a local seasonal vegetable eaten frequently.

Keywords: mercury, monomethylmercury, exposure assessment, mercury contaminated area

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Role of funding source

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1 Introduction

Idrija is a small town in Slovenia that is well known for its Hg mine. Hg was discovered in 1490 and mining was taken over by the government in 1575. Over the years, generations of miners excavated over 12 million tons of ore and an estimated total of about 153,000 tons of commercial mercury was extracted. The mine operated effectively until the 1970s, when information about the harmful effects and mass poisoning with Hg (Minamata 1956, Iraq 1965) become widely known. Consequently, the price of Hg on the world market fell and in 1977 mining in Idrija was temporally suspended. The mine re-opened in 1983 and worked with minimal capacity until 1994 (Miklavčič, 1999).

As a result of 500 years of mining and smelting activities, intense pollution of Hg of nearly all environmental compartments occurred in the Idrija area. Hg measured in water, sediments and soil from the mercury mine area showed very high levels, the values of THg content in soil samples taken in the wider Idrija area varying between 8.4 and 415 mg/kg, up to 40-fold higher than the maximum permissible (10 mg Hg / kg) set by Slovenian legislation (Kocman et al., 2004). These high levels are in part due to the natural geological occurrence of Hg, as well as anthropogenic activities. The concentrations of MeHg in soils were up to 32 μg/kg. The lowest levels of MeHg were found on the riverside and the highest levels on hill slopes, which was the opposite that for THg determined in the same soil samples (Tomiyasu et al., 2012). Moreover, the distribution of MeHg was correlated with the total organic content (Tomiyasu et al., 2012). THg in the river Idrijca increased downstream of the mine from a few ng/L to up to 500 ng/L with MeHg accounting for 1.5 % above the town of Idrija, 0.2 to 0.7 % below Idrija and 2-3 % in river reservoirs. Hg in sediments and flood plain soils also increased by several fold downstream, with MeHg ranging from 0.01 to 0.1% (Horvat et al., 2002).

Hg can be harmful even at very low levels, especially for the most susceptible population comprising pregnant women and children. Importantly, the toxicokinetics of Hg is dependent on the route of exposure (inhalation, oral or dermal exposure) and on the form of mercury to which a person is exposed (Horvat et al., 2011a). Organic Hg compounds are more readily absorbed in the gastrointestinal tract than inorganic Hg (IHg) compounds. While 95 % of MeHg is thought to be absorbed in the gastrointestinal tract, animal studies indicate that the absorption of IHg is approximately 10-30 % (Piotrowski et al., 1992; Morcillo and Santamaria, 1995, ASTDR, 1999). The major toxic effect of MeHg is on the central nervous system and the developing foetus is the most vulnerable target (NRC, 2000). Inorganic species of Hg can give rise to kidney damage, including in some cases the nephritic syndrome. Inhaled Hg vapour can also cause damage to the central nervous system due to its ability to cross the blood-brain barrier (Clarkson and Magos, 2006).

Because most people from this region eat home and locally produced food, special attention should be given to vegetables grown in this area. Therefore, 27 different kinds of vegetables were collected in 10 individual domestic gardens selected randomly in Idrija town and its surroundings. Besides checking the present situation in these samples, levels of THg and MeHg were also determined in mushrooms and fish from the Idrija Hg mine area, since in the past elevated levels of Hg were found in these kinds of foodstuffs collected in this area (Kosta et al., 1974; Byrne and Kosta, 1970; Stegnar et al., 1973; Byrne et al., 1979; Byrne et al., 1976; Horvat et al., 2004). In addition, Cd and Se concentrations were determined in selected vegetable samples in order to assess the exposure levels of Cd and Se. Determination of selenium was also included in the analysis due to its detoxifying role as an essential component of antioxidative enzymes and in forming insoluble complexes with metals and metalloids, either in food, especially

of animal origin, or after ingestion in humans (Raymond and Ralston, 2004; Levander, 1977; Zeng., 2001; Zeng et al., 2005).

Thus the aim of this study was to assess the potential exposure to Hg species of the population living in the contaminated area through consumption of local food items. Moreover, the results presented in this study are important in checking the present situation regarding the contents of Hg in various selected local vegetables, mushrooms and fish samples, and in following any changes resulting from closure of the mine and environmental remediation measures.

2 Methods

2.1 Study area

The Hg mine is located in the town of Idrija, which lies on the alluvial deposit at the confluence of the Nikova and the Idrijca Rivers. The location can be taken as marking the dividing line between the Alps to the north and the Karst plateau to the south (Kocman, 2008). This study area is shown in the Figure 1. Ten sampling sites were selected in individual domestic gardens in the town and its surroundings (Figure 1), from which vegetables and soil samples were collected. Samples of fish were collected at 4 different locations on the river Idrijca (Figure 2).

2.2 Collection and storage of samples

2.2.1 Vegetables and mushrooms

10 sampling sites in individual domestic gardens were selected in the town of Idrija and its surroundings (Figure 1) in which the sampling of different vegetables was performed. Samples of cabbage (n=5), kale (n=5), chicory (n=7), parsley leaves (n=6), carrot (n=5), leek (n=3), cucumber (n=3), zucchini (n=1), pumpkin (n=1), pepper (n=2), chili pepper (n=1), Brussels sprouts (n=1), mangold (n=1), broccoli (n=1), red beet (n=2), egg plant (n=1), red cabbage (n=2), celery (n=1), tomato (n=1), corn salad (n=1) and mushrooms (*Hydrum Repandum* (n=1), *Lactarius deliciosus* (n=2), *Kuehneromyces mutabilis* (n=1), *Macrolepiota procera* (n=1)) were collected in August 2008, while samples of cabbage (n=5), kale (n=1), chicory (n=5), parsley leaves (n=4), carrot (n=4), leek (2), cucumber (2), zucchini (n=2), pepper (n=1), lettuce (n=2), mangold (n=3), melon (n=1), corn (n=1), beans (n=3), chives (n=3), celery (n=2), tomato (n=4) and onion (n=2) were collected in August 2009. In addition, samples of parsley leaves (n=1), chicory (n=1), cabbage (n=2), onion (n=1) and mushrooms *Kuehneromyces mutabilis* (n=1) were collected in Orle village, outside the city of Ljubljana, where the soil is not contaminated with Hg and which served as a background or control area. The cleaning procedure was selected to resemble domestic food preparation. Samples were washed with cold tap water and frozen at -18°C. Before analysis samples were freeze dried (ALPHA 1-4 Freeze drier) at -47 ºC (0,05 mbar) for 72 hours and homogenized in an agate micro mill (Fritsch, Pulverisette 7).

2.2.2 Fish samples

44 fish samples comprising the 8 different species Mediterranean barbel (*Barbus meridionalis*), three different kinds of trout (*Salmo marmoratus*, *Salmo trutta*, *Salmo sp.*), rainbow trout (*Oncorhynchus mykiss*), grayling (*Thymallus thymallus*), souffia (*Leuciscus souffia*) and bullhead (*Millerigobius macrocephalus*) were collected at 4 different locations on the river Idrijca in 2009 (Figure 2).

From sampling sites 1, 2, 3 and 4 we collected 10, 13, 6 and 16 different fish samples respectively (see Table 1).

Fish were cleaned, gutted, scaled and skinned and the residue stored in a freezer at -18°C. Only the edible part (muscle) of the fish was used for analysis.

2.2.3 Soil samples

1 kg of each soil sample was collected from the selected domestic gardens in the town of Idrija in 2011 at the depth of 20 cm (Figure 1). A soil sample from Orle village was collected in 2010, at the same time as vegetables were collected from this reference garden. All soil samples were dried to constant weight at 35°C in a drying oven for approximately 3 days. Samples were then ground and homogenized in an agate mortar, and sieved through a mesh of 200 μm pore size. In addition, a separate aliquot of each fresh sample was dried at 105°C to constant weight for the determination of moisture content, and results were expressed on a dry weight basis.

2.3 Study methods

2.3.1 Determination of THg in fish

0.030 g to 0.200 g of fish tissue was weighed into 50 ml volumetric flask, to which 1 ml of distilled water, 2 ml of a mixture of 65 % HNO_3 (Merck, Germany, p.a.) – $HClO_4$ (Merck, Germany, Suprapur) (1:1, v/v) and 5 ml of 96 % H_2SO_4 (Merck, Germany, Suprapur) were added. The open flasks were heated on a hotplate for 20 minutes. After cooling, the digested samples were filled up to the 50 ml mark with distilled water. A

semi-automated Mercury Analyser based on cold vapour atomic absorption spectrometry was used for determination of THg in the digested samples (Akagi, 2007).

The accuracy of the results was checked by analysing Dogfish Muscle CRM (Certified Reference Material) for Trace Metals DORM-2 from the National Research Council Canada. Levels of THg determined in the CRM (4510 ± 140 ng/g) were in good agreement of the certified level (4640 ± 260 ng/g). All samples were prepared and measured in duplicate (except CRMs) and the deviation between them was less than 10 %. The limit of detection (LOD) calculated as 3 times the standard deviation of the blank sample was 0.1 ng/g and the limit of quantification (LOQ) was estimated at 1 ng/g.

Akagi (1997) described the procedure in detail.

2.3.2 Determination of THg in vegetables and mushrooms

THg in vegetables and mushrooms was determined by thermal combustion at 650 °C, amalgamation and atomic absorption spectrometry using a Direct Mercury Analyzer (Milestone, USA). The procedure, using about 0.020 g of freeze dried sample has been described in detail elsewhere (EPA Method 7473, 1998). BCR-679 CRM (No 165 (white cabbage) from the Institute for Reference Materials and Measurements in Belgium was used to check the accuracy of the results for THg in vegetables and mushrooms and the values found (6.0 ± 0.7 ng/g, n=16) were in good agreement with the certified reference value (6.3 ± 1.4 ng/g). All other samples were measured in duplicate and the deviation between them was less then 10%.

The LOD of the method calculated as three times the standard deviations of the blank sample was 0.2 ng/g sample, while the LOQ calculated as ten times the standard deviation of the blank sample was 0.7 ng/g sample.

2.3.3 Determination of MeHg in fish

0.030-0.100 g of fish tissue was weighed into 50 mL Teflon distillation vial to which 9 mL of distilled water, 1 mL of 4M H_2SO_4 (Merck, Germany, Suprapur) and 50 µL of 20% KCl (analytical grade, Merck) solution were added. Distillation was then carried out with a Tekran 2750 distillation apparatus at 145 °C for about 1h 15 minutes until 85-90% of the vial content was distilled. The procedure has been described in detail elsewhere (Horvat et al., 1993).

After distillation MeHg was determined by aqueous phase ethylation and gas chromatography followed by cold vapour atomic fluorescence detection using a Tekran 2700 Methyl Mercury Analysis System (EPA 1630).

The accuracy of the results was checked by analysing the Fish Protein CRM for Trace Metals DORM-3 from the National Research Council of Canada. Levels of MeHg found in the CRM (344 ± 65 ng/g) were in good agreement with the certified level (355 ± 56 ng/g). All the other samples were measured in duplicate and the deviation between them was less than 10 %. The LOD calculated as 3 times the standard deviation of the blank sample was 0.02 ng/g of fish sample and the LOQ was estimated at 0.06 ng /g of fish sample.

2.3.4 Determination of MeHg in vegetables and mushrooms

Approximately 0.030 g of freeze dried vegetable or mushroom was weighed into 50 mL Teflon distillation vial to which 9 mL of distilled water, 1 mL of 4M H_2SO_4 (Merck, Germany, Suprapur) and 50 µL of 20% KCl (analytical grade, Merck) solution were added. Distillation and MeHg determination was then carried out as described previously (Section 2.2.3).

The accuracy of the results was checked by analysing the Seaweed (*Fucus sp.*) reference material (RM) 140/TM from the IAEA, Vienna. Levels of MeHg determined in this RM (0.52 ± 0.10) ng/g were in agreement with the reference level (0.626 ± 0.107). All the samples were measured in duplicate and the deviation between them was less than 10 %. The LOD calculated as 3 times the standard deviation of the blank sample was 0.03 ng/g of foodstuff and the LOQ was estimated at 0.09 ng/g.

2.3.5 Determination of Se and Cd in vegetables by ICP-MS

About 0.2 g of sample was weighed into a quartz tube. 3 mL 65 % HNO_3,(Merck, Germany, suprapur) and 1 mL 30 % H_2O_2, ,(Merck, Germany, suprapur) were added and the sample subjected to closed vessel microwave digestion (Microwave system ETHOS 1, Milestone 130471) at max. power of 1500 W: ramp to 130 °C 10 min, ramp to 200 °C 10 min, hold 20 min, cooling 20 min. Digested sample solutions were diluted to 20 mL. Before measurements, samples were further diluted if necessary. Measurements of concentrations of elements in digested solutions were made by an Octapole Reaction System (ORS) Inductively Coupled Plasma Mass Spectrometer (7500ce, Agilent) equipped with an ASX-510 Autosampler (Cetac). Two different modes of ORS (hydrogen and helium) were used. Quantification on all isotopes was performed using three central points of the spectral peaks. Instrumental conditions: nebulizer Micro Mist, spray chamber Scott-type, spray chamber temperature 5 °C, plasma gas flow rate 15 L/min, carrier gas flow rate 0.8 L/min, make-up gas flow rate 0.1 L/min, nebulizer pump 0.1 rps, RF power 1500 W, reaction cell gases: H_2 4 mL/min and He 4 mL/min, tuning of the instrument was made daily, and the isotopes monitored were ^{78}Se, ^{111}Cd.

CRM-679 (White Cabbage) from the Institute of Reference Materials and Measurements in Belgium and Standard Reference Material (SRM) 1570a (Spinach Leaves) from the National Institute of Standards & Tehnology of the United States of America were used to check the accuracy of the results and the values found were in agreement with the certified reference values.

2.3.6 Determination of THg by k_o INAA

0.17-0.19 g of soil was sealed into a pure polyethylene ampoule (SPRONK system, Lexmond, The Netherlands). Sample and standard (Al-0.1%Au IRMM-530R disc 5 mm in diameter and 0.2 mm high) were stacked together and fixed in the polyethylene ampoule in sandwich form and irradiated for 13 hours in the carousel facility (CF) of the Institute's 250 kW TRIGA Mark II reactor at a thermal neutron flux of 1.1×10^{12} cm^{-2} s^{-1} (Jaćimović, 2003; Jaćimović et al., 2003a).

Following irradiation, the samples were measured after 3, 10-13 and 25-28 days cooling time on two absolutely calibrated HPGe detectors (40 and 45% relative efficiency). The HyperLab (HyperLab 2002 System 2002) program was used for peak area evaluation, whereas for determination of f (thermal to epithermal flux ratio) and α (epithermal flux deviation from the ideal 1/E distribution), the "Cd-ratio" method for multi monitor was applied (Jaćimović *et al*. 2003a). The values f = 28.6 and α = -0.001 were used to calculate the elemental concentrations. The THg concentrations and effective solid angle calculations were carried out on the software package Kayzero for Windows (User's Manual Kayzero for Windows Version 2, 2005). The method applied is in accordance with the ISO 17025 standard and also accredited by the Slovenian Accreditation system (Accreditation certificate LP-090).

For quality control purposes BCR-320R Channel Sediment was used. The results were in good agreement with the certified values.

2.3.7 Statistical analysis

All data obtained were analysed using STATA11/SE software.

The distribution of THg, and MeHg values in fish and vegetables, and Se and Cd values in vegetable samples was assessed using the Shapiro-Wilk test. Since the data were not normally distributed, the Wilcoxon-Mann Whitney test was used for comparison of two different groups and the Kruskal-Wallis test was used for comparison of more than two groups. The level of statistical significance was set to $p< 0.05$.

2.3.8 Exposure assessment

To estimate the potential exposure to MeHg and IHg (obtained as the difference between THg and MeHg) of the population from the consumption of local food items, the equations 1 and 2 were used to calculate the probable daily intake (PDI):

$$PDI_{intakeMeHg}\ (\mu g/kg\ body\ weight/\ day) = food\ intake\ (g/day) \cdot [MeHg]/\ body\ weight \quad \ldots 1$$

$$PDI_{intakeIHg}\ (\mu g/kg\ body\ weight/\ day) = food\ intake\ (g/day) \cdot [IHg]/\ body\ weight \quad \ldots 2$$

Data on the average annual intake of various foodstuffs by the Slovenian population was taken from the Statistical Office of the Republic of Slovenia (SORS), estimated for the year 2010.

3 Results and discussion

3.1 Mercury in fish

The concentrations of THg and MeHg found in fish from different locations on river Idrijca are shown in Table 1.

Fish from the most polluted sampling site (sampling site 2) contained on average higher levels of THg and MeHg than fish from other sampling sites. A statistical test (Wilcoxon-Mann Whitney test) showed that these differences were significant ($Z=-3.732$, $p < 0.001$ for THg levels from sampling site 2 compared to THg levels from other sampling sites; and $Z=-3.193$, $p = 0.001$ for MeHg levels from sampling site 2 compared to MeHg levels from other sampling sites). Furthermore, fish from the most polluted site contained the lowest percentage of MeHg and the difference between the percentages of MeHg in fish from sampling site 2 and other sampling sites was significant (Wilcoxon-Mann Whitney test: $Z=2.692$, $p=0.007$). The average levels of Hg in fish from the least polluted sampling site (sampling site 1) are comparable with the average levels of Hg in fish available on the Slovenian market (Miklavčič et al., 2011a).

On comparing the concentrations of THg and MeHg according to species at the same sampling site, we did not find any statistically significant differences with the exception of differences in MeHg levels between rainbow trout (*Oncorhynchus mykiss*) and grayling (*Thymallus thymallus*) at sampling site 2 (Wilcoxon-Mann Whitney test: $Z=2.309$, $p=0.0209$). The lack of significant differences in Hg levels between fish species could also be due to the low number of samples of the same species of fish.

The Hg levels in fish found in this study are comparable with the levels from the same sampling sites measured in 2003 (Horvat et al., 2004), with the exception of fish from

sampling site 4. The higher levels in fish from sampling site 4 measured in 2003 could be due to a difference in the species sampled and analysed. In 2003 higher levels were found especially in grayling (X=1350 ng/g, Med= 1560 ng/g, n=4), which in the present study was not included in the samples available from sampling site 4. Moreover, the higher levels in grayling could be due to the weight of fish being above average.

The data available from previous studies (Kosta et al.., 1974; Horvat et al., 2004) and the present work show that the levels of Hg in fish have not changed in the past decades, while the levels of Hg in air after closure of Hg mine in 1994 have diminished significantly (Kotnik et al., 2004).

Based on the provisional tolerable weekly intake (PTWI) for MeHg (1.6 μg/kg body weight) of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (WHO, 2004) and based on the 95th percentile level of MeHg in fish from the sampling sites 2, 3 and 4 on the river Idrijca (1170 ng/g), it can be estimated that the MeHg in one portion of fish (150g) for a 70 kg man exceeds the PTWI by approximately 60%. However, a 70 kg man could eat a portion of fish from the least polluted sampling site 1 approximately twice a week. Unfortunately, there is no data on the consumption of fish from the river Idrijca by the population of Idrija. Therefore, it is impossible to estimate the potential exposure from fish caught in the Idrijca. Nevertheless, because the population of Idrija town are well informed about the threat from Hg and people buy fish mostly from the fish market, a considerable exposure to MeHg from consumption of fish from the Idrijca can be excluded.

3.2 Mercury in vegetables and mushrooms

THg levels in vegetables were not very high (Table 2), but elevated compared to the levels in vegetables from non-contaminated areas which are usually very low. The average background level of THg determined in parsley leaves, chicory, cabbage and onion samples from Orle village near Ljubljana city was 6.6 ng/g d.w. (Min=1.2 ng/g d.w., Max=17 ng/g d.w.) and these results are in accordance with the literature (Falnoga et al., 2003). Our study showed that Hg accumulates in mushrooms and chicory (Table 4). These higher results found in chicory or mushrooms are in the same range as THg concentrations found in some plants in an abandoned cinnabar mining area in Spain or in the Almadén mining area in Spain (García-Sánchez et al., 2009; Millán et al., 2006). From past measurements it is also known that Hg accumulates in mushrooms from the Idrija mine area (Kosta et al., 1974; Byrne et al., 1979; Byrne et al., 1976), partly as MeHg (Stegnar et al., 1973). Moreover, the levels of THg we found in chicory are comparable with the levels measured in 2001 in the Idrija Hg mine area (Table 2). Therefore, the data indicate that the levels of Hg in vegetables have not diminished in the past 6 years.

Overall, the high concentrations of Hg in vegetables such as chicory and in mushrooms should not be overlooked, as in season these are consumed daily or frequently. Further and more regular monitoring of Hg levels in different species of mushrooms and chicory from the Hg mining area should be carried out in the future.

As mentioned, the vegetable samples were collected from 10 different gardens located above the smelter, near the smelter, in the other parts of Idrija town and in its surroundings. In addition, soil samples from these gardens and from the reference garden located in the village of Orle near the city of Ljubljana were sampled and THg levels were determined (Table 3). The THg levels from the contaminated Idrija area were much higher than the levels found in the reference soil. Importantly, THg levels in soils varied according to the location of the garden. The range of THg levels (Table 3) in soils from

the Idrija Hg mine area is in accordance with previously measured levels (Kocman et al,. 2004) and comparable with THg in soils measured in Hg polluted areas of the province of Guizhou in China (Horvat et al., 2003).

Although THg in soil varied according to garden position (Table 3), there were no significant differences observed in THg levels in vegetables from the 10 different gardens (Kruskal-Wallis test: $p=0.43$). This is in accordance with the results of studies where THg and MeHg levels determined in contaminated soils were not predictive of the THg and MeHg levels in rice (Rothenberg et al., 2012; Horvat et al., 2003). Not only is THg in soil not the most favorable predictor for MeHg levels in soil due to the number of biotic and abiotic factors influencing Hg(II)-methylation (Kelly et al., 1995; Ullrich et al., 2001), but also the contamination of Hg in vegetables is affected by factors such as the specific binding form in soil (Quevauviller, 2002), and the different ability of various vegetables species or different ability of different varieties of a foodstuff species (Rothenberg et al., 2012) to accumulate Hg from the soil. The levels of THg in soil can only be one, and not the most important of the variables. Furthermore, the situation in Idrija is complicated by the inhomogeneous natural occurrence and anthropogenic inputs of elemental Hg and Hg as cinnabar. In our study Hg levels determined in vegetables can additionally reflect surface contamination due to the deposition of Hg on the surface of the leaves from the atmosphere or due to other factors.

3.3 MeHg, Se and Cd levels in certain vegetables higher in THg

After finding higher THg levels in some vegetables and mushroom samples, MeHg and some other elements were additionally determined in all chicory, cabbage, parsley and mushroom samples (Table 4). Surprisingly, in some samples MeHg levels were sometimes up to 100 times higher than those previously determined in vegetables (Kobal

et al., 2005) from the Hg contaminated Idrija region. The highest levels of MeHg found in some of the vegetable samples were of the same order of magnitude as the levels in polluted areas of the Guizhou province in China (Horvat et al., 2003). Moreover, the mean overall percentage of MeHg in vegetables found in our study (X=13.5%, STD=17%) is comparable with the mean percentage of MeHg (X=10.8 %, STD=21.1%) found in vegetables (cabbage, salad, fennel, tomato, beet, basil, parsley, garlic) grown near a chlor-alkali plant (Gibičar et al., 2009). But it must be pointed out that the levels of THg in vegetables were much lower in the vicinity of the chlor-alkali plant than the levels found in the Idrija mine area, and moreover the mean percentage of MeHg found in cabbage and parsley near the chlor-alkali plant was much lower and less variable than that found in cabbage and parsley from the Idrija mine area. Therefore, further monitoring of vegetables from the Idrija mine area should be conducted and further studies should be made to confirm this result and to assure the safety of local vegetable consumption.

No significant differences were observed in MeHg levels in vegetables among the 10 different gardens in the contaminated area (Kruskal-Wallis test: p=0.18), while differences were observed if the levels of MeHg in vegetables from the reference garden were included (Kruskal-Wallis test was marginally significant p=0.038). In addition, no significant differences were observed for As (Kruskal-Wallis test: p=0.60), Se (p=0.18) and Cd (p=0.10) in vegetables from all 11 different gardens. It must be pointed out that the study design (including the sample preparation) was not intended to study the uptake of Hg or other elements. Therefore, it is impossible to conclude anything about the elemental uptake mechanisms. Furthermore, the uptake of Hg is influenced by a number of factors such as the element species and binding form in soil, the soil type and conditions, etc. To evaluate all these factors extensive studies are needed with large numbers of samples of a certain species, grown in varying soil conditions. A recent study (Rothenber at al., 2012) showed that MeHg concentrations in polished white rice were

strongly associated with the rice genotype. Therefore, future research should be focused on foodstuff varieties with low Hg-accumulating ability.

In terms of identifying the presence of elevated levels of some contaminants in vegetables, our results indicate elevated Cd levels in mushrooms compared to Cd levels found in vegetables (Table 4). However, these levels are still slightly below the threshold level for Cd in mushrooms set by European Commission Regulation ES no. 1881/2006. Se is sometimes present at elevated levels in chicory and cabbage from Idrija town and its surroundings. It is well known that Se is essential in small concentrations, but can be toxic at larger ones, and the range between essentiality and toxicity is only about one order of magnitude. According to the highest levels of Se found in chicory or cabbage in this study and according to the tolerable upper intake set by the Word Health Organization (WHO, 1996), approximately 400 g of chicory or cabbage may be consumed daily. At high levels antagonistic effects or mutual detoxification between Hg and Se have been confirmed in animal species (Parizek, 1967; Ng et al., 2001). Se could have an antagonistic effect on Hg in vegetables from the contaminated site of Idrija. However, not many studies have attempted to elucidate the influence of Se on biological processes involving Hg in plants and further studies are needed to investigate this.

Since this study was focused mainly on estimation of Hg exposure in the contaminated area due to the contamination of local food items, the 95th percentile of MeHg levels (46 ng/g w.w.) found in vegetables and the 95th percentile of IHg (466 ng/g w.w.) were used to calculate the PDIs (Equations 1 and 2). On the assumption that the population of Idrija does not consume local fish, Hg contamination of fish was neglected. The calculated PDIs for MeHg and IHg were 166 ng of MeHg/ kg day and 1.66 µg of IHg/kg day, respectively. It was calculated from our results that the weekly intake of MeHg did not exceed the PTWI of 1.6 µg/kg body weight (WHO, 2004). Nevertheless, the PDI for

MeHg estimated in this study is about 7 times higher than the PDI estimated for a population of pregnant women mainly from the Ljubljana region (Miklavčič et al., 2011b). Although the PTWI is relatively high, in the literature it is suggested that the absorption of IHg is only approximately 10%-30% (Piotrowski et al., 1992; Morcillo and Santamaria, 1995). Further, it must be pointed out that our PDI's are only rough exposure assessments, since it must be taken into account that we did not have accurate data on the consumption of local food items and only a few species of vegetables were included. Moreover, the PDIs assume the worst case scenario that the population in Idrija eat all year round only vegetables from their gardens and only vegetables higher in THg levels. Based on the data collected in the PHIME project for Slovenian pregnant women in 2008, about 33 % of subjects eat home-grown cabbage, Savoy cabbage or broccoli, only 12 % eat mushrooms collected in the forest and 51 % of the population eat home grown green or red lettuce. Overall, the worst case scenario estimated in this study is hardly probable. However, special attention should be given to foodstuffs such as chicory that represent a seasonal vegetable eaten daily.

4 Conclusions

Levels of Hg in food from the Idrija mine area were elevated compared to non-contaminated sites. Based on previous data, we can conclude that the levels of Hg in local food have not diminished significantly during the past 15 years after the closure of the Hg mine. Importantly, our study showed that Hg is accumulated in mushrooms and chicory and these high levels should not be overlooked, because chicory in particular represents a seasonal vegetable eaten daily. Levels of Cd and Se determined in vegetables with elevated Hg contents were below the threshold levels.

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REFERENCES

Akagi, H., 1997. Analytical method for evaluating human exposure to mercury due to gold mining. In: Proceedings of the international workshop on health and environmental effects of mercury due to mining operations. National Institute for Minamata Disease, Manila, pp. 131-141.

ATSDR, 1999. Toxicological Profile for Mercury, Atlanta, GA: Agency for Toxic Substances and Disease Registry.

Byrne, A.R., Dermelj, M., Vakselj, T., 1979. Silver accumulation by fungi. Chemosphere 10, 815-821.

Byrne, A.R., Kosta, L. 1970. Studies on the distribution and uptake of mercury in the area of the mercury mine at Idrija, Slovenia (Yugoslavia). Vestnik SKD 17, 5-11.

Byrne, A.R., Kosta, L., Ravnik, V., 1976. Trace element concentrations in higher fungi. Sci Total Environ. 6, 65-78.

EPA Method 7473. 1998. Mercury in solids and solutions by thermal decomposition amalgamation and atomic absorption spectrometry.

EPA Method 1630. 2001. Methyl Mercury in Water by Distillation, Aqueous Ethylation, Purge and Trap, and CVAFS.

Falnoga, I., Jereb, V., Smrkolj, P., 2003. Hg and Se in foodstuffs grown near a Hg mining area. J. Phys. 107, 447-450.

García-Sánchez, A., Murciego, A., Álvarez-Ayuso, E., Santa Regina, I., Rodríguez-González, M.A. 2009. Mercury in soils and plants in an abandoned cinnabar mining area (SW Spain). J. Hazard. Mater. 168, 1319-1324.

Gibičar, D., Horvat, M., Logar, M., Fajon, V., Falnoga, I., Ferrara, R., Lanzillotta, E., Ceccarini, C., Mazzolai, B., Denby, B., Pacyna, J., 2009. Human exposure to mercury in the vicinity of a chlor-alkali plant. Environ. Res. 109, 355-367.

Gosar, M., Šajn, R. 2005. Arsenic in the environment: enrichments in Slovenian soils. Geologija. 48(2), 253-262.

Horvat, M., Bloom, N.S., Liang, L. 1993. Comparison of distillation with other current isolation methods for the determination of methyl mercury compounds in low level environmental samples Part 1. Sediments. Anal. Chim. Acta 281, 135-152.

Horvat, M., Jereb, V., Fajon ,V., Logar, M., J. Kotnik, Faganeli, J., Hines, M.E., Bonzongo, J.-C. 2002. Mercury distribution in water, sediments and soil in the Idrijca and Soča river systems. Geochem. Explor. Environ. Anal. 2, 287-296.

Horvat M., Nolde N., Fajon V., Jereb V., Logar M., Lojen S., Jacimovic R., Falnoga I., Liya Q., Faganeli J., Drobne D., 2003. Total mercury and selenium in merury polluted areas in the Guizhou province, China. Sci. Total Env. 304, 231-256.

Horvat, M., Toman, M., Stergaršek, J., Kotnik, J., Fajon, V., Gibičar, D. 2004. Mercury and selenium in fish species in the Idrijca river polluted due to past mercury mining. Mat. and Geoenv., 51, 1073-1077 A.

Horvat, M., Snoj Tratnik, J., Miklavčič, A. 2011a. Mercury: Biomarkers of exposure and human biomonitoring. In: Knudsen, L.E., Merlo D.F. (Eds.), Biomarkers and Human Biomonitoring, Royal Society of Chemistry, Cambridge, pp 381-417.

Hughes, M. F., Thomas, D.J., Kenyon, E.M., 2009. Toxicology and epidemiology of arsenic and its compounds. In: Henke, E.K. (Ed.), Arsenic. Environmental Chemistry, Health Threats and Waste Treatmnet. John Wiley & Sons Ltd, West Sussex, pp. 237-277.

Kelly, C.A., Rudd, J.W.M., St. Louis, V.L., Heyes, A. 1995. Is total mercury concentration a good predictor of methyl mercury concentration in aquatic systems. Water Air Soil Pollut. 80, 715-724.

Kobal, A.B., Barbone, F., Horvat, M., Osredkar, J., Tamburlini, J., Križaj, B., 2005. Final Report of Research Project: Exposure of Women in the Fertile Period to Inorganic and Methyl-Mercury and Potential DNA damage from Polluted Areas in the Town of Idrija in Slovenia and in the Coastal Friuli-Venezia-Giulia Region of Italy. 11 p.

Kocman, D., 2008. Mass balance of mercury in the Idrijca river catchment. Jožef Stefan International Postgraduate school, Ljubljana, 152p.

Kocman, D., Horvat, M., Kotnik, J., 2004. Mercury fractionation in contaminated soils from the Idrija mine region. J. Environ. Monit. 6, 696-703.

Kosta, L., Byrne, A. R., Zelenko, V., Stegnar, P., Dermelj, M., Ravnik, V., 1974. Studies on the uptake, distribution and transformation of mercury in living organisms in the Idrija region and comparative areas. Vest. Slov. Kem.Druš., 21, 49-76.

Kotnik, J., Dizdarovič, T., Horvat, M., 2004. Current and past mercury distribution in air over the Idrija region, RMZ.

Levander, O.A., 1977. Metabolic interrelationships between arsenic and selenium. Environ. Health Perspect. 19, 159-164.

Miklavčič, V., 1999. Mercury in the town of Idrija (Slovenia) after 500 years of mining and smelting. In: Ebinghaus, R.(Ed.), Turner, R.R.(Ed.), de Lacerda, L.D. (Ed.), Vasiliev, O.(Ed), Salomons W.(Ed.) Mercury Contaminated Sites. Springer-Verlag, Berlin, pp. 259-269.

Miklavčič, A., Stibilj, V., Heath, E., Polak, T., Snoj, Tratnik, J., Klavž, J., Mazej, D., Horvat, M., 2011a. Mercury, selenium, PCBs and fatty acids in fresh and canned fish available on the Slovenian market. Food Chem. 124, 711-72.

Miklavčič, A., Cuderman, P., Mazej, D., Snoj Tratnik, J., Krsnik, M., Planinšek, P., Osredkar, J., Horvat, M. 2011b. Biomarkers of low-level mercury exposure through fish consumption in pregnant and lactating Slovenian women. Environ. Res. 111, 1201-1207.

Millán, R., Gamarra, R., Schmid, T., Sierra, A.J., Quejido, D.M., Sánchez, A.I., Cardona, M., Fernández, M., Vera, R. 2006. Mercury content in vegetation and soils of the Almadén mining area (Spain). Sci. Total Environ. 368, 79-87.

Magos, L., Clarkson, T.W., 2006. The toxicology of mercury and its chemical compounds. Crit Rev Toxicol. 36, 609-662.

Morcillo, M.A., Santamaria, J., 1995. Whole-body retention, and urinary and fecal excretion of mercury after subchronic oral exposure to mercuric chloride in rats. BioMetals. 4, 301-308.

Ng, P.S., Li, H., Matsumoto, K., Yamazaki, S., Kogure, T., Tagai, T., Nagasawa, H., 2001. Striped dolphin detoxificates mercury as insoluble Hg(S, Se) in the liver. Proc. Jpn. Acad., Ser. B: Phys. Biol. Sci. 77, 178–183.

NRC (National Research Council), 2000. Toxicological Effects of Methylmercury. National Academy Press, Washington, DC.

Parizek, J., 1967. The protective effect of small amounts of selenite in sublimate intoxication.
Experientia. 23, 142–143.

Piotrowski, J.K., Szymanska, J.A., Skrzypinska-Gawrysiak, M., Kotelo J., Sporny S., 1992. Intestinal absorption of inorganic mercury in rats. Pharmacol. Toxicol. 70, 53-55.

Raymond, L., Ralston, V.C.N., 2004. Mercury-selenium interactions and health implications. SMDJ. 7, 1, 72-75.

Rothenberg, S.E., Feng , X., Zhou, W., Tu, M., Jin, B., You, J., 2012. Environment and genotype controls on mercury accumulation in rice (*Oryza sativa* L.) cultivated along a contamination gradient in Guizhou, China. Sci Total Environ. In press.

Quevauviller P. H., 2002. Methodolologies in Soil and Sediment Fractionation Studies, The Royal Society of Chemistry, UK.

Sakurai, T., Kaise, T., Ochi, T., Saitoh, T., Matsubara, C., 1997. Study of in vitro cytotoxicity of a water soluble organic arsenic compound, arsenosugars, in seaweed. Toxicology. 122, 205-212.

Schoof, R.A., Yost, L.J., Eickhoff, J., Crecelius, E.A., Cragin, D.W., Meachernic, D.M., Menzel, D.B., 1999. A market basket survey of inorganic arsenic in food. FACT. 37, 839-846.

Stegnar, P., Kosta, L., Byrne, A.R., Ravnik, V., 1973. The accumulation of mercury by, and the occurrence of methyl mercury in some fungi. Chemosphere 2, 57-63.

Tomiyasu, T., Mutsuyama, A., Imura, R., Kondamatani, H., Miyamoto, Y., Kono, Y., Kocman, D., Kotnik, J., Fajon, V., Horvat, M., 2011. The distribution of total and methylmercury concentrations in soils near the Idrija mercury mine, Slovenia, and the dependence of the mercury concentrations on the chemical composition and organic carbon levels of the soil. Environ Earth Sci. 65, 1309-1322.

Ullrich, S., Tanton, T., Abdrashitova, S., 2001. Mercury in the aquatic environment: a review of factors affecting methylation. Crit Rev Environ Sci Technol 2001; 31; 241-293.

Van der Weijden, C.H., 2002. Pitfalls of normalization of marine geochemical data using a common divisor. Mar Geol. 184, 167-187.

Vreča, P., Pirc, S., Šajn, R., 2001. Natural and anthropogenic influences on the geochemistry of soils in terrains of barren and mineralized carbonate rocks in the Pb-Zn mining district of Mežica, Slovenia. JGE. 74, 99-108.

WHO, 2004. Technical Report Series 922. Sixty-First Report of the Joint FAO/WHO Expert Commitee on Food Additives (JEFCA), 133.

WHO, 1996. Trace Elements in Human Nutrition and Health. Geneva, World Health Organization.

Zeng, H., 2001. Arsenic suppresses necrosis induced by selenite in human leukemia HL-60 cells, Biol. Trace Elem. Res. 83, 1-14.

Zeng, H., Uthus, E.O., Combs, G.F., 2005. Mechanistic aspect of the interaction between selenium and arsenic. J. Inorg. Biochem. 99, 1269-1274.

Table 1: Mean (X) ± standard devation (STD), Minimum (Min) and Maximum (Max) (in parentheses) concentrations of THg and MeHg in fish collected at four different locations on the river Idrijca.

Location	Species	n	THg (ng/g wet weight)	MeHg (ng/g wet weight)	MeHg (%)
Sampling site 1	*Salmo trutta*	7	182±97 (92-330)	153±84 (92-301)	84±17 (92-330)
	Oncorhynchus mykiss	2	166, 137	126, 125	76, 91
	Barbus meridionalis	1	247	179	72
	Total	**10**	**183±83 (92-330)**	**148±68 (92-301)**	**83±15 (61-100)**
Sampling site 2	*Oncorhynchus mykiss*	4	882±469 (463-1440)	610±230 (350-834)	74±14 (58-93)
	Salmo marmoratus	2	1450, 205	1044, 205	72, 100
	Salmo trutta	3	439±113 (336-559)	320±55 (319-421)	89±25 (57-100)
	Thymallus thymallus	4	478±119 (321-571)	277±68 (190-349)	58±3 (54-61)
	Total	**13**	**647±412 (205-1450)**	**452±264 (190-1044)**	**74±18 (54-100)**
Sampling site 3	*Salmo sp.*	1	410	410	100
	Salmo marmoratus	1	755	246	33
	Oncorhynchus mykiss	4	237±85 (149-350)	211±113 (78-410)	84±22 (52-100)
	Total	**6**	**352±218 (149-755)**	**250±118 (78-410)**	**84±22 (33-100)**
Sampling site 4	*Leuciscus souffia*	4	147±37 (112-189)	146±36 (112-189)	99±3 (94-100)
	Millerigobius macrocephalus	2	96, 203	203	100
	Barbus meridionalis	5	295±75 (180-381)	301±78 (180-381)	102±3 (100-106)
	Salmo marmoratus	5	604±382 (105-1150)	581±379 (105-1172)	95±12 (75-102)
	Total	**16**	**337±309 (96-1150)**	**346±312 (105-1172)**	**99±7 (75-106)**

Table 2: Comparison of THg levels in vegetables collected from different gardens in the Idrija mine area in 2001 (Falnoga et al., 2003) and in 2008, 2009 (this work).

	1	2	3	4	5	6	7	8	9	10	11
	above the smelter	**near smelter**	**Idrija town and its surroundings**								**Orle reference area**
THg (mg/kg d.w.)	121	254	33.0	46.5	484	421	310	364	41.4	48.8	0.26

Table 3: THg levels in soil samples from different gardens in Idrija.

	n	X (ng/g d.w.)	Med (ng/g d.w.)	Min (ng/g d.w.)	Max (ng/g d.w.)
cabbage 2008, 2009	10	228	174	53	627
chicory 2008, 2009	12	**1950**	**603**	**86**	**12700**
parsley 2008, 2009	10	373	378	125	759
mangold 2008, 2009	12	362	236	196	781
mushrooms 2008	5	**5680**	**1370**	**346**	**17100**
lettuce 2009	2	336, 642			
vegetables without chicory, cabbage, parsley, mushrooms, mangold and lettuce 2008, 2009	59	87	31	2	860
onion, pumpkin, tomato, carrot 2001	12	240	109	4,7	737
parsley 2001	8	3950	2300	242	13900
chicory 2001	6	1960	1280	374	6430

Table 4: Results (X, Min, Max levels) of THg, MeHg, As, Se and Cd levels in cabbage, chicory, parsley and mushrooms.

	Cabbage			**Chicory**			**Parsley**			**Mushrooms**	
	Smelting facility n=2	Idrija town n=8	Ref. area n=1	Smelting facility	Idrija Town n=10	Ref. area n=1	Smelting facility n=2	Idrija Town n=5	Ref. n=1	near Idrija area	Ref. area
THg (ng/g d.w.)	280 (164-393)	215 (53-627)	2.4	762 (656-869)	2188 (86-12713)	16	510 (471-548)	376 (125-760)	11	346	75
MeHg (ng/g d.w.)	26 (7-44)	25 (3-88)	0.4	21 (18-23)	136 (0.9-623)	0.2	248 (206-291)	28 (1.2-127)	0.5	44	20
Se (ng/g d.w.)	1124 (94-2153)	261 (23-1450)	26	116 (65-170)	287 (9.5-2186)	19	35 (11-60)	16 (8.1-29)	22	58	76
Cd (ng/g d.w.)	123 (58-189)	150 (79-390)	80	447 (309-586)	228 (105-497)	55	80 (51-109)	109 (63-188)	52	1990	1206

**The table shows mean values, while the Max and Min values are shown in parentheses.*

3.6 Economic benefits of methylmercury exposure control in Europe: Monetary value of neurotoxicity prevention

In this section, the article (Bellanger et al., 2013) entitled: "Economic benefits of methylmercury exposure control in Europe: monetary value of neurotoxicity prevention" by Martine Bellanger, Céline Pichery, Dominique Aerts, Marika Berglund, Argelia Castaño, Mája Čejchanová, Pierre Crettaz. Fred Davidson, Marta Esteban, Marc E Fischer, Anca Elena Gurzau, Katarina Halzlova, Andromachi Katsonouri, Lisbeth E Knudsen, Marike Kolossa-Gehring, Gudrun Koppen, Danuta Ligocka, Ana Miklavčič, M Fátima Reis, Peter Rudnai, Janja Snoj Tratnik, Pál Weihe, Esben Budtz-Jørgensen and Philippe Grandjean is presented. The article was published in Environmental Health.

Based on harmonised protocols developed in EU FP6 COPHES, the hair-Hg concentrations in women of reproductive age groups in 17 European countries from the DEMOCHOPHES project (LIFE09 ENV/BE/00410) were used in conjunction with literature data to generate estimates of the economic impacts of MeHg exposures in Europe. An assessment of the economic benefits of preventing developmental neurotoxicity is necessary for any cost-benefit analysis (Bellanger et al., 2013).

My contribution was to review the published data on MeHg exposure.

Economic benefits of methylmercury exposure control in Europe: Monetary value of neurotoxicity prevention

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Abstract

Background

Due to global mercury pollution and the adverse health effects of prenatal exposure to methylmercury (MeHg), an assessment of the economic benefits of prevented developmental neurotoxicity is necessary for any cost-benefit analysis.

Methods

Distributions of hair-Hg concentrations among women of reproductive age were obtained from the DEMOCOPHES project (1,875 subjects in 17 countries) and literature data (6,820 subjects from 8 countries). The exposures were assumed to comply with log-normal distributions. Neurotoxicity effects were estimated from a linear dose-response function with a slope of 0.465 Intelligence Quotient (IQ) point reduction per μg/g increase in the maternal hair-Hg concentration during pregnancy, assuming no deficits below a hair-Hg limit of 0.58 μg/g thought to be safe. A logarithmic IQ response was used in sensitivity analyses. The estimated IQ benefit cost was based on lifetime income, adjusted for purchasing power parity.

Results

The hair-mercury concentrations were the highest in Southern Europe and lowest in Eastern Europe. The results suggest that, within the EU, more than 1.8 million children are born every year with MeHg exposures above the limit of 0.58 μg/g, and about 200,000 births exceed a higher limit of 2.5 μg/g proposed by the World Health Organization (WHO). The total annual benefits of exposure prevention within the EU were estimated at more than 600,000 IQ points per year, corresponding to a total economic benefit between €8,000 million and €9,000 million per year. About four-fold higher values were obtained when using the logarithmic response function, while adjustment for productivity resulted in slightly lower total benefits. These calculations do not include the less tangible advantages of protecting brain development against neurotoxicity or any other adverse effects.

Conclusions

These estimates document that efforts to combat mercury pollution and to reduce MeHg exposures will have very substantial economic benefits in Europe, mainly in southern countries. Some data may not be entirely representative, some countries were not covered, and anticipated changes in mercury pollution all suggest a need for extended biomonitoring of human MeHg exposure.

Keywords

Economic evaluation, Methylmercury, Prenatal exposure, Neurodevelopmental deficits

Background

Methylmercury (MeHg) is a well-documented neurotoxicant, and prenatal exposures are therefore of particular concern [1,2]. The main sources of exposure are seafood and freshwater fish [3]. Thus, MeHg exposures vary with dietary habits, contamination levels, and species availability. While the distribution of MeHg exposures has been studied in substantial detail in the United States [4], only scattered information is available on MeHg exposures in Europe.

Because the critical effect of MeHg exposure is developmental brain toxicity, exposures among women of reproductive age groups are of primary concern [5,6]. As has previously been determined in regard to lead exposure [7], developmental MeHg exposure is linked to a loss in Intelligence Quotient (IQ), with associated lower school performance and educational attainment, thereby leading to long-term impacts on societal benefits of pollution abatement [8]. These consequences may be expressed in terms of economic impacts, as has been demonstrated in United States [9,10]. However, few economic evaluations have been performed in Europe [8,11,12], primarily because of the lack of exposure data.

Based on harmonised protocols developed in COPHES [13], the DEMOCOPHES project has just completed a multi-country study of hair-mercury concentrations in women of reproductive age groups in 17 European countries. In conjunction with literature data, we now utilise the exposure data to generate estimates of economic impacts of MeHg exposures in Europe.

The economic assessment relies on several assumptions. The hair-Hg concentrations is used as the main exposure indicator in this study, and any blood-based measurements also considered are expressed in terms of hair-mercury using a conversion factor of 250 [14,15]. In regard to the dose-response function (DRF), a linear model is usually the default [14], although it may not necessarily provide the best statistical fit to the data [16]. We therefore used the linear slope as the primary DRF and then conducted a sensitivity analysis using the log function, where each doubling of exposure above the background causes the same deficit of 1.5 IQ points [10].

With regard to background exposures and the possible existence of a threshold, the U.S. EPA's Reference Dose (RfD) of 0.1 μg/kg body weight/day corresponds to a hair-Hg concentration of about 1 μg/g hair [14]. Updated calculations [17] resulted in an adjusted biological limit about 50 % below the recommended level, corresponding to 0.58 μg/g hair. The validity of this lower cut-off point below the RfD is supported by recent studies of developmental neurotoxicity at exposure levels close to the background [18-21]. We assumed that, below the 0.58 μg/g cut-off point, only negligible adverse effects would exist. As additional reference point, we use a tolerable limit proposed by the World Health Organization (WHO), which corresponds to a hair-Hg concentration of approximately 2.5 μg/g [22]. This limit takes into account the possible compensation of MeHg toxicity by beneficial nutrients in seafood [22].

Methods

Exposure information

DEMOCOPHES is a cross-sectional survey of European population exposure to environmental chemicals. The human exposure biomarkers included the hair-mercury concentration and was collected in 17 European countries based on children aged 6–11 years and their mothers. A common European protocol, developed by the COPHES project, was followed in each country. The main inclusion and exclusion criteria were (1) residence in the study area for at least five years, and (2) not having metabolic disturbances. The period of sampling was September 2011 to February 2012. A total of 1,875 child-mother pairs were recruited from urban and rural communities in the participating countries, while excluding exposure hot-spots. Major efforts were carried out to achieve high quality and comparability of data. Standard operational procedures for total mercury concentrations in hair were developed and validated by the Laboratory of Environmental Toxicology in Spain, to ensure comparable measurements, which included a strict quality assurance programme, in which seventeen European laboratories participated. Each DEMOCOPHES partner contributed information to allow estimation of the underlying distribution of exposures in the population, where rural and urban results were merged. In addition, each partner provided the frequencies of results above the cut-off levels of 0.58 μg/g, 1.0 μg/g, and 2.5 μg/g. The latter corresponds to WHO's tolerable limit, which takes into account likely toxicity compensation by beneficial nutrients in seafood [22].

Additional information on MeHg exposures in Europe was obtained to complement the DEMOCOPHES data. Thus, information of similar quality was extracted from published articles (Miklavčič, unpublished data), and distribution information from comparable studies was obtained from Belgium, Denmark, France, Norway, Slovenia, and the United Kingdom.

As explained below, missing information was calculated assuming a log-normal distribution of the exposures.

Exposure distributions

Using the number of births in 2008 and the observed hair-Hg concentrations, we estimated the number of births exceeding the three exposure limits for each country and obtained the sum for all of the EU. For missing EU member states, MeHg exposures were assumed to be the same as a neighbouring country. The year 2008 was chosen as the closest to the time during which the exposure data had been collected, and it allowed complete information for the calculations envisaged. Due to the existence of sampling uncertainty, "smoothed" proportions exceeding the three limits were calculated assuming log-normal distributions. Because log-transformed concentrations would follow a normal distribution, the parameters in the log-normal distributions could be estimated by standard normal distribution methods. Each data set included probabilities (prob) for being below specific percentiles (perc). The parameters in the logarithmic distributions were therefore obtained as the intercept and slope when regressing log(perc) on Φ^{-1}(prob), where Φ is the cumulative distribution function of the standard normal distribution. Using the total numbers of births in 2008, numbers of births exceeding the three cut-off limits in each country were calculated from observed and smoothed distributions.

Calculation of IQ benefits

A linear dose-response function was applied as the default model [14]. Thus, as a 1 μg/L increase of the cord-blood mercury concentration is associated with an average adverse impact on IQ of 0.093 times the standard deviation (which is standardised to be 15), each increase in the maternal hair-mercury by 1 μg/g is associated with an average loss of 0.465 IQ points [10]. This slope is based on a range of neuropsychological tests and subtests administered in the Faroe Islands study at age 7 years [23]. As some recent studies [18-21] suggest MeHg-associated deficits close to or below the cut-off level of 0.58 μg/g hair, the calculations may represent an underestimate. In addition, the slope may be steeper at low exposure levels. Thus, a log model was used for sensitivity analyses. In this model, a doubling in prenatal MeHg exposures is associated with a delay in development of 1.5–2 months at age 7, which corresponds to about 10 % of the standard deviation, i.e. 1.5 IQ points [1]. Again, we applied this slope for exposures above the 0.58 μg/g the cut-off point.

To estimate the benefits at exposures above the cut-off point, we calculated the average hair-mercury concentration in women exceeding 0.58 μg/g based on 1,000,000 simulations from the estimated log-normal distribution (as described above). After deduction of the 0.58 μg/g and multiplication by the slope factor, an average IQ benefit was obtained. This amount was then multiplied by the annual number of births exceeding the cut-off level. A similar calculation was made in the logarithmic dose-response model except that here we calculated the average log-transformed mercury concentration in women exceeding 0.58 μg/g, deducted log(0.58) and multiplied by the slope factor of the logarithmic dose-response model (1.5/log(2)).

Annual benefits of exposure reduction

The major component of the social costs incurred by an IQ reduction is loss of productivity and thus a lower earning potential [9,24]. The economic consequence of prenatal exposure to

MeHg is valued as the lifetime earning loss per person. We assumed singleton births only, so that the number of women was equal to the cohort size. We also assumed that IQ deficits present at age 7 years or preschool ages are permanent [25]. The estimated individual benefits are the avoided lifetime costs using 2008 data (slightly lower benefits are obtained if referring to more recent years, and benefits are only minimally affected by subsequent membership of the Euro zone). The benefit estimates originate from the 2008 figure of €17,363 per IQ point as recently calculated for France based on data from the United States [24]. For the various European countries involved, this value is adjusted for differences in purchasing power. While simple currency exchange conversion and Gross Domestic Product (GDP) per capita do not adjust for price differences, Purchasing Power Parity (PPP) conversion rates allow for comparison based on a common set of average international prices [26,27]. We also carried out the calculations after adjustment for productivity as the ratio of PPP-adjusted real GDP/capita in each country in relation to the US as a reference. The estimated value of an IQ point then takes into account the impact of labour costs and productivity (Additional file 1).

Results

Table 1 and Additional file 2 show summary information on MeHg exposures in the European countries covered by DEMOCOPHES or other exposure studies. There is a clear trend from north and east to southern countries, most likely due to differences in dietary habits and availability of large fish species from the Mediterranean (the sources of exposure were not considered in the present study). In Table 1, exposures in Austria were assumed to be similar to those in Germany, as suggested by available data [28]. Exposure information from the Flemish part of Belgium [29,30] do not differ much from the national data obtained in DEMOCOPHES, which were therefore used for the calculations. The Flemish data were used to represent exposures in The Netherlands. In the absence of exposure data from Estonia, Finland, Latvia, and Lithuania, the DEMOCOPHES exposure information from Sweden was applied. National data from France are available [31] and have been used in recent economic calculations [8]. Data for Croatia and Greece were obtained from a recent birth cohort study [32]. Two exposure studies had been carried out in Italy, one in the northeast [32] and one in Naples [33], and a joint distribution was therefore used to obtain national exposure distributions that would also apply to Malta. Thus, a log-normal distribution was first fitted to each Italian data subset, and then the parameters of a joint log-normal distribution were determined as the mean of the parameters for the two distributions. Recent results from the Norwegian national birth cohort were used for this country [34]. As DEMOCOPHES data from the United Kingdom covered only a small rural sample, we relied on data on blood-mercury in pregnant women obtained from the ALSPAC birth cohort study in the 1990s [35]. Additional exposure data from Ukraine [36] supported the notion that MeHg exposures in Eastern Europe are low, with only small percentages exceeding the cut-off level, but this study was considered too small to be used for detailed calculations. The same applied to several other sources identified (Miklavčič, unpublished data).

Table 2 presents the estimated IQ losses associated with the MeHg exposures using the linear model, along with the estimates of economic impacts. We used both the observed data and the modelled distributions, and only small differences were seen, thus supporting the notion that the log-normal exposure distribution has an appropriate fit. The greatest benefits accrue for the largest countries with the highest proportions of subjects with exposures above the cut-off level. The total benefit from control of MeHg exposure was the highest for Spain and the lowest for Hungary. On a per capita basis, the calculated benefits are the greatest in the Faroe Islands and the southern countries, Spain, Greece, Portugal, Italy, and Croatia. The total annual benefits in terms of IQ points within the EU were estimated to be in excess of 600,000 per year for the linear DRC. With an average benefit of €13,579 per IQ point, the total economic benefits are estimated to exceed €9,000 million per year. When adjustment for productivity is included, the benefits are somewhat lower for several countries, and the EU total is slightly less than €8,000 million per year (Additional file 3).

For comparison, Table 3 shows the estimated IQ losses and economic benefits using the log transformed DRF. Due to the steeper curve shape at exposures close to the cut-off point of 0.58 μg/g, the estimated benefits are about 4-fold greater, at about 2.7 million IQ points per year, which correspond to total benefits for the EU of approximately €39,000 million or, after productivity adjustment, €33,000 million.

Discussion

This study provides for the first time regional European data on economic benefits of controlling MeHg exposure in relation to prevention of developmental neurotoxicity. It relies on data from a multi-country study of hair-Hg concentrations with a high level of quality assurance and with similar population sampling criteria. In addition, available data from other studies have been taken into consideration to provide supplementary information, thereby allowing EU-wide estimates to be calculated. Given the low MeHg exposures in Eastern Europe and the relatively small contributions from Croatia, the Faroe Islands, Norway, and Switzerland, the results suggest that benefits for all of Europe will not be substantially above the benefits calculated for the EU.

Several assumptions and caveats must be acknowledged. The hair-Hg concentration is an established biomarker of human MeHg exposure and is generally considered reliable [14]. We used available data from DEMOCOPHES and other sources, with most studies including only about 120 subjects. The sampling size and strategy may have underestimated the occurrence of uncommon high-level exposures, which would weigh more in the calculation of IQ benefits. Adjustment for this bias is obtained in the modelled distributions, which tended to show slightly greater benefits. Although these calculations rely on an assumption of a log-normal distribution of the exposures, the concurrence of the two sets of estimates support the validity of this assumption.

In calculating the IQ benefits, we used a linear dose-response function for the decrease in IQ at increased prenatal MeHg exposures, and this curve shape is an approximation of unknown validity. As has been documented for lead [37], a logarithmic DRF may be plausible, and a log curve shows a slightly better fit [16]. As the results for the log curve (Table 3) are about 4-fold higher than those obtained for the linear curve, the benefits calculated in Table 2 must be considered likely underestimates. In recent calculations using French data using similar methods [8], the logarithmic curve shape also resulted in substantially higher estimates.

The cut-off level assumed to be 0.58 μg/g hair may also result in underestimated benefits. Recent data from Poland [20], Japan [21] and the United States [18,19] suggest that a lower threshold is likely. If the threshold is indeed lower than we have assumed, the benefits of controlling MeHg exposures will likely be greater, although an additional effort may be required to achieve such lower exposures. Further, given that the much higher tolerable limit of 2.5 μg/g is likely exceeded by 200,000 births in the EU per year, clear benefits will accrue already from controlling the very highest exposures.

The IQ benefits from controlling mercury pollution were translated into economic impacts based on the calculated current life-time income benefits from a higher IQ level. These benefits are mainly based on studies carried out in the United States [24,38], and it is possible that IQ-linked differences in life-time incomes may not be the same in Europe. Adjustment for differences in purchasing power has been included to take this issue into partial account. We used data from 2008 to secure complete data sources; the use of more recent records would change the estimates only slightly. An alternative approach might be to calculate benefits from prevention of specific diseases, e.g. for mental retardation or autism, associated with MeHg exposure. However, the attributable risks associated with increases in MeHg exposure are unknown, and such calculations are therefore uncertain [10,39].

Some sources of imprecision in exposure estimates must be emphasized. Thus, in several cases when exposure information was not available for an EU member state, data from a neighbouring country were used as a proxy. Further, the results reported in DEMOCOPHES and in published reports may not be representative for each country. Although high fish consumers may possibly have been oversampled, it is more likely that the avoidance of known exposure hot-spots resulted in lowered exposure estimates. In addition, especially for small studies, an element of uncertainty exists with regard to the frequencies of the highest exposures, although this problem was addressed by modelling a log-normal distribution of exposures. Temporal variation and time trends may also play a role, especially in regard to older data. We have assumed stable diets, so that any seasonal or other time trends as well as the time dependence of MeHg sensitivity during brain development would not matter for the calculation of impacts.

Our focus on the loss in life-time earnings is similar to the avoidable costs previously calculated in relation to lead exposure [24]. Other costs were ignored, such as direct medical costs linked to treatment or interventions for children with neurodevelopmental disorders. We also neglected indirect costs, such as those related to special education or additional years of schooling for children as a consequence of these disorders, as well as intangible costs. In addition, our study did not consider other avoided direct health care costs in the longer term, such as those potentially related to the treatment of cardiovascular or neurodegenerative effects of MeHg exposure, which could be important for high fish consumers [2], but would be difficult to estimate. Any compensation of the IQ benefit due to special education and other remedies was not taken into account. Overall, the estimates presented in Table 2 are likely underestimates of the total benefits of MeHg exposure abatement.

Clear differences are apparent between European countries. Seafood and freshwater fish constitute the main source of exposure, but countries with high fish consumption levels, such as Spain and Norway, clearly show great differences in MeHg exposure that are undoubtedly related to the choice of fish species consumed as well as the contamination level. The high exposure levels observed in Spain are in accordance with other studies [40,41]. The elevated exposures in the Faroes are likely related to the occasional consumption of pilot whale meat [23].

Calculations from the United States have resulted in several greatly varying estimates, depending on the DRF assumptions. One comparable estimate put the aggregate economic benefit for each annual birth cohort in the US at $8.7 billion (range: $0.7–$13.9 billion for year 2000) [10]. We recently calculated the annual benefit for the US at about 264,000 IQ points, which would correspond to benefits of approximately $5 billion [42]. The EU benefits of over 600,000 IQ points are much higher. However, in comparing the figures for the US and the EU, note should be taken that annual number of births in the EU (5.4 million) are 27 % greater than the 4.2 million births in the US per year. In addition, MeHg exposures in parts of Europe are higher than in the US [4]. On a global scale, benefit estimates can be extended on the basis of GDP values adjusted for PPP and productivity, but the validity of such calculations is limited by the lack of exposure assessments [43]. However, the present study leaves little doubt that global benefits substantially exceed $20 billion.

The present study did not aim at calculating annual costs of investments in pollution abatement due to the paucity of available data. Relevant investment costs would consider mercury emissions controls in coal-fired power plants, reduction of mercury usage in the chlorine industry, measures taken in dentistry, plus expenses for recycling and treatment of

mercury releases. Some information is available and suggests that one-time expenses may be quickly balanced by the cumulated annual benefits from exposure abatement [9]. However, mercury emissions control needs to be carried out on a global level due to the regional and hemispherical dispersion of mercury releases [43]. These costs would likely have additional socioeconomic yields from better control of mercury emissions, e.g. job creation and modernization of capital equipment.

The control of inorganic mercury emissions will only result in diminished MeHg exposure in the long term, and the benefits will therefore be delayed. As MeHg exposure mainly originates from seafood and freshwater fish, public health advice on dietary choices is an important element of the intervention [6,44]. Due to the essential nutrients present in seafood [3], a reduction in MeHg exposure should not be sought through a decrease or replacement of fish in the diet. A prudent advice would be to maintain fish consumption and minimise the MeHg exposure by consumption of fish known to have lower MeHg concentrations, e.g., smaller species, younger fish, and catches from less polluted waters. Such advice should be directed toward women during pregnancy as the most cost-effective preventive action. Restricted consumption of large, piscivorous fish species may also benefit overfished populations of pelagic fish, such as tuna [45].

The successful completion of the DEMOCOPHES project and the complements from other exposure studies in Europe illustrate the feasibility and usefulness of biological monitoring approaches, in particular when relying on hair samples that may be easily obtained, stored and transported. While such studies have become a routine function in the United States through the National Health And Nutrition Examination Survey [4], and the biomonitoring reports from the Centers for Disease Control and Prevention have become key resources for research on human exposures to environmental chemicals, Europe has lagged behind. Following international policy decisions to decrease global mercury pollution, such human biomonitoring studies will be crucial to monitor the effects of the interventions.

Conclusions

Annual benefits of removing Hg exposure can be estimated to be approximately €9 billion in Europe. While our results support enhanced public policies for the prevention of MeHg exposure, the economic estimates are highly influenced by uncertainties regarding the dose-response relationship. Thus, a logarithmic response curve results in 4-fold higher benefit estimates. In addition, benefits might be underestimated because costs linked to all aspects of neurotoxicity and long-term disease risks have not been considered. These European data and the calculated economic benefits support the need for interventions to minimize exposure to this hazardous pollutant.

Abbreviations

DRF, Dose-response Function; EPA, Environmental Protection Agency; EU, European Union; GDP, Gross Domestic Product; hair-Hg, Mercury concentration in hair; MeHg, Methylmercury; IQ, Intelligence Quotient; perc, Percentile; PPP, Purchasing Power Parity; prob, Probability; RfD, Reference Dose; US, United States; WHO, World Health Organization

Competing interests

PG is an editor of this journal but did not participate in the editorial handling of this manuscript. The authors declare that they have no competing interests.

Authors' information

National guarantors of the DEMOCOPHES data are listed as coauthors. The DEMO/COPHES Consortium that established and tested harmonised human biomonitoring on a European scale (www.eu-hbm.info) also included Jürgen Angerer, Pierre Biot, Louis Bloemen, Ludwine Casteleyn, Milena Horvat, Anke Joas, Reinhard Joas and Greet Schoeters.

Authors' contributions

MB, CP, EBJ and PG planned the economic evaluation, carried out the calculations, and drafted the manuscript. AM reviewed published data on MeHg exposure. DA coordinated the contributions of the 17 DEMOCOPHES countries. AC and ME were responsible for the development and follow-up of the Standard Operating Procedures and Quality Assurance for hair sampling and mercury analyses in support to comparability of DEMOCOPHES measurements. DA, MB2, AC, MČ, PC, FD, MEF, AEG, KH, AK, LEK, MK-G, GK, DL, AM, MFR, PR, JST, and PW contributed unpublished exposure data from European countries and act as guarantors of the data applied. All authors commented on the draft manuscript, and all authors read and approved the final version.

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3.7 Mercury: Biomarkers of Exposure and Human Biomonitoring

In this section entitled: »Mercury: Biomarkers of Exposure and Human Biomonitoring« by Prof. Dr. Milena Horvat, Janja Snoj Tratnik and Ana Miklavčič a contribution published in the book: Biomarkers and Human Biomonitoring, Volume 1: Ongoing Programs and Exposures, edited by Lisbeth E. Knudsen and Domenico Franco Merlo is presented.

Overall, this work represent a review about biomarkers of exposure and human biomonitoring. Hg is one of the most toxic metals and occurs throughout the environment as a consequence of natural sources and human activity. Hg species are broadly classified into three categories (the metallic or elemental form, divalent inorganic forms and organic Hg compounds), having different toxicokinetic properties.The selection of biological media to assess human exposure depends on the Hg compounds, the exposure pattern (e.g. chronic, acute) and time of sampling after the exposure. Exposure to elemental Hg is well represented by the presence of Hg in urine. Scalp hair has been widely used as a good indicator of MeHg exposure through the diet. As a biomarker of prenatal exposure, Hg inumbilical cord blood and not maternal blood is preferentially measured. In blood, MeHg accumulates mainly in the red blood cells, while exposure to elemental Hg leads to an increased concentration of Hg in the plasma. Therefore, through the analysis of total Hg in these blood samples it is possible to differentiate between exposure to elemental and MeHg. Although the toxic nature of acute exposure to Hg is well acknowledged, less known are the effects of Hg on humans as a consequence of long term chronic exposure to low concentrations. In many cases the use of biomarkers, such as Hg concentrations in blood and urine, are not sufficient to assess the internal doses and potential effects on the central nervous system, kidney, the immune system, and other possible targets. Therefore, a better understanding of the risks to human health, especially to persons living close to potentially contaminated sites and those exposed to low levels over a long period of time, is needed (Horvat et al., 2011a).

As a co-author of this work I contributed in reviewing the exposure pathways and toxicokinetic of Hg.

Mercury – biomarkers of exposure and human biomonitoring

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Contents

1 BACKGROUND

1.1 Chemical structures, compound classes

Mercury in the environment exists in several forms. These forms can be grouped under three headings:

metallic mercury (also known as elemental mercury),

inorganic mercury

organic mercury.

Several forms of mercury *occur naturally in the environment*. The most common natural forms of mercury found in the environment are metallic mercury, mercuric sulfide (cinnabar ore), mercuric chloride, and methyl mercury. Some microorganisms and natural processes can change mercury in the environment from one form to another. The most common organic mercury compound that microorganisms and natural processes generate from other forms is methyl mercury. Methyl mercury is of particular concern because it can build up in certain edible freshwater and saltwater fish and marine mammals to levels that are many times higher than the levels in the surrounding water, thus resulting in exposure among fish eating populations, often at levels exceeding what is regarded as safe.

1.1.1 Metallic mercury (or elemental mercury)

Metallic mercury is a heavy, shiny, silver-white metal that is a liquid at room temperature. Metallic mercury is the elemental or pure form of mercury. Metallic mercury is the liquid metal used in thermometers and some electrical switches. At room temperature, some of the metallic mercury will evaporate and form mercury vapour. Mercury vapour is colourless and odourless.

1.1.2 Inorganic mercury

Many salts of divalent mercury (Hg^{2+}) are readily soluble in water, such as mercury sublimate ($HgCl_2$), and thus highly toxic. In contrast, the water solubility of HgS (cinnabar) is extremely low, and, correspondingly, HgS is much less toxic than $HgCl_2$. The extremely high affinity of Hg^{2+} for sulfhydryl groups of amino acids such as cysteine and methionine in enzymes explains its high toxicity.

Monovalent mercury is found only in dimeric salts such as Hg_2Cl_2 (calomel), which is sparingly soluble in water and, again correspondingly, much less toxic than $HgCl_2$ (sublimate).

1.1.3 Organic mercury compounds

When mercury combines with carbon, the compounds formed are called **"organic"** mercury compounds or organomercurials. There is thus a potentially large number of organic mercury compounds; however, by far the most common organic mercury compound in the environment is monomethyl mercury (known as methyl mercury). The term "methyl mercury" is used throughout this text to represent monomethyl mercury compounds. In many cases, the complete identity of these compounds is not known except for the monomethyl mercury cation, CH_3Hg^+, which is associated either with a simple anion, like chloride, or a large charged molecule (e.g. a protein). In the past, the aryl organic mercury compound phenyl mercury was used in some commercial products. *Ethyl mercury* is used as a preservative in vaccines. Another organic mercury compound called *dimethyl mercury* is also used in small amounts as a reference standard for some chemical tests. Dimethyl mercury is very harmful to humans and animals.

1.2 Production and use

Mercury is available as a *commodity* on the world market from several sources:

Mine production of primary mercury (extracted from ore) is still carried out in Algeria, Kyrgyzstan, and until only recently in Spain and China. There are also reports of small-scale artisanal mining of mercury in China, Russia (Siberia), Outer Mongolia, Peru and Mexico, mainly serving local demand.

Mercury occurs as a *by-product of mining or refining* of other metals (such as zinc, gold, and silver) or minerals, as well as refining of natural gas.

- Reprocessing or secondary mining of historic mine tailings containing mercury.

Recycled mercury recovered from spent products and waste from industrial processes.

Private *stocks* (such as mercury used in the chlor-alkali and other industries).

Examples **of the uses of** mercury, in no particular order, include:

As a metal (among others):

- For extraction of gold and silver (for centuries)

- As a cathode in the mercury-cell process for chlor-alkali production
- In electrical and electronic switches
- In fluorescent lamps
- In discharge lamps (e.g. streetlights and some automobile headlights)
- In thermometers
- In thermostats
- In manometers for measuring and controlling blood pressure (sphygmomanometers)
- In barometers
- In dental amalgam fillings

Metallic mercury is still used in some herbal or religious remedies in Latin America and Asia, and in rituals or spiritual practices in some Latin American and Caribbean religions such as Voodoo, Santeria, and Espiritismo. These uses may pose a health risk from exposure to mercury both for the user and for others who may be exposed to elemental mercury vapour in contaminated air.

As a chemical compound (among others):

- In batteries
- Vaccines (as a preservative in the form of ethyl mercury in thimerosal)
- Biocides/fungicides in the paper industry, paints and on seed grain
- In pharmaceutical antiseptics
- Laboratory analysis reagents
- Catalysts (e.g. to produce vinyl chloride monomer)
- Pigments and dyes (may be historical)
- Detergents (may be historical)
- Soaps and creams (as a bactericide and/or whitening agent)
- Explosives (mercury fulminate detonators; may be historical)

Most of these uses have been reduced significantly in many industrialised countries, particularly during the last two decades because of their adverse impacts on humans and the environment. However, many of the uses discontinued in OECD countries, are still alive in other parts of the world.

1.3 Sources of mercury in the environment

Mercury is a **naturally** occurring element found throughout the environment. It enters the environment as the result of the normal breakdown of minerals in rocks and soil from exposure to wind and water, forest fires, and from volcanic activity. Mercury releases from natural sources have remained relatively constant in recent history, resulting in a steady rise in environmental mercury. However, it should be noted that a part of today's emissions from soil and water surfaces is composed of previous deposition of mercury from both anthropogenic and natural sources. The mercury found today in air, water, soil and biota may come from both natural sources and human activity.

Human activities since the start of the industrial age (e.g., mining, burning of fossil fuels) have resulted in additional release of mercury to the environment. Estimates of the total annual mercury release that result from human activities range from one-third to two-thirds of the total mercury releases.

A major uncertainty in these estimates is the amount of mercury that is released from water and soils that were previously contaminated by human activities as opposed to new natural releases.

The level of mercury in the atmosphere is very low and does not pose a health risk; however, the steady release of mercury has resulted in current levels that are *three to six times higher* than the estimated levels in the preindustrial era.

Approximately 80 % of the mercury released from human activities is elemental mercury emitted to the air, primarily from fossil fuel combustion, mining, and smelting, and from solid waste incineration. About 15 % of the total is released to the soil from fertilizers, fungicides, and municipal solid waste (for example, from waste that contains discarded batteries, electrical switches, or thermometers). An additional 5 % is released from industrial wastewater to water bodies in the environment.

With the exception of mercury ore deposits, the amount of mercury that naturally exists in any one place is usually very low (Figure 1). In contrast, the amount of mercury that may

be found in soil at a particular hazardous waste site because of human activity can be very high (over 200,000 times natural levels).

1.4 Environmental fate

Most of the mercury found in the environment is in the form of **metallic mercury** and **inorganic mercury** compounds. Metallic and inorganic mercury enters *the air* from mining deposits of ores that contain mercury, from the emissions of coal-fired power plants, from burning municipal and medical waste, from the production of cement, and from uncontrolled releases in factories that use mercury. Metallic mercury is a liquid at room temperature, but some of the metal will evaporate into the air and can be carried long distances. In air, mercury vapour can be changed into other forms of mercury, and can be further transported to water or soil in rain or snow. Inorganic mercury may also enter *water or soil* from the weathering of rocks that contain mercury, from factories or water treatment facilities that release water contaminated with mercury, and from incineration of municipal waste that contains mercury (for example, in discarded thermometers, electrical switches, or batteries). Inorganic or organic compounds of mercury may be released to water or soil if mercury-containing fungicides are used.

Microorganisms (bacteria, phytoplankton in the ocean, and fungi) convert inorganic mercury to **methyl mercury**. Methyl mercury released from microorganisms can enter water or soil and remain there for a long time, particularly if the methyl mercury becomes attached to small particles in the soil or water. Mercury usually stays on the surface of sediments or soil and does not move through the soil to underground water. If mercury enters the water in any form, it is likely to settle to the bottom where it can remain for a long time.

Mercury can enter and *accumulate in the food chain.* The form of mercury that accumulates in the food chain is methyl mercury. Inorganic mercury accumulates up the food chain to a much smaller degree. When small fish eat the methyl mercury in food, it accumulates in their tissues. When larger fish eat smaller fish or other organisms that contain methyl mercury, most of the methyl mercury originally present in the small fish will then be stored in the tissues of the larger fish (bio-magnification). As a result, the larger and older fish living in contaminated waters build up the highest amounts of methyl mercury in their bodies. Seawater fish (especially sharks and swordfish) and marine mammals (e.g. dolphins, whales, seals) that live a long time and can grow to a very large size tends to have the highest levels of mercury in their tissues.

Plants (such as corn, wheat, and peas) have very low levels of mercury, even if grown in soils containing mercury at significantly higher than background levels. Rice, however, grown in contaminated sites can contribute a large portion of the daily dose of inorganic and methyl mercury in countries dependent on a diet rich in rice[1]. Also, mushrooms can accumulate high levels if grown in contaminated soils.

1. EXPOSURE PATHWAYS

This global cycling of mercury results in its distribution in the most remote regions of the planet such us Arctic waters[2,3]. Microorganisms in the aquatic environment are capable of converting inorganic mercury to methyl mercury, which bio-accumulates and biomagnifies in food webs. As a consequence, the organisms higher organisms such as sharks and fish-eating marine mammals contain highest mercury concetrations. This implies that humans must have consumed methyl mercury in fish dating back to times before *Homo sapiens* evolved[4].

Because mercury is mostly deposited in the environment in its inorganic form of Hg (II) and methyl mercury is more toxic than other stable forms of mercury, the biogeochemical cycling of mercury in the environment plays a key role in modulating mercury toxicity. Direct transformation processes involving methyl mercury in the environment are the methylation of inorganic mercury and the degradation of methyl mercury. The reduction of inorganic mercury to elemental mercury and the oxidation of elemental mercury to inorganic mercury affect methyl mercury formation indirectly by controlling levels of inorganic mercury, the substrate for methylation[5].

2.1 Exposure to elemental or inorganic mercury

People may be exposed to elemental or inorganic mercury from dental amalgams and through inhalation of ambient air during occupational activities where mercury and mercury compounds are produced[6]. Occupational exposures have been reported from chlor-alkali plants, mercury mines, mercury-based small-scale gold and silver mining, refineries, thermometer factories, dental clinics with poor mercury handling practices and production of mercury-based chemicals. Furthermore, small scale or artisanal mining, using gold-mercury amalgamation to extract gold from ore, is a significant source of exposure for the workers and nearby populations[7].

Amalgams release mercury vapour that can be inhaled. Concentrations of mercury vapour in the air in the oral cavity were shown to exceed occupational health standards. However, the quantity of vapour is small because the volume of the cavity is small. Furthermore, the vapour retained is much less than that inhaled under conditions of occupational exposure. Levels of mercury vapour in ambient air are low and the intake from this source is negligible. Therefore, with the exception of certain occupational exposures, dental amalgam is the main source of human exposure to mercury vapour[3].

Exposures to elemental mercury or inorganic mercury can also occur due to the use of some skin-lightening creams and soaps, the presence of mercury in some traditional medicines, use of mercury in cultural practice, and due to various accidental mercury spills in homes, school or other locations. Moreover the use of mercuric compounds as fungicides in latex paint and to disinfect grain seeds can result in exposure to inorganic mercury, but such use is prohibited in many countries[7].

2.2 Exposure to organic mercury

People are exposed to methyl mercury mainly through their diet, especially through the consumption of freshwater and marine fish and consumption of other animals that consume fish (such as marine mammals). The highest levels are found in fish of older age that are apical predators such as the king mackerel, pike, shark, swordfish, walleye, barracuda, large tuna, scabbard, marlin and fish-consuming mammals such as seals and toothed whales[8-12]. Trimming, skinning and cooking mercury-contaminated fish does not reduce the mercury content of the fillet portion. However people that consume moderate amounts of a variety of fish are not at risk[0]. For example, based on the levels of MeHg in fish available on the Slovenian market and the JECFA provisional tolerable weekly intake (PTWI) for methyl mercury, a 70 kg man can eat a portion (150 g) of fish at the top of the food chain approximately once per week or approximately three portions (150 g) of fish lower on the food chain[12].

Minor exposure to other forms of mercury may result from the use of thimerosal (ethyl mercury thiosalicylate) as a preservative in some vaccines and other pharmaceuticals. However, the use of thimerosal in vaccines is being discontinued, or significantly reduced in many countries, especially in vaccines intended for children[7].

2. TOXICOKINETICS

The toxicokinetics of mercury is dependent on the route of exposure (inhalation exposure, oral exposure or dermal exposure) and on the form of mercury to which the person has been exposed.

3.1 Absorption

Metallic mercury is highly lypophilic, and absorption of the inhaled vapour, followed by rapid diffusion across the alveolar membranes of the lungs into the blood, has been reported to be substantial. Exposure to 0.1-0.4 mg/m^3 elemental mercury vapour can result in approximately 70-80 % of inhaled mercury vapor[13,14].

Ingesting small amounts of metallic mercury such as contained in a standard thermometer does not produce symptoms of intoxication, because the absorption of ingested metallic mercury is negligible[15,16]. Animal studies indicate, that the absorption of inorganic mercury such as mercuric chloride is approximately 10-30 %[17,18]. The rate of oral absorption of inorganic mercury compounds in rats is dependent on intestinal pH, compound dissociation, age and diet[19].

Organic mercury compounds are more readily absorbed in the gastrointestinal tract than inorganic mercury compounds. About 95 % of methyl mercury ingested is thought to be absorbed by the oral route. However, the absorption and bio-availability of methyl mercury may be affected by dietary components in food such as dietary fibre found in cereal products or selenium in fish[20,21]. Depending on the species of fish a low percentage of mercury in the fish is bio-available (less than 20 %) in both simulated stomach and intestinal digestion. The low methyl mercury absorption could be attributed to the low ability of enzymes in the *in vitro* method to release mercury contained the samples, perhaps due to the fact that mercury is complexed by selenium rather than a lack of bio-availability of methyl mercury itself. In fact, a recovery of 89 % from in vitro enzymolysis of a sample spiked with methyl mercury was obtained[22]. Higher faecal extraction and lower tissue accumulation of mercury in rats from contaminated fish than from methyl mercury chloride added to fish was demonstrated[23].

3.2 Distribution

Because of its lypophilic nature, metallic mercury is distributed throughout the body and can cross the blood-brain and placental barriers very easily. It is distributed in all tissues and reaches peak levels within 24 hours, except in the brain where peak levels are

achieved within 2-3 days[13]. The mercury concentration in red blood cells in humans is twice that measured in the plasma[0]. The kidney is the major organ of mercury deposition after inhalation exposure of rats to metallic mercury vapour[25,26]. In chronic long-term occupational exposure to mercury vapour by mercury miners, the organs accumulating and retaining the highest concentrations were thyroid and brain, and this was associated with a one-to-one molar mercury:selenium ratio[27].

In contrast to metallic mercury, inorganic mercury hardly crosses the placental barrier. In the plasma the mercuric ion binds to albumin and globulins. After administration of mercuric salts levels of mercuric ions in the plasma are similar to mercuric ions in red blood cells. Binding also occurs in tissues, with the brain retaining mercury the longest[20]. After oral exposure to mercuric chloride, animal studies showed the highest level in the kidneys[28,29]. Renal uptake of mercury salts occurs from luminal membranes in the renal proximal tubule in the form of the cysteine S-conjugates (Cys-S-Hg-S-Cys), or from the baso-lateral membrane through organic anion transporters[30].

In humans, the distribution of methyl mercury is similar to that of metallic mercury, because is readily transferred to all tissues, including the brain and foetus. Methyl mercury is bound to thiol-containing molecules such as cysteine, which mimic methionine to cross the blood-brain barrier and placenta through the neutral amino acid carrier[30]. Approximately 90 % of methyl mercury was found in red blood cells[31,0]. The highest levels of organic mercury are found in the kidneys[33-35]. Concentrations of methyl mercury in hair are proportional to simultaneous concentrations in blood and are in average 250 times higher. They are also proportional to concentrations in the target tissue, the brain. Levels in cord blood are proportional to but slightly higher than levels in maternal blood[36]. Methyl mercury is also excreted in breast milk. However, unlike placenta, where methyl mercury moves more easily across the placental barrier than inorganic mercury, inorganic mercury is more readily eliminated from breast milk than methyl mercury[37].

The distribution, metabolism, media or possible media for biological monitoring in the case of exposure to elemental mercury, inorganic mercury and methyl mercury are shown in Figures 3 and 4

3.3 Metabolism

When metallic mercury vapour is inhaled through the lungs and rapidly enters the bloodstream, the dissolved vapour can undergo rapid oxidation to its inorganic divalent

form by hydrogen peroxide-catalase[39]. Because ethanol is a competitive substrate for the hydrogen peroxide catalase, the oxidation of metallic mercury can be inhibited by ethanol[40]. The oxidation of metallic mercury may occur in the brain, liver, lungs and probably other tissues to some degree[41,42,43]. Some studies suggest that the divalent inorganic mercury cation might be reduced by mammalian tissue to metallic mercury after its oxidation[44,45].

After absorption, methyl mercury can be converted into inorganic mercury in tissues, specially the divalent cation[45]. After methyl mercury exposure several studies have reported high levels of inorganic mercury in tissues and faeces[46-48]. Intestinal flora can also convert methyl mercury into inorganic mercury[49,50].

3.4 Elimination and excretion

The main excretory pathways of metallic and inorganic mercury in humans are the urine and faeces, with a half life of approximately 1-2 months[41]. In a study of former chlor-alkali workers long term exposed to metallic mercury, the elimination of mercury in urine was well characterized by a one-compartment model, with an estimated half-life of 55 days. A tendency toward longer half lives with shorter duration exposure compared to long term exposure was also observed[51]. Therefore the excretion of mercury depends on the duration of exposure. Furthermore, after acute mercury exposure in humans, urinary excretion accounts for 13 % of the total body burden, while after long-term exposure urinary excretion increases to 58 %. Elimination of metallic mercury also occurs through expired air. After human exposure to mercury vapour for less than an hour, excretion through expired air accounted for 7 %. The half life for this elimination pathway was estimated to be 14-25 hours[13,0]. In a group of chlor-alkali workers long term exposed to metallic mercury, a two compartment model was used to estimate the half lives of whole blood and plasma. In whole blood the half-lives were 3.8 and 45 days for the fast and slow phase, respectively, while for plasma the half lives were 2 and 36 days for the fast and slow phase, respectively.

After an acute exposure to a high level of mercuric chloride, the elimination half life in urine was estimated to be 25.9 days[52]. The overall half life of inorganic mercury from the body was estimated to be 60 days and is eliminated with the same rate as from the kidneys, where most of the body burden is localized[20].

The predominant excretory route for methyl mercury is the faecal pathway, with less than one-third of total mercury excretion occurring through the urine[53]. In a study that included

four Japanese people, the extraction of methyl mercury into faeces was confirmed, but they found similar concentrations of methyl mercury in the urine compared to concentrations in faeces[54].

Animal studies showed that methyl mercury is secreted in the bile and can be reabsorbed in the intestine. Clearance half-times are longer with methyl mercury than with inorganic compounds. The half-time in the blood is estimated to be 50 days[20]. Elimination of methyl mercury compounds generally follows first order kinetics because excretion, it is directly proportional to body burden and independent of the route of administration[55]. Duration of exposure may affect the extraction process of mercury. A two compartment model was establish for a single oral dose in monkeys, while following repeated dosing for two years a one-compartment model was considered a more reasonable fit for the data. Therefore the average study-state blood levels of mercury after chronic-duration exposure should not be estimated on the basis of short-term exposure data[33].

3. SAMPLING, SAMPLE HANDLING AND ANALYSIS

Hundreds of papers have been published in recent years on the development and validation of sensitive analytical methods for total mercury and mercury speciation. Several review papers have also been published summarizing the advantages and disadvantages of various analytical protocols[56], therefore only brief notes are provided in this chapter. While much has been done on the measurement techniques, relatively little is known about the effects of storage on the stability of methyl mercury in biological samples. Significant external contamination of samples with methyl mercury is unlikely to occur; however extreme precautions are necessary to avoid contamination by inorganic mercury.

Blood and hair samples are often analysed in order to estimate exposure of humans to mercury and its compounds. Blood should be taken by venipuncture. Since some commercial containers may contain mercury compounds added as preservatives, it is advisable to check each commercial batch before use. Samples should be refrigerated but not frozen, as it is sometimes useful to measure mercury in plasma and red blood cells separately. The separation of plasma and red blood cells should be performed as soon as possible to avoid haemolysis of the sample. If extensive haemolysis has occurred, the sample should be homogenized before an aliquot is taken for analysis. Blood samples may also be heparinised for total blood, serum, and red blood cell analyses. If

unavoidable, samples may be stored deep frozen. However, repeatedly frozen and unfrozen blood samples showed a remarkable decrease in methyl mercury concentrations[57]. There is some evidence that methyl mercury may be destroyed during lyophilisation of blood samples.

Analysis of human hair offers several advantages over analysis of blood samples: e.g. ease of sampling and sample storage, the concentration of methyl mercury is approximately 250 times higher than in blood, and analysis of different longitudinal sections of hair can give information on the history of exposure to methyl mercury ingested through food. Adhering dust and grease should be removed by one of the following solvents: hexane, alcohol, acetone, water, diethylether, or detergents. IAEA and WHO recommend the use of only water and acetone[58]. Long-term storage of human hair samples has shown that methyl mercury is stable for a period of a few years if stored dry and in darkness at room temperature.

Biological samples are preferably analysed fresh or after lyophilisation. Deep-freezing of fresh samples, especially with long storage, should be avoided, since it has been noticed that in some organisms methyl mercury may decompose with repeated freezing and thawing (particularly in bivalves and blood)[57]. Methyl mercury and total mercury in lyophilized biological samples, such as biological certified reference materials (CRMs), are stable for years. CRMs are, however, sterilized either by autoclaving or by gamm ray-irradiation. This important step prevents bacteriological activity, which may otherwise lead to methylation/demethylation processes. In general, very little is known about the effects of sterilization on the stability of methyl mercury compounds. More studies are needed to investigate the stability of organic mercury compounds in biological samples, particularly under various sample preparation and long-term storage conditions.

4.1 Analytical methods

4.1.1. Total mercury

Most methods for the determination of total mercury in solid samples require preliminary digestion of the sample. They are classified as wet (oxidizing digestion) and dry (combustion/pyrolysis) decomposition methods.

For the determination of low level mercury concentrations, a number of instrumental analytical methods can be used. Among the most frequently employed are the following:

CV AAS, CV AFS, ICP-MS, electrochemical methods, and neutron activation analyses (NAA). During the last two decades CV AAS and CV AFS have replaced most of other techniques. The relative detection limits of some techniques are presented in Table 1. It should be noted, however, that the detection limits reported are dependent on the overall analytical procedure including sample preparation prior to the final quantification step.

4.1.2 Speciation of mercury

In general, methods are classified according to the isolation technique and the detection system. Most methods for the isolation/separation of organic mercury compounds are based on solvent extraction, differential reduction, difference calculations between "total" and "ionic" mercury, derivatization, or on paper- and thin layer chromatography. The most common approaches to organic mercury separation and detection are schematically presented in Figure 5.

There are also a few methods that are based on differential reduction. In the method developed by Magos[59] the inorganic mercury in an alkaline digested sample is selectively reduced by stannous chloride, while organic mercury compounds are reduced to elemental mercury by a stannous chloride-cadmium chloride combination. Elemental mercury released can be measured by CV AAS.

Quality assurance refers to those procedures that ensure that analytical results are valid, traceable, reproducible, representative, complete and accurate, i.e. close to the “true value”. It also includes measures developed to assess performance. It is generally accepted that mercury analysis and speciation must be done by well trained staff who, in principle, should be involved in the measurement process from sampling to the production of final results, particularly if speciation of mercury is intended. The use of reference materials certified for mercury and its compounds plays an important role in method validation and demonstration of traceability. At present there are many reference materials certified for total mercury concentrations in various matrices (sediment, soil, ash, water, plants, and tissues) of different origin. Unfortunately, only a few reference materials are certified for methyl mercury compounds[56,60].

4. HUMAN BIOMONITORING OF MERCURY EXPOSURE

Exposure to mercury is estimated by measuring mercury in human tissues (hair, blood, urine), which are considered forms of biomonitoring. These measurements are also known as biomarkers of exposure. The selection of media depends on mercury compounds, exposure pattern (e.g. chronic, acute) and time of sampling after exposure[0]. The presence of mercury in blood indicates recent or current exposure to mercury and does not give information on the historical exposure and seasonal (or other peak) variations. In the general population, methyl mercury from fish and mercury vapour from dental amalgam fillings influence mercury concentrations measured in blood, but this is not true for occupationally exposed people – in this case mercury vapour is the major contributor to blood mercury concentration[62,0]. Exposure to elemental mercury is well represented by the presence of mercury in urine. As inorganic mercury is slowly excreted from kidneys through urine, urinary mercury reflects not only recent exposure to elemental mercury, but also exposure that occurred some time in the past[62,64]. In the non-occupationally exposed population, the number of amalgam surfaces was found to be the best predictor for urinary mercury. Interestingly, in previously exposed chlor-alkali workers, more than one year after the exposure ceased no association between the number of amalgam surfaces and urinary mercury concentration was found[65]. Since the concentration of waste product in urine can vary significantly due to dilution with water, mercury in urine is preferably adjusted for creatinine or specific gravity.

Scalp hair is a matrix of choice in studies assessing environmental mercury exposure, as it provides a simple, integrative, and non-invasive sample for estimating long-term average exposure to methyl mercury. Assessment of methyl mercury exposure through measuring total mercury in hair is based on the assumption that methyl mercury in hair is proportional to the methyl mercury blood concentration and that almost 100 % of total mercury in hair is methyl mercury[66,0]. However, the exact relationship between mercury exposure and mercury excretion in hair follicles is not known, and can vary highly between individuals and ethnic groups.

5.1 Human biomonitoring of occupational exposure

In certain working environments workers are exposed to mercury by inhaling metallic mercury vapour. Exposures in the working environment are monitored by measuring inorganic mercury in urine or blood. Urine has been used as the prefered matrix because metallic mercury is mostly excreted through urine (see section 3.4) and also because collection of urine is non-invasive. The maximum urine mercury concentration set by

WHO (1991)[67] is 50 μg/g creatinine. Mercury urine levels rarely exceed 5 μg/g creatinine in persons who are not occupationally exposed to mercury[0]. Individual human biomonitoring studies in eight Swedish chlor-alkali plants showed that urinary mercury excretion in workers has declined from about 200 μg/L in the 1950s to 150 μg/L in the 1960s and less than 50 μg/L in 1990s[62]. Decrease in exposure has also been observed in the UK since the 1980s and 1990s[64]. In 1990s, workers from chlor-alkali plants had in general an approximately 10 times higher Hg concentration in urine, as well as in blood, than non-exposed controls[0,68]. The occupational mercury contribution from dental work is generally low; however, individuals can show urinary mercury concentrations close to a level of 15 μg/L[69]. Peak exposures have been reported to occur mainly during the insertion and removal of amalgams and during polishing of amalgam surfaces[0].

However, as mentioned above, urinary mercury reflects average long-term exposure, while blood mercury is a good indicator of recent exposure peaks[62,64]. Urinary mercury is an integrating marker of exposure to metallic and inorganic mercury over many previous months. In long-term workers, 60-70 % of mercury was found in urine, while in workers exposed less than 6 months, only 20-25 % of mercury contributed to urinary mercury. Moreover, previous weeks of exposure contributed only 10 % to urinary mercury[64]. When monitoring **temporal** occupational exposure, it should be born in mind that peak urinary mercury may occur a few weeks after exposure. It has been reported that there was a 2-3 weeks delay in peak urinary mercury in people without previous exposure, which was explained by the time taken for renal uptake and subsequent excretion[70,64]. In contrast to urinary mercury, blood mercury increased immediately after temporal exposure - urine-to-blood ratios for the temporally exposed workers (maintenance engineers) were <0.02 compared with ~0.4 usually found in those chronically exposed[64].

When blood total mercury is used for monitoring exposure to mercury vapour, methyl mercury from fish consumption can be a disturbing factor[62]. The results of the above described studies[0,68] have demonstrated clearly that inorganic mercury in blood is a better marker for occupational exposure than total or organic mercury. Furthermore, in the case of measuring total mercury in blood, significantly higher difference between exposed and non-exposed groups were observed in serum samples than in whole blood samples (3-fold vs. 11-fold).

If the exposure is stable, natural intra-individual variation for mercury in blood is moderate (about 10 %), but rapid changes caused by change in exposure can occur. In

urine, the day-to-day variation is relatively high, even when 24-h samples are used under stable exposure conditions[62]. Variability in individual urinary mercury results was reported to be due to different methods of correction, diurnal variation of mercury excretion and levels of laboratory analytical precision[64]. A consistent diurnal variation of urinary mercury concentration (creatinine corrected) has been demonstrated in 36 occupationally exposed workers, the concentration being highest in the morning and lowest in the late evening[71]. A fixed time point for sample collection has been suggested to remove the effects of diurnal variation in excretion of mercury[64]. Creatinine correction of mercury concentration significantly reduced the mean intra-individual variation, both between and within days, to ~50 % of the variation in uncorrected urine values[64]. When a correction for specific gravity or creatinine is made, typical coefficients of variation are 15-20 %, and the levels on two consecutive days often differ by 25-50 %[62].

5.2 Human biomonitoring of environmental exposure

In most epidemiological studies, total mercury in hair is used as a biomarker of methyl mercury exposure, as it provides a long-term biomarker of exposure to methyl mercury through fish consumption. Mercury concentrations in hair of populations consuming no fish are normally below 0.5 µg/g; in populations with low to moderate fish consumption, hair total mercury varies from below 1 to 2 µg/g, while people with frequent consumption (once or more per day) may have total mercury levels in hair exceeding 10 µg/g[0].

It is usually assumed that total mercury in hair is about 250 times higher than the blood mercury concentration at the moment hair is formed and that almost 100 % of total Hg in hair is methyl mercury[66,0]. The latter is only true in populations with high fish consumption, whereas in populations with relatively low fish consumption, inorganic mercury might constitute a larger fraction of hair mercury[Error! Reference source not found.]. Relatively high inter-individual variability in the hair-to-blood MeHg ratio was reported, ranging from 140 to 370[0] and from 150 to 690[72], related with low-to-medium exposure levels. Furthermore, high variability in the relation between mercury levels in hair and methyl mercury exposure through fish consumption was reported by Canuel et al[73]. Therefore, it is suggested that in the general population hair mercury to be used as an approximate indicator of long-term MeHg exposure from fish consumption, while blood mercury as a more accurate indicator of recent exposure to mercury (organic and/or inorganic).

Mercury in blood is present as methyl mercury and inorganic mercury, therefore it is important to perform speciation analyses for accurate assessment of specific exposure. Especially in cases of low-to-moderate level of exposure to methyl mercury from fish consumption, inorganic mercury from dental amalgam fillings or other sources (vaccines, food,…) might constitute a substantial proportion of total mercury – from 0 to 80 %[74,72]. However, there is no need to perform mercury speciation at very low exposure levels, as such exposure presents little risk. The NRC identifies 2 μg/L as the normal mean concentration for populations with little or no fish consumption in the US[66]. It should also be mentioned that speciation of mercury at levels below ~ 1 μg/L is demanding and requires additional clean-up steps. Therefore, in terms of cost-effectiveness, it is sufficient to measure total mercury. Speciation analysis is required when total mercury levels above 1 μg/L are detected.

Levels of mercury found in biological samples of various populations worldwide are presented in Table 2.

Mixed occupational and environmental exposure

A good example of a mixed occupational and environmental exposure is the gold mining area in the Tapajos river basin of the Amazon, where inhabitants are exposed to metallic mercury by direct inhalation of Hg vapour (gold miners and gold shop workers) and to methyl mercury in fish from rivers downstream of the gold-mining sites. Akagi et al[84] reported that total mercury levels in blood were not correlated with total mercury levels in urine, but inorganic mercury levels were. On average 73 % of the total mercury in blood was in the form of methyl mercury, not associated with occupational exposure. Abnormally high levels of mercury mostly in inorganic form were found in the urine of gold shop workers (162 ± 95 μg/g creatinine), while hair total mercury in gold shop workers was significantly lower (4.1 ± 1.3 mg/kg) than in the inhabitants of the fishering villages (around 30 mg/kg).

5.3 Human biomonitoring of prenatal exposure

Methyl mercury and metallic mercury move freely across the placenta from maternal to foetal blood, while inorganic mercury is prevented from being transferred to the foetus and accumulates in the placenta[52,72,75,0]. Various studies reported cord blood methyl mercury to be almost twice as high as maternal blood methyl mercury[72,74,86,87], which is a

consequence of higher haematocrit levels[88] and also higher haemoglobin concentrations in cord red blood cells.

As a biomarker of prenatal exposure, mercury in umbilical cord blood and not maternal blood mercury is preferentially measured. Despite the strong association between cord blood methyl mercury concentration and maternal blood methyl mercury concentration, high variation in the cord blood-to–maternal blood ratio methyl mercurywas found in several studies: 1.0-3.5)[72], 1.8 (0.88-3.1, 95 % CI)[74], 1.7 ± 0.56[86] and 1.72 (1.44-2.01, 95% CI)[87]. As concluded by Murata et al[0], the high variation indicates that cord blood mercury should be used as a more useful and valid biomarker of prenatal exposure to methyl mercury than maternal blood mercury.

In addition to cord blood mercury, which was demonstrated as the best biomarker of prenatal methyl mercury exposure[89], the suitability of umbilical cord tissue mercury was assessed and discussed. These studies showed a strong correlation between cord blood and cord tissue mercury, while the correlation between maternal hair and cord tissue mercury was not as strong. In addition, cord tissue mercury was almost as good a predictor of methyl mercury-associated neuropsychological deficits at 7 years of age as was cord blood mercury[89].

Infant exposure to methyl mercury and inorganic mercury via breast-feeding is low compared with late foetal exposure[90]. Based on the reported milk-to-plasma ratios of about 0.2 for methyl mercury and 0.6-1.0 for inorganic mercury, it seems that inorganic mercury is more easily transported to breast milk than is methyl mercury. The association between inorganic mercury in maternal blood and mercury in breast milk indicates transport of inorganic mercury into milk as well[90].

5. PREDICTORS AND IDENTIFICATION OF HIGH RISK POPULATIONS

There are two general types of susceptible subpopulations: (1) those that are **more sensitive to the effects of mercury** and (2) those that are **exposed to higher levels of mercury**, such as those that frequently consume locally caught fish from mercury-contaminated water bodies or long-lived predatory oceanic species, individuals with dental amalgams, and occupationally exposed workers (miners, workers in chlor-alkali plants, dentistry and industries for the production of batteries, thermometers and fluorescent lamps)[0].

A sensitive population is a group that may experience more severe adverse effects at comparable exposure levels or adverse effects at lower exposure levels than the general population. For mercury, the most sensitive subpopulations are developing organisms, particularly the foetus, because of the **sensitivity of the developing nervous system**[20]. Among mercury species, methyl mercury and metallic mercury reach the foetus during pregnancy by passing the placenta[91]. Trans-placental passage may occur by mimicry of essential compounds or by binding to such compounds for which a transport mechanism exists. Prenatal exposures to environmental chemicals interfere with the above described processes, adversely affecting the structural integrity of the nervous system, which results in altered functioning[92,93]. Exposure to methyl mercury and metallic mercury during early foetal development can cause sub-clinical brain dysfunction at doses much lower than those affecting adult brain functions. Although these chemicals might have caused impaired brain development in million of children worldwide, these latent impairments could not be unmasked until the natural neuronal attrition associated with ageing occured. Numerous studies have shown that Parkinson's disease and Alzheimer's disease are of purely genetic origin in only a minority of cases and appear in most instances to arise through interactions among genetic and environmental factors. Exposure of the developing brain to environmental toxic agents during windows of vulnerability in early life may be an important contribution to causation of these diseases[94].

The susceptibility of infants and children is further enhanced by their increased exposures, increased absorption rates and diminished ability to detoxify many exogenous compounds relative to those of adults[95]. Selective susceptibility may be due to differences in metabolizing enzymes, rates of excretion and binding affinity to target proteins. Partial lack of a blood-brain barrier in the foetus[91] is also of great importance, because it fails to prevent some selected molecules from entering the nervous system[93]. Small lypophilic particles (e.g. metallic mercury) can be easily passed transcellularly[93], while methyl mercury can enter the brain by conjugating with L-cysteine (methyl mercury-L-cysteine complex), and thus exploiting the L-methionine uptake pathway (mimicry) to traverse the protective blood-brain barrier[95]. A methyl mercury-glutathione complex was proposed, although it is expected that this complex be water soluble[66]. The blood-brain barrier itself can be subject to damage by mercury - it accumulates in endothelial cells and damages their membranes, leading to brain haemorrhage and oedema[93]. It was observed that high-dose exposure disrupts the blood-brain barrier.

Individuals with diseases of the liver, kidneys, nervous system and lungs are at higher risk of suffering from the toxic effects of mercury. Individuals with a dietary insufficiency of zinc, glutathione, antioxidants or selenium and those who are malnourished may be more sensitive to the toxic effects of mercury because of the diminished ability of these substances to protect against mercury toxicity. There is also limited population variability in regard to elimination of MeHg[0,66,].

The sensitivity of individuals is further influenced by alterations in chromosomal structure and **polymorphism of enzyme activities** involved in the metabolism of mercury[0]. Glutathione S-transferase is a gene family involved in the detoxification of electrophilic compounds by conjugation with glutathione, therefore enhancing the excretion of mercury via the bile or urine. In higher organisms at least five glutathione S-transferases gene classes have been well studied. It was shown that glutathione deficiency is associated with sensitivity to both mercury chloride and methyl mercury[0]. As was observed in two tribes living in the same area of the Amazonian region subjected to intensive gold prospecting, and with the same dietary habits based on fish, the GSTM1 gene could be involved in mercury metabolism or could be associated with reduced mercury levels: in the study an association between a high frequency of the GSTM1+ allele and lower mercury contamination was found[0].

Furthermore, the processes of cysteine and glutathione synthesis were shown to be reduced in autistic children[98]. Autistics have 20 % lower plasma levels of cysteine and 54 % lower levels of glutathione, which, among other things, adversely affect their ability to detoxify and excrete metals like mercury. Decreased capacity of mercury excretion in autistic children was supported by the finding that mercury levels in the hair of the first haircut of autistic children were about 8-fold lower than in normal children, although autistic children had been exposed to significantly higher mercury levels through maternal dental amalgams and thiomersal[98].

6. ASSOCIATIONS OF BIOMONITORING DATA AND ADVERSE EFFECTS

A number of adverse health effects associated with environmental exposure to mercury have been identified in humans and in animal studies. Most extensive are the data on neurotoxicity, particularly in developing organisms. At high exposures, mercury can cause cerebral palsy, seizures, microcephaly, mental retardation and also death. The most

severe effects were seen in Minamata and Niigata, Japan, where children were born with severe cerebral palsy in a population that consumed seafood contaminated with methyl mercury from industrial discharges[99]. Neurological disorders were also observed in populations exposed chronically to low doses of methyl mercury, especially in children of mothers whose diet included substantial quantities of fish and other seafood.

As most toxicological studies aim at deriving information that may be used to establish a virtually safe dose for regulatory purposes, the U.S. EPA derived a Reference Dose (RfD) in 2001[21] based on analysis of the health effects of methyl mercury by the National Research Council[66]. The RfD is the dose below which no unacceptable risk of adverse effect is expected[100]. It was evaluated based on three epidemiological studies suitable for quantitative analysis. These longitudinal prospective developmental studies were conducted in the Seychelles Islands, the Faroe Islands and New Zealand. 779, 900 and 237 mother-child pairs, respectively, were selected from fish-eating populations living in these Islands. Children were assessed at different ages on a number of standardized neuropsychological endpoints, which were compared with maternal hair mercury concentration or cord blood mercury concentration (Faroe Islands) as the biomarker of exposure. Investigators in the Seychelles Islands study reported no evidence of impairments related to prenatal methyl mercury exposure, whereas the other two studies found exposure-related effects in a number of neuropsychological endpoints[101]. In the assessment described by the NRC, emphasis was placed on the results of the Faroe Islands study, the larger of the two studies that identified methyl mercury -related developmental neurotoxicity.

To identify a biomarker concentration that is associated with a dose affecting a certain proportion of a selected population, the relationship between the **neuropsychological performance (endpoint)** and **exposure** has to be modelled. The NRC used a linear dose-effect relationship using five endpoints that had been reported as significantly affected by methyl mercury exposure[102]. The benchmark dose ($BMDL_{05}$) was established, which represents an estimate of the lower confidence limit (5^{th} percentile) of the dose that affects a small percentage of the population compared to the control group[103]. That methyl mercury concentration was than translated into the RfD. That determination requires the back-calculation of dose using a pharmacokinetic model. A one-compartment pharmacokinetic model was used to convert cord blood (or maternal hair) mercury to maternal intake. A one-compartment model is a significant simplification of the pharmacokinetics of methyl mercury in the maternal body and maternal-foetal unit, which

does not address the variance in the parameters (e.g. body weight, blood volume, hair-blood partition coefficients). Each of the model parameters is a variable whose possible values in a population can be described by a probability distribution. The ingested dose of methyl mercury corresponding to a measured biomarker concentration, therefore, should also be described by a probability distribution. The ingested dose used as the basis for the RfD should be at the lower range of doses that could result in a given biomarker concentration. In order to address inter-individual toxicokinetic variability in the derivation of the RfD, an uncertainty factor of the central-tendency estimate of the ingested dose was applied. The dose was divided by an uncertainty factor of 10, which was derived from probabilistic analysis of the distributions of the parameters of the one-compartment pharmacokinetic model[66,101].

Based on integrative analysis of all three studies and all endpoints, a $BMDL_{05}$ of 32 ng/g methyl mercury in maternal whole blood was calculated, giving an RfD of **0.1 µg/ kg body weight /day** as an exposure without recognized adverse effects[21]. Cord blood total mercury level equivalent to the the RfD is **5.8 ng/mL**. The corresponding maternal hair total mercury concentration would than be about 1 µg/g.

Reassessing each of the input parameters in the one-compartment model, taking into account cord blood-to-maternal blood ratio and reducing the uncertainty in the estimate of the central tendency of the maternal dose, Stern[104] has revised the U.S. EPA reference dose. The cord blood-to-maternal blood ratio had a significant influence on the estimate of a maternal dose: maternal intake dose corresponding to the fetal cord blood mercury concentration of 58 ng/mL was assessed to be half the value previously reported by the U.S. EPA.

Other levels of daily or weekly methyl mercury or mercury intakes estimated to be safe have also been established by several countries and international organizations based on the available information. All levels represent exposure that protects the most susceptible part of population. The estimates are given in Table 3.

The expected constant and linear relation between methyl mercury oral dose and body burden is used by government officials to establish guidelines on safe levels of methyl mercury exposure. However, the mercury level in hair, which is used in most epidemiological studies as the only indicator of human exposure, does not always reflect the reported level of methyl mercury intake via fish consumption. As reported by Canuel et al[73], the expected constant and linear relation between methyl mercury oral dose and

body burden seemed to vary among ethnic groups, most probably due to differences in specific genetic characteristics and/or interactive effects of other dietary components. The reported difference between expected and actual hair concentration was up to 14-fold[73]. Moreover, Budtz-Jørgensen et al[103] have demonstrated that imprecision in the exposure variable is likely to lead to underestimation of the exposure effect.

To summarize, all these studies lead us to the conclusion that the accurate assessment of exposure itself is crucial in risk assessment studies. For example, hair mercury can provide a good assessment of exposure to methyl mercury, but excretion of methyl mercury from blood to hair depends significantly on the metabolism of individuals. In this case blood methyl mercury is the preferred matrix of choice. Also, a considerable body of literature exists focusing on the effects of prenatal mercury exposure through fish consumption on neurodevelopment. Yet, findings have been inconsistent, particularly when assessing the effects of exposures to low mercury levels.

7. GEOGRAPHICAL VARIATIONS - IDENTIFICATION OF "HOT SPOTS"

Depending on the mercury source and how it was released into the environment, mercury may be present in concentrated "hot spots" or dispersed over extensive areas. “Hot spots” are defined as sites containing high mercury concentrations relative to the local levels in soils and sediments. Hot spots may result from a single well-delimited pollution source. The area concerned may be small – a few hundred square metres – but the potential consequences in terms of human exposure are significant for the local population. Hot spots may also result from several associated single-source releases. The area concerned is much more extensive than in the previous case, and the associated pollution impact can be regional with a radius exceeding the tens of kilometre scale. The contamination is more diffuse and may affect the whole population living in the area.

Altogether, more than 1200 sites have currently been identified[109] where mercury ore was mined and/or processed, 220 sites where mercury has been used in the chlor-alkali industry (both active and converted plant were taken into account), approx. 500 locations where precious metals (gold and silver) are processed in large-scale mining activities and more than 600 locations where non-ferrous metals ore is processed (including zinc, copper, lead and nickel). As Artisanal Small-scale Gold Mining (ASGM) activities are

conducted at hundreds of small sites they are considered as point sources, and cannot be individually identified. Estimates of mercury releases from ASGM activities based on country data were made in the report of Telmer and Veiga[110].

From a global perspective, most mercury contaminated sites identified (>70 %) are concentrated in industrial regions of Europe and North America that are adjacent to the Atlantic Ocean and Mediterranean Sea. In contrast to Europe and North America, the number and extent of mercury contaminated sites in other parts of the world (especially Asian countries and India) are increasing due to rising use of mercury in various products and processes.

The recent UNEP/WHO publication "Guidance for identifying populations at risk from mercury exposure"[0], summarizes the risks of mercury exposure to public health in general and provides guidelines for identification of populations at risk.

People living in contaminated sites are exposed to elevated levels of mercury, frequently exceeding safe levels. The release of mercury to the environment from mineral deposits enriched in mercury can impact humans and biota through direct and indirect pathways. Direct pathways include ingestion of tailings and soils contaminated with mercury and respiration of mercury vapour and enriched particles. Ingestion is primarily of concern for young children who may eat soil directly, or be exposed by a high level of hand-mouth activity. Indirect pathways that impact humans are more important and include consumption of fish and, more rarely, edible plants that have been contaminated with methyl mercury.

The factors that determine the occurrence and severity of adverse health effects include the chemical form of mercury, the dose, the age or developmental stage of the person exposed (the foetus is considered to be the most susceptible), the duration of exposure, and the route of exposure (inhalation, ingestion, and dermal contact). Dietary patterns can increase exposure, e.g. in a fish-eating population when fish and seafood are contaminated with mercury.

The primary targets of toxicity of mercury and mercury compounds are the nervous system, the kidneys, and the cardiovascular system. It is generally accepted that developing organ systems (such as the foetal nervous system) are the most sensitive to the toxic effects of mercury. At the same time, studies have indicated that levels of mercury in the foetal brain appear to be significantly higher than those in maternal blood. Other

systems that may be affected include the respiratory, gastrointestinal, haematological, immune and reproductive systems.

Although the toxic nature of mercury is well acknowledged, little is known of the effects of mercury on humans as a consequence of long-term exposure to low concentrations, which are often seen in contaminated sites. In many cases, the use of biomarkers, such as mercury concentrations in blood and urine, are not sufficient to assess the internal doses and potential effects on the central nervous system, kidney, the immune system, and other possible effects. Therefore, better scientific understanding of risks to human health, especially to those people living close to potentially polluted sites, is needed.

In addition, there are many important gaps in knowledge defining the benchmark dose for significant effects due to uncertainties associated with epidemiological studies carried out so far:

- Exposure assessment is imprecise. In the case of methyl mercury, calculation of the intake is complex because it is based on the conversion of biomarker data such as hair levels into daily intake.
- The effect of a single factor has been assumed (in this case a single form of mercury) in a situation where many covariates may affect the final impact. There are a large number of potential confounding factors in major epidemiological studies on methyl mercury, such as the source and pattern of methyl mercury exposure (or elemental mercury exposure), the nature of the populations, the influence of nutrition, and the presence of other pollutants such as PCBs, which make comparison of studies and interpretation of the data difficult.
- In numerous contaminated sites/regions, humans are simultaneously exposed to elemental mercury through inhalation, and to inorganic mercury and methyl mercury through food consumption. So far, very little is known of the effects of these combined exposures on adults and children. Further studies are needed to develop safe limits of exposure for the most vulnerable groups.

Most human health related studies in contaminated sites were carried out in mercury mining areas[111], artisanal gold mining sites (GEF-UNIDO project)[112,113] and chlor-alkali sites (Kazakhstan, Albania, Italy and Sweden)[114-116]. Unfortunately, these studies mainly addressed exposure. Risk characterization, however, is the integration of the hazard identification, dose-response assessment, and exposure assessments to describe the nature

and magnitude of the health risk. Risk characterisation of the relevant populations in the above mentioned studies have not been addressed adequately. This prevented the development and implementation of effective programmes to protect the populations.

Only limited information on exposure to mercury in contaminated sites is available. Exposure to methyl mercury has recently been addressed by numerous groups as a consequence of long-range transport, deposition and availability. A few studies implemented in contaminated sites indicate that direct exposure to elemental, inorganic and methyl mercury in contaminated sites significantly exceed that in background areas and contributes to the exposure of wildlife. It is documented that wild fish, mammals and birds that live on fish may be at risk from elevated dietary methyl mercury intake and toxicity. In controlled feeding studies, a diet that contained mercury (as methyl mercury) at environmentally realistic concentrations resulted in a range of toxic effects in fish, birds, and mammals, including behavioural, neurochemical, hormonal, and reproductive changes. A limited number of field-based studies support laboratory-based results demonstrating significant relations between methyl mercury exposure and various indicators of methyl mercury toxicity, including reproductive impairment[117].

Very limited information is available on the effects of mercury released from contaminated sites on candidate wildlife species. Limited (but useful) guidelines for ecosystem response to mercury contamination is available[118], but these are commonly not included and implemented in the monitoring strategies, particularly for existing contaminated sites. Further development of monitoring strategies is needed to improve our knowledge and prevent negative impacts.

REFRENCES

1 M. Horvat, N. Nolde, V. Fajon, V. Jereb, M. Logar, S. Lojen, R. Jaćimović, I. Falnoga, Q. Liya, J. Faganeli, D. Drobne. Total mercury, methylmercury and selenium in mercury polluted areas in the province Guizhou, China. *Sci. Total Environ.*, 2003, **304**, 231-256.

2 W.F. Fitzgereld and T.W.Clarkson, Mercury and monomethylmercury: present and future concerns, *Environ. Health Perspect*., 1991, **96**, 159-166.

3 T.W. Clarkson, The three modern faces of mercury, *Environ. Health Perspect.*, 2002, **110**, 11-22.

4 T.W. Clarkson, Health effects of metals: a rule of evolution, *Environ. Health Perspect.*, 1995, **96**, 159-166.

5 T. Barkay and I. Wagner-Döbler, Microbial transformation of mercury: potentials, challenges, and achievements in controlling mercury toxicity in the environment, *Advanced In Applied Microbiology*, 2005, **57**, 1-54.

6 N. Pirrone, M. Wichmann-Fiebig, R. Ahrens, J.M. Pacyna, A. Borowiak, P. Boffetta, J.C. Hansen, P. Grandjean, H.C. Ehrlich, G. Petersen and M. Horvat, Exposure to mercury in Europe. In: Ambient Air Pollution by Mercury (Hg). Position Paper. Luxembourg, Belgium 2002.

7 UNEP. Global Mercury Assessment. UNEP Chemicals Mercury Programme. (www.chem.unep.ch/mercury/report/Final_Assessment_report.htm). 2008.

8 US EPA a. Mercury study report to congress. Volume I: Executive Summary. EPA-452/R-97-007. U.S. Environmental Protection Agency, Office of Air Quality Planning and Office of Reaseach and Development. 1997. Washington, DC.

9 US EPA b. Mercury Study Report to Congress, Volume IV: Assessment of Exposure to Mercury in the United States. EPA-452/R-97-007. U.S. Environmental Protection Agency, Office of Air Quality Planning and Office of Reaseach and Development. 1997. Washington, DC.

10 US EPA. Fact Sheet. Update: National Listing of Fish and Wildlife Advisories. EPA 540-R-01-005. U.S. Environmental Protection Agency, Office of Water. 2003. Washington, DC.

11 UNEP. Global Mercury Assessment. UNEP Chemicals Mercury Programme. (www.chem.unep.ch/mercury/report/Final_Assessment_report.htm), 2002.

12 A. Miklavčič, V. Stibilj, E. Heath, T. Polak, J. Snoj Tratnik, J. Klavž, D. Mazej and M. Horvat, Mercury, selenium, PCBs and fatty acids in fresh and canned fish available on the Slovenian market, *Food Chem.*, 2010, **124**, 711-720.

13 J.B. Hursh, T.W. Clarkson and M.G. Cherian, Clearance of mercury (Hg-197, Hg-203) vapor inhaled by human subjects, *Arch. Environ. Health*, 1976, **31**, 302-309.

14 J. Sundberg and A. Oskarsson, Placental and lactational transfer of mercury from rats exposed to methylmercury in their diet: Speciation of mercury in the offspring, *J. Trace Elem. Exp. Med.*, 1992, **5**, 47-56.

15 Y.J. Sue, Mercury. In: L.R. Goldfrank, N.E. Flomenbaum, N.A. Lewin (Eds.) Toxicologic Emergencies, Fifth Edition, Norwalk, Connecticut. Appleton and Lange, 1992, 1051-1062.

16 N. Wright, W.B. Yeoman and G.F. Carter, Massive oral ingestion of elemental mercury without poisoning, *Lancet*, 1980, **1**, 8161-206.

17 J.K. Piotrowski, J.A. Szymanska, M. Skrzypinska-Gawrysiak et al., Intestinal absorption of inorganic mercury in rats, *Pharmacol. Toxicol.*, 1992, **70**, 53-55.

18 M.A. Morcillo and J. Santamaria, Whole-body retention, and urinary and fecal extraction of mercury after subchronic oral exposure to mercuric chloride in rats, *BioMetals*, 1995, **4**, 301-308.

19 T. Endo, S. Nakaya and R. Kimura, Mechanisms of absorption of inorganic mercury from rat small intestine: III. Comparative absorption studies of inorganic mercury compounds *in vitro*, *Pharmacol. Toxicol.*, 1990, **66**, 347-353.

20 ATSDR. Toxicological Profile for Mercury. 1999. Atlanta, Georgia, Agency for Toxic Substances and Disease Registry.

21 US EPA. Methylmercury (MeHg) (CASRN 22967-92-6). Reference Dose for Chronic Oral Exposure (RfD), (http://www.epa.gov/iris/subst/0073.htm). U.S. Environmental Protection Agency. 2001. Washington, DC.

22 A.I. Cabañero, Y. Madrid and C. Cámara, Mercury-selenium species ratios in representative fish samples and their bioaccessibility by an in vitro digestion method, *Biol. Trace Elem. Res.*, 2007, **119**, 195-211.

23 M.H.G. Berntssen, K. Hylland, A. Lundebye et al, Higher faecal excration and lower tissue accumulation of mercury in Wistar rats from contaminated fish than from methylmercury chloride added to fish, *Food Chem. Toxicol.*, 2004, **42**, 1359-1366.

24 M.G. Cherian, J.G. Hursh, T.W. Clarkson et al, Radioactive mercury distribution in biological fluids and excretion in human subjects after inhalation of mercury vapor, *Arch. Environ. Health,* 1978, **33**, 190-214.

25 A. Rothstein and A.L. Hayas, The turnover of mercury in rats exposed repeatedly to inhalation of vapour, *Health Phys.*, 1964, **10**, 1099-1113.

26 R. Eide and G.R. Wesenberg, Mercury contents of indicators and target organs in rats after long-term, low-level, mercury vapor exposure, *Environ. Res.*, 1993, **2**, 212-222.

27 L. Kosta, A.R. Byrne, V. Zelenko, Correlation between selenium and mercury in man following exposure to inorganic mercury, *Nature*, 1975, **254**, 238-239.

28 J.B. Nielsen and O. Andersen, Disposition and retention of mercuric chloride in mice after oral and parenteral administration, *J. Toxicol. Environ. Health.*, 1990, **68**, 167-180.

29 Y.M. Sin, Y.F. Lim and M.K. Wong, Uptake and distribution of mercury in mice from ingesting soluble and insoluble mercury compounds, *Bull. Environ. Contam. Toxicol*., 1983, **31**, 605-612.

30 C.C Bridges, K. Z. Rudolfs, Molecular and ionic mimicry and the transport of toxic metals, *Toxicol. Appl. Pharmacol.*, 2005, **204**, 274-308.

31 T.G. Kershaw, T.W. Clarkson and P.H. Dhahir, The relationship between blood levels and dose of methylmercury in man, *Arch. Environ. Health*, 1980, **56**, 565-575

32 M. Berglund, B. Lind, K.A. Björnberg B. Palm, Ö. Einarsson and M. Vahter, Inter-individual variations of human mercury exposure biomarkers: a cross-section assessment, *Environ. Health*, 2005, **20**, 1-11.

33 D.C. Rice, Blood mercury concentrations following methylmercury exposure in adult and infant monkeys, *Environ. Res.*, 1989, **49**, 115-126.

34 D.M. Ryan, Y.M. Sin, M.K. Wong, Uptake, distribution and immunotoxicological effects of mercury in mice, *Environ. Monit. Assess.*, 1991, **19**, 507-517.

35 F.F. Farris, R.L. Dedrick, P.V. Allen, J.C. Smith, Physiological model for the pharmacokinetics of methyl mercury in the growing rat, *Toxicol. Appl. Pharmacol*. 1993, **119**, 74-90.

36 E. Cernichiari, R. Brewere, G.J. Myers, D.O. Marsh, L.W. Lapham, C. Cox, C.F. Shamlaye, M. Berlin, P.W. Davidson and T.W. Clarkson, Monitoring methylmercury during pregnancy: maternal hair predicts fetal brain exposure, *Neurotoxicology*, 1995, **16**, 705-710.

37 J. Sundberg, B. Ersson, B. Lönnerdal, and A. Oskarsson, Johanna Sundberg, Bo Ersson, Bo, Agneta Oskarsson Protein binding of mercury in milk and plasma from

mice and man — a comparison between methylmercury and inorganic mercury, *Toxicology*, 1999, **137**, 169-184.

38 C.G. Elinder, L. Gerhardsson and Oberdorster, Biological monitoring of toxic metals-Overview. In: T.W. Clarkson, L. Friberg, G.F. Nordberg and P.R. Sager (Eds), Biological Monitoring of Toxic Metals, Plenum press. New York. 1988.

39 S. Halbach and T.W. Clarkson, Enzymatic oxidation of mercury vapor by erythrocytes, *Biochem. Biophys. Acta*, 1978, **523**, 522-531.

40 F. Nielsen-Kudsk, The influence of ethyl alcohol on the absorption of methylmercury vapor from the lungs of man, *Acta Pharmacol. Toxicol.*, 1965, **23**, 263-274.

41 T.W. Clarkson, Mercury, *J. Am. Coll. Toxicol*., 1989, **8,** 1291-1296.

42 L. Magos, S. Halbach and T.W. Clarkson, Role of catalase in the oxidation of mercury vapor, *Biochem. Pharmacol.*, 1978, **27**, 1373-1377.

43 J.B. Hursh, T.W. Clarkson, M.G. Cherian, Clearance of mercury (Hg-197, Hg-203) vapor inhaled by human subject, *Arch. Environ. Health*, 1976, **31**, 302-309.

44 T.W. Clarkson and A. Rothstein, The excretion of volatile mercury by rats injected with mercuric salts, *Health Phys.*, 1964, **10**, 1115-1121.

45 J.D. Dunn and T.W. Clarkson, Does mercury exalation signal demethylation of methylmercury? *Health Phys.*, 1980, **38**, 411-414.

46 L. Magos and W.H. Butler, Cumulative effects of methylmercury dicyandiamide given orally to rats, *Food Cosmet. Toxicol.*, 1972, **10**, 513-517.

47 WHO. Methyl mercury. Vol. 101. Word Health Organization, International Programe on Chemistry Safety. 1990. Geneva, Switzerland.

48 M.D. Turner, J.C. Smith, R.W. Kilpper et al, Absorption of natural methylmercury (MeHg) from fish, *Clin. Res.*, 1975, **23**, 2.

49 I. Nakamura, K. Hosokawa, H. Tamra et al, Reduced mercury extraction with faeces in germfree mice after oral administration of methylmercury chloride. *Bull. Environ. Contam. Toxicol.*, 1977, **17**, 528-533.

50 I. Rowland, M. Davies and J. Evans, Tissue content of mercury in rats given methylmercury chloride orally: Influence of intestinal flora, *Arch. Environ. Heath*, 1980, **35**, 155-160.

51 G. Sallsten, L. Barregard and A. Schutz, Clearance half life of mercury in urine after the cessation of long term occupational exposure: influence of a chelating agent (DMPS) on eycretion of mercury in urine, *Occup. Environ. Med.*, 1995, **51**, 337-342.

52 T. Suzuki, T. Hongo, N. Matsuo, H. Imai, M. Nakazawa, T. Abe, Y. Yakamura, M. Yoshida, H. Aoyama, An acute mercuric mercury poisoning: chemical spciation of hair mercury shows a peak of inorganic mercury value, *Hum. Exp. Toxicol.*, 1992, **11(1)**, 53-57.

53 T. Norseth and T.W. Clarkson, Intestinal transport of Hg-203 labeled metylmercury chloride: Role of biotransformation in rats, *Arch. Environ. Health*, 1971, **22**, 568-577.

54 N. Ishihara, Extraction of methylmercury in human feces., *Arch. Environ. Health*, 2000, **55**, 44-47.

55 J.B. Nielsen and O. Andersen, Methyl mercuric chloride toxicokinetics in mice: I. Effects of strain, sex, route of administration and dose, *Pharmacol. Toxicol.*, 1991, **68**, 201-207.

56 M. Leermarkers, W.F.J. Baeyens, P. Quevauviller, M. Horvat. Mercury in environmental samples: speciation, artifacts and validation. *TrAC, Trends Anal. Chem.*, 2005, **24,** 383-393.

57 M. Horvat and A.R. Byrne, Preliminary study of the effects of some physical parameters on the stability of methylmercury in biological samples, *Analyst*, 1992, **117**, 665-668.

58 J. Angerer, E. Ulrich, W. Michael, Human biomonitoring: state of the art. *Int. J. Hyg. Env. Health*, 2007, **210**, 201-228.

59 L. Magos, Selective Atomic-absorption determination of inorganic mercury and methylmercury in undigested biological samples, *Analyst,* 1971, **96**, 847-852.

60 M. Horvat. Determination of mercury and its compounds in water, sediment, soil and biological samples. V: Pirrone, Nicola (Ed.), Mahaffey, Kathryn R. (Ed.). Dynamics of Mercury Pollution on Regional and Global scales: Atmospheric Processes and Human Exposures Around the World. New York: Springer, 2005, 154-190.

61 UNEP and WHO. Guidance for identifying populations at risk from mercury exposure. United Nations Environment Programme and World Health Organization, 2008. Geneva, Switzerland.

62 L. Barregård, Biological monitoring of exposure to mercury vapour, *Scand. J. Work. Environ. Health,* 1993, **19**, 45-49.

63 S. Langworth, O. Almkvist, E. Söderman, B-O. Wikström, Effects of occupational exposure to mercury vapour on the central nervous system, *British J. Ind. Med.*, 1992, **47**, 545-555.

64 H.J. Mason, P. Hindell and N.R. Williams, Biological monitoring and exposure to mercury, *Occup. Med*., 2001, **51**, 2-11.

65 D.G. Ellingsen, R.I. Holland, Y. Thomassen M. Landro-Olstad, W. Frech, H. Kjuus, Mercury and selenium in workers previously exposed to mercury vapour at a chloralkali plant, *British J. Ind. Med.*, 1993, **50**, 745-752.

66 National Research Council (NRC). Toxicological Effects of Methylmercury. 2000. National Academy Press, Washington.

67 IPCS/WHO, 'Environmental Health Criteria 118', "Inorganic Mercury", WHO, Geneva, Switzerland, 1991.

68 L. Piikivi, U. Tolonen, EEG findings in chlor-alkali workers subjected to low long term exposure to mercury vapour, *British J. Ind. Med.*, 1989, **46**, 370-375.

69 I. Skare, T. Bergström, A. Engqvist, J.A. Weiner, Mercury exposure of different origins among dentists and dental nurses, *Scan. J. Work Environ. Health*, 1990, **16**, 340-347.

70 G. Sällsten, L. Barregård, A. Schütz, Decrease in mercury concentration in blood after long term exposure: a kinetic study of chloralkali workers, *British J. Ind. Med.*, 1993, **50**, 814-821.

71 I.M. Calder, G.R. Kelman, H. Mason, Diurnal variations in urinary mercury excretion, *Hum. & Exp. Toxicol.*, 1984, **3**, 463-467 (abstract).

72 M. Horvat, A. Prosenc, J. Smrke, D. Konda, A.R. Byrne, P. Stegnar and I. Begič, Trace elements in the hair, blood, cord blood and placenta of pregnant women from Central Slovenia. In: A. Aitio, A. Aro, J. Järvisalo, H. Vainio (Eds.), Trace Elements in Health and Disease. The Royal Society of Chemistry. 1988.

73 R. Canuel, S.B. de Grosbois, L. Atikesse, M. Lucotte, P. Arp, C. Ritchie, D. Mergler, H.M. Chan, M. Amyot and R. Anderson, New evidence on variations of human body burden of methylmercury from fish consumption, *Environ. Health Perspect.*, 2006, **114**, 302-306.

74 M. Vahter, A. Åkesson, B. Lind, U. Björs, A. Schütz and M. Berglund, Longitudinal study of methyl mercury and inorganic mercury in blood and urine of pregnant and lactating women, as well as in umbilical cord blood, *Environ. Research, Section A*, 2000, **84**, 186-194.

75 M.Horvat, P. Stegnar, A.R. Byrne and M. Dermelj, A study of trace elements in human placenta, blood and hair from the Yugoslav Central Adriatic. In: P. Brätter, P. Schramel (Eds.) Trace element analytical chemistry in medicine and biology, Proceedings of the Fifth International Workshop Neuherberg, Germany. Walter de Gruyter, Berlin, New York. 1988.

76 A. Lindberg, K. Ask Björnberg, M. Vahter, M. Berglund, Exposure to methylmercury in non-fish-eating people in Sweden, *Environ. Res.,* 2004, **96**, 28-33.

77 P. Grandjean, E. Budtz-Jørgensen, P.J. Jørgensen, P. Weihe, Umbilical cord blood concentration as biomarker of prenatal exposure to methylmercury, *Environ. Health Perspect.*, 2005, **113**, 905-908.

78 D. Gibičar, M. Horvat, S. Nakou, J. Sarafidou, J. Yager, Pilot study of intrauterine exposure to methylmercury in Eastern Aegean Islands, Greece, *Sci.Total Environ.*, 2006, **367**, 586-595.

79 E. Unuvar, H. Ahmadov A.R. Kiziler, B. Aydemir, S. Toprak, V. Ulker, C. Ark, Mercury levels in cord blood and meconium of healthy newborns and venous blood of their mothers: Clinical, prospective study, *Sc. Total Environ.*, 2007, **374**, 60-70.

80 A. Batáriová, V. Spěváčková, B. Beneš, M. Čejchanová, J. Šmíd, M. Černá, Blood and urine levels of Pb, Cd and Hg in the general population of the Czech Republic and proposed reference values, *Int. J. Hyg. Environ. Health*, 2006, **20/4**, 359-366.

81 German Environmental Survey 1998 (GerES III), http://www.umweltbundesamt.de/gesundheit-e/survey/us98/blut.htm.

82 K. Becker, M. Müssig-Zufika, A. Conrad, A. Lüdecke, C. Schulz, M. Seiwert, M. Kolossa-Gehring, German Environmental Survey for children 2003/06 (Ger ES IV).

Levels of selected substances in blood and urine of children in Germany. Federal Environmental Agency (UBA), Dessau-Roβlau, 2008.

83 Centers for Disease Control and Prevention (CDC), Fourth national report on human exposure to environmental chemicals, Department of Health and Human Services, Centers for Disease Control and Prevention (http://www.cdc.gov/exposurereport/), 2009.

84 H. Akagi, F.J.P. Branches, Y. Kinjo, Y. Kashima, J.R.D. Guimaraes, R.B. Oliveira, K. Haraguchi, W.C. Pfeiffer, Y. Takizawa and H. Kato, Human exposure to mercury due to goldmining in the Tapajos river basin, Amazon, Brazil: speciation of mercury in human hair, blood and urine, *Water, Air, and Soil Pollution*, 1995, **80**, 85-94.

85 K. Ask, A. Åkesson, M. Berglund and M. Vahter, Inorganic mercury and methylmercury in placentas of Swedish women, *Environ. Health Perspect.*, 2002, **110**, 523-526.

86 A.H. Stern and A.E. Smith, An assessment of the cord blood: maternal blood methyl mercury ratio: implications for risk assessment, *Environ. Health Perspect.,* 2003, **111**, 1465-1470.

87 K. Murata, M. Dakeishi, M. Shimada, H, Satoh, Assessment of intrauterine methyl mercury exposure affecting child development: messages from the newborn, *The Tohoku Journal Experim. Med.*, 2007, **213**, 187-202.

88 Greener Y, Kochen JA: In vitro studies on methyl mercury distribution in human blood. *Teratology,* 1983, **28,** 375-387.

89 P. Grandjean and P.J. Landrigan, Developmental neurotoxicity of industrial chemicals. *The Lancet*, 2006. Published Online 8-11-2006.

90 K.A. Björnberg, M. Vahter, M. Berglund, B. Niklasson, Blennow and G. Sandborgh-Englund, Transport of methyl mercury and inorganic mercury to the fetus and breast-fed infant, *Environ. Health Perspect.,* 2005, **113**, 1381-1385.

91 H.R. Andersen, J.B. Nielsen and P. Grandjean, Toxicological evidence of developmental neurotoxicity of environmental chemicals, *Toxicology*, 2000, **144**, 121-127.

92 P.M. Rodier, Developing brain as a target of toxicity, *Environ. Health Perspect.*, 1995, **103**, 73-76.

93 B. Blake, Toxicology of the nervous system. In: E. Hodgson (Ed). A Textbook of Modern Toxicology. 3rd ed., John Willey & Sons, Inc., New Jersey. 2004.

94 P.J. Landrigan, B. Sonawane, R.N. Butler, L. Trasande, R. Callan and D. Droller, Early environmental origins of neurodegenerative disease in later life, *Environ. Health Perspect.*, 2005, **113**, 1230-1233.

95 M. Aschner and J.L. Aschner, Mercury neurotoxicity: mechanisms of blood-brain barrier transport, *Neurosc. Biobehav. Rev.*, 1990, **14**, 169-176.

96 F. Gil and A. Pla, Biomarkers as biological indicators of xenobiotic exposure, *J. Appl. Toxicol.,* 2001, **21**, 245-255.

97 M.N. Klautau-Guimarães, R.D. Ascenção, F.A. Caldart, C.K. Grisolia, J.R. de Souza, A.C. Barbosa, C.M.T. Cordeiro and I. Ferrari, Analysis of genetic susceptibility to mercury contamination evaluated through molecular biomarkers in at-risk Amazon Ameridian populations, *Gen. Molecul. Biol.*, 2005, **28**, 827-832.

98 J. Mutter, J. Naumann, R. Schneider, H. Walach and B. Haley, Mercury and autism: accelerating evidence? *Neuroendocrinol. Lett.*, 2005, **26**, 439-446.

99 K.S. Crump, C.V. Landingham, C. Shamlaye, C. Cox, P.W. Davidson, G.J. Myers and T.W. Clarkson, Benchmark concentrations for methyl mercury obtained from the Seychelles child development study, *Environ. Health Perspect.*, 2000, **108**, 257-263.

100 W. Krewitt, D.W. Pennington, S.I. Olsen, P. Crettaz and O. Jolliet, Indicators for human toxicity in Life Cycle Impact Assessment. Position paper for SETAC-Europe, WAI2 Task Group on Human toxicity. 2002.

101 D.C. Rice, The US EPA reference dose for methyl mercury: sources of uncertainty, *Environ. Res.*, 2004, **95**, 406-413.

102 US EPA. Use of the Benchmark Dose Approach in Health Risk Assessment. EPA/630/R-94/007. U.S. Environmental Protection Agency, Risk Assessment Forum. 1995. Washington, DC.

103 E. Budtz-Jørgensen, N. Keiding, P. Grandjean. Effects of exposure imprecision on estimation of the benchmark dose. *Risk Anal.,* 2004, **24(6),** 1689-96.

104 A. Stern, A revised probabilistic estimate of the maternal methyl mercury.intake dose corresponding to a measured cord blood mercury concentration, *Environ. Health Perspect.,* 2005, **113**, 155-163.

105 WHO, Environmental Health Criteria 70, Principles of the safety assessment of food additives and contaminants in food (www.inchem.org/documents/ehc/ehc/ehc70.htm), 1987.

106 WHO, WHO Food Additive Series 52, Safety evaluation of certain food additives and contaminants. International Programme on Chemical Safety (IPCS), (whqlibdoc.who.int/publications/2004/9241166052X.pdf), 2004.

107 WHO, Evaluation of certain food additives and contaminants (Sixty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 940 (whqlibdoc.who.int/trs/WHO_TRS_940_eng.pdf), 2007.

108 US EPA, Integrated Risk Information System (IRIS) for Mercury, Elemental (CASRN 7439-97-6), Washington, DC: National Centre for Environmental Assessment, Office of Research and Development (www.epa.gov/iris/subst/0370.htm), 1995.

109 D. Kocman, M. Horvat, N. Pirrone, S. Cinnirella, Contribution of contaminated sites to the global mercury budget, *Global Biogeochem. Cycl.*, 2011, *in press*.

110 K.H. Telmer, and M.M. Veiga, World emissions of mercury from artisanal and small scale gold mining, Springer, New York, 131–172, 2009.

111 A.B. Kobal, M. Horvat, M. Prezelj, A. Sešek-Briški, M. Krsnik, T. Dizdarevič, D. Mazej, I. Falnoga, V. Stibilj, N. Arnerić, D. Kobal Grum, J. Osredkar. The impact of long-term past exposure to elemental mercury on antioxidative capacity and lipid peroxidation in mercury miners. *J. Trace Elem. Med. Biol.*, 17, 2004, 261-274.

112 S. Bose-O'Reilly, B. Lettmeier, R. Matteucci Gothe, C. Beinhoff, U. Siebert, G. Drasch**,** Mercury as a serious health hazard for children in gold mining areas. *Environ. Res.* 2008, **107**, 89-97.

113 J.R. Ikingura and H. Akagi, Monitoring of fish and human exposure to mercury due to gold mining in the Lake Victoria goldfields, Tanzania, *Sci. Tot. Environ.*, 1996, **191**, 59-68.

114 D. Gibičar, M. Horvat, M. Logar, V. Fajon, I. Falnoga, Ingrid, R. Ferrara,E. Lanzillotta, C. Ceccarini, B. Mazzolai, B. Denby, J. Pacyna. Human exposure to mercury in the vicinity of chlor-alkali plant. *Environ. Res.*, 2009, **109**, 355-367

115 D. Jarosińska, M. Horvat, G. Sällsten, B. Mazzolai, B. Dąbkowska, A Prokopowicz, M. Biesiada, L. Barregård, Lars. Urinary mercury and biomarkers of early renal dysfunction in environmentally and occupationally exposed adults: a three-country study, *Environ. Res.*, 2008, **108**, 224-232.

116 L. Barregård, M. Horvat, B. Mazzola, G. Sällsten, D. Gibičar, V. Fajon, S. Di Bona, J. Munthe, I. Wängberg, H. Eugensson. Urinary mercury in people living near point sources of mercury emissions. *Sci. Total Environ.*, 2006, **368**, 326-334.

117 D. Mergler, H. Anderson, L. Man Chan, K. Mahaffey, M. Murray, M. Sakamoto, A. Stern Methylmercury Exposure and Health Effects in Humans: a Worldwide Concern*, AMBIO,* 2006*.*

118 R. Harris, M. W. Murray, T. Saltman, R. Mason Ecosystem Responses to Mercury Contamination: Indicators of Change. *Soc. Environ. Toxicol. Chem.*, 2007.

4. Conclusions

1. Based on our results and the current JECFA PTWIs for MeHg, it can be concluded that the levels of MeHg in fish available on the Slovenian market consumed by Slovenian pregnant women do not represent a health risk to the woman and their susceptible developing foetuses. Our study also confirmed that fish lower in the food chain contain relatively low levels of MeHg. The concentrations of THg in cord blood (median (Med)=1.5 ng/g), hair (Med=297 ng/g) and breast milk (Med=0.2 ng/g) in Slovenian women are low, due to the low frequency of fish consumption and probable prevalent consumption of farmed fish with lower levels of THg and MeHg compared to wild fisheries (Miklavčič et al., 2011a,b).

2. The calculated PDI based on the frequency of fish consumption and MeHg levels in the most frequently consumed fish (0.023 µg/day kg) (Miklavčič et al., 2011b) was comparable with the PDI calculated from THg levels in hair (0.029 µg/day kg). However, when we included only THg levels determined in fish from supermarkets that were probably mostly aquacultured and excluded the levels of MeHg in tuna, the PDI intake was round 4 times lower (0.0054 µg/day kg), indicating that MeHg levels in tuna fish comprised the majority of the PDI.

3. Compared to pregnant women from Italy, Croatia and Greece, pregnant women mainly from central Slovenia have the lowest MeHg exposure levels. The highest levels of Hg were found in cord blood and breast milk from Greek women. The differences in Hg exposure between the countries were probably due to the different amounts of fish consumption and the consumption of different species of fish of different origin (Miklavčič et al., 2013a).

4. The percentages of MeHg in biological samples did not differ between the four countries, with the exception of the percentages of MeHg in breast milk. The lower percentages of MeHg in breast milk from Greece could be due to different sources of Hg exposure, or might be a consequence of a more efficient metabolism of MeHg elimination in women from Greece (Miklavčič et al., 2013b).

5. It was confirmed that fish available on the Slovenian market represent an important source of Se and n-3 fatty acids. The concentrations of n-3 fatty acids in fish lower on the food chain tend to be higher compared to the levels of n-3 fatty acids in fish at the top of the food chain (Miklavčič et al., 2011a).

6. Considering the PCB tolerance limit recommended by the FDA, we conclude that edible fish from the Slovenian market pose a minimal health risk for humans (Miklavčič et al., 2011a).

7. Fish consumption should not be discouraged, but it should be advised that the most vulnerable population such as pregnant women consume fish low in MeHg. Furthermore, regular monitoring of Hg levels in fish, particularly fresh fish, should be performed and the results communicated to the public, together with trustworthy advice for various population groups on the safety of fish consumption.

8. At low levels of exposure a number of factors can influence the strength of the correlation between biomarkers of exposure and intake of MeHg through fish consumption. The important factors are the accuracy of the data contained in the questionnaire, the high variability of MeHg in fish and the presence of inorganic Hg from sources other than fish (Miklavčič et al., 2011b).

9. Inter-individual differences might play an important role, especially at these low Hg exposure levels. In terms of science-based questions related to the safety of fish consumption for the most susceptible groups of the population, more studies are needed to better understand the role of genetic factors influencing the use of different biomarkers of MeHg exposure through fish consumtion (Miklavčič et al., 2011b).

10. THg in hair and THg or MeHg in cord blood are suitable biomarkers of Hg exposure from fish consumption. Moreover, for large epidemiological studies the THg level in hair is the most appropriate biomarker for exposure assessment, because of its simple and practical collection, ease of storage of the samples, and the relatively simple, fast and precise analytical determination of THg in hair (Miklavčič et al., 2011b).

11. Significant linear correlations were found between logarithmic transformation (ln) of THg levels and ln Se levels, ln As and ln Se levels, and ln As and ln THg levels in cord blood, mother's blood and breast milk. In addition, significant correlations were found between the frequency of fish consumption and different biomarkers of exposure. Fish consumption, the possible common source of As, Hg and Se, could explain these correlations between the elements determined in cord blood, mother's blood or breast milk (Miklavčič et al., 2013a).

12. The highest levels of As were found in cord blood and breast milk from Greek women, while the highest Se levels were found in cord blood from Italy. The differences in As exposure between the countries were probably due to the different amounts of fish consumption and the consumption of different species of fish of different origin. Since speciation analyses of As were not included in this study, it is still not clear whether As levels came mainly from fish or in part also from some sources other than fish, which could also influence the differences in As levels between countries. Because Se levels in different food items also depend on the origin of

food, the highest levels of Se in Italy are probably the consequence of more frequent consumption of various food items in Italy that have higher Se levels (Miklavčič et al., 2013a).

13. The levels of As found were far below the threshold levels for acute or chronic toxicity even assuming it was all inorganic As. However, it is still unknown if such low long-term exposure levels could affect the most susceptible population (Miklavčič et al., 2013a).

14. Levels of Hg in food from the Idrija mine area were elevated compared to non-contaminated sites. Moreover, the levels of Hg in local food have not diminished significantly over the past 15 years since the closure of the Hg mine. Importantly, our study confirmed that Hg is accumulated in mushrooms and chicory (*Cichorium intybus*), and these high levels should not be overlooked, because chicory in particular represents a seasonal vegetable eaten daily. In addition, higher levels of MeHg in some vegetables were found compared to those previously found (Miklavčič et al., 2013b). However, the exposure levels assessed through biomarkers of exposure in this area during pregnancy were on the level of the population that live sin uncontaminated area (see the Appendix).

15. Overall, next step is to better understand the bioavailability of different Hg species and the role of genetic factors influencing the use of biomarkers of Hg exposure (Miklavčič et al., 2011a, 2011b, 2013a). A further monitoring of vegetables from the Idrija mine area should be conducted and additional studies made to confirm these results and to assure the safety of local vegetable consumption (Miklavčič et al., 2013b).

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No one can whistle a symphony. It takes a whole orchestra to play it. —H.E. Luccock

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6. References

Abernathy, C.O.; Thomas, D.J.; Calderon, R.L. Health effects and risk assessment of arsenic. *J Nutr* 133, 1536S-38S (2003).

Akesson, I.; Schutz, A.; Attewel, R.; Skerfving, S.; Glantz, P.O. Status of mercury and selenium in dental personnel: Impact of amalgam work and fillings. *Arch Environ Health* **46**, 102-109 (1991).

Ask, K.; Åkesson, A.; Berglund, M.; Vahter, M. Inorganic mercury and methylmercury in placentas of Swedish women. *Environ Health Perspect* **110**, 523-526 (2002).

ATSDR Toxicological Profile for Mercury (update). Agency for Toxic Substances and Disease Registry, Atlanta, Georgia, 1999, 1-485 (1999).

ASTDR Toxicological Profile for Arsenic, GA: Agency for Toxic Substances and Disease Registry (2007). http://www.atsdr.cdc.gov/toxprofiles/tp2.pdf

Ball, L.K.; Ball, R.; Pratt, RD. An assessment of thiomerosal use in childhood vaccones. *Pediatrics* **107**, 1147-1154 (2001).

Bakir, F.; Damluji, S.F.; Amin-Zaki, L.; Murtadha, M.; Khalidi A.; al-Rawi, N.Y.; Tikriti, S.; Dhahir, H.I.; Clarkson, T.W.; Smith, J.C. et al. Methylmercury poisoning in Iraq. *Science* **181**, 230-241 (1973).

Ballatori, N.; Clarkson, T. W. Biliary secretion of glutathione and of glutathione-metal complexes. *Fundamental and Applied Toxicology* **5**, 816-831 (1985).

Barbone, F.; Valent, F; Pisa, F; Horvat, M; Daris, F. Maternal fish consumption, markers of mercury exposure and neurodevelopment in a cohort of Italian newborns. *Epidemiology* **5**, 14 (2003).

Barbone, F; Valent, F; Pisa, F; Daris, F.; Fajon, V.; Gibičar, D.; Logar, M.; Horvat, M. Prenatal low-level methyl mercury exposure and child development in an Italian coastal area. *Seychel Med Dent J* **7**, 149–154 (2004).

Barcelos, G. R. M.; de Marco, K.C.; Grotto, D.; Valentini, J.; Garcia, S.C.; Braga, G.Ú. L.; Barbosa J.F. Evaluation of glutathione S-transferase GSTM1 and GSTT1 polymorphisms and methylmercury metabolism in an exposed amazon population. *J Toxicol Environ Health Part A* **75**, 960-970 (2012).

Barkay, T.; Wagner-Döbler, I.; 2005. Microbilal transformation of mercury: potentials, challenges, and achivements in controlling mercury toxicity in the environment. *Advan Appl Microbiol* **57**, 1-54 (2005).

Barregård, L. Biological monitoring of exposure to mercury vapour. *Scand J Work Environ Health* **19**, 45-49 (1993).

Barregård, L. Exposure to mercury in the general population of Europe and the Arctic. In: Pirrone, N.; Mahaffey, K.R. (eds) *Dynamics of mercury pollution on regional and global scales.* (386-403 Springer Science, New York, 2005).

Barregård, L.; Horvat, M.; Mazzolai, B.; Sällsten, G.; Gibičar, D.; Fajon, V.; diBona, S.; Munthe, J.; Wängberg, I.; Eugensson, M.H. Urinary mercury in people living near point sources of mercury emission. *Sci Tot Environ* **368,** 326-334 (2006).

Barregård, L.; Horvat, M.; Schutz, A. No Indication of in vivo methylation of inorganic mercury in chloralkali workers. *Environ Res* **67(**2), 160-167 (1994).

Barregård, L.; Rekić, D.; Horvat, M.; Elmberg, L.; Lundh, T.; Zachrisson, O. Toxicokinetics of mercury after long-term repeated exposure to thimerosal-containing vaccine. *Toxicol Scien* **120**, 499-506 (2011).

Beck, M.A.; Levandert, O.A.; Handy J. Selenium deficiency and viral infection. *Journal of Nutrition* **133**(5), 1463S-1467S (2003).

Beijer, K.; Jernelov, A. Ecological aspects of mercury-selenium interactions in the marine environment. *Environ Health Perspect* **25**, 43-45(1987).

Berglund, M.; Lind, B.; Björnberg, K.A.; Palm, B.; Einarsson, Ö.; Vahter, M. Inter-individual variations of human mercury exposure biomarkers: a cross-section assessment, *Environ Health* **20**, 1-11 (2005).

Berlin, M.; Blomstrand, C.; Grant, C. A.; Hamberger, A.; Trofast, J. Tritiated methylmercury in the brain of squirrel monkeys. *Archives of environmental health* **30**(12), 591(1975).

Berntssen, M.H.G.; Hylland, K.; Lundebye, A.; Julshamn, K. Higher faecal excretion and lower tissue accumulation of mercury in Wistar rats from contaminated fish than from methylmercury chloride added to fish. *Food and Chemical Toxicology* **42**, 1359-1366 (2004).

Bird, G.M. Biomonitoring. In: Greim H, Snyder R. (eds.) *Toxicology and risk assessmant: a comprehensive introduction.* 418-426 (Wiley, West Sussex, 2008).

Björkman, L; Lundekvam, B.F.; Lægreid, T.; Bertelsen, B.I.; Morild, I.; Lilleng, P.; Lind, B.; Palm, B.; Vahter, M. Mercury in human brain, blood, muscle and toenails in relation to exposure: an autopsy study *Environ Health* **6**, 1-14 (2007).

Björnberg, A.; Vahter, M.; Berglund. M.; Niklasson, B.; Blennow, M.; Sandborgh-Englund, G. Transport of methyl mercury and inorganic mercury to the fetus and breast-fed infant. *Environ Health Perspect* **113**, 1381-1385 (2005).

Boffetta, P.; Garcia-Gómez, M.; Pompe-Kirn, V.; Zaridze, D.; Bellander, T.; Bulbulyan, M.; Caballero, J.D.; Ceccarelli, F.; Colin, D.; Dizdarevic, T.; Español, S.; Kobal, A.; Petrova, N.; Sällsten, G.; Merler, E. Cancer occurrence among European mercury miners. *Cancer Causes and Control* **9**(6), 591-599 (1998).

Boffetta, P.; Sällsten, G.; Garcia-Gomez, M.; Pompe-Kirn, V.; Zaridze, D.; Bulbulyan, M.; Caballero, J.D.; Ceccarelli, F.; Kobal, A.B.; Merler, E. Mortality from cardiovascular diseases and exposure to inorganic mercury. *Occupational and environmental medicine* **58**, 461-466 (2001).

Bridges, C.C.; Zalups, R.K. Molecular and ionic mimicry and the transport of toxic metals, *Toxicol Appl Pharmacol* **204**, 274-308 (2005).

Bridges, C.C.; Zalups, R.K. Transport of inorganic mercury and methylmercury in target tissues and organs. *J Toxicol Environ Health*, **13**, 385-410 (2010).

Brown, T.A.; Shrift, A. Selenium: toxicity and tolerance in higher plants. *Biol Rev* **57**, 59–84 (1982).

Burk, R.F. Selenium, an antioxidant nutrient. *Nutrition in clinical Care* **5**(2), 75-79 (2008).

Byrne, A. R.; Škreblin, M.; Falnoga, I.; Al-Sabti, K.; Stegnar, P.; Horvat, M. Mercury and selenium: perspectives from Idrija. *Acta chim slov* 42, 175-198 (1995).

Cabañero, A.I.; Carvalho, C.; Madrid, Y.; Batoréu, C.; Cámara, C. Quantification and speciation of mercury and selenium in fish samples of high consumption in Spain and Portugal. *Biological Trace Element Research* 103, 17-34 (2005).

Cabañero, A.I.; Madrid, Y.; Cámara, C. Mercury-selenium species ratios in representative fish samples and their bioaccessibility by an in vitro digestion method. *Biol Trace Elem Res* **119**, 195-211 (2007).

Cace, I.B.; Milardovic, A.; Prpic, I.; Krajina, R.; Petrovic, O.; Vukelic, P.; Spiric, Z.; Horvat, M.; Mazej, D.; Snoj, J. Relationship between the prenatal exposure to low-level of mercury and the size of a newborn's cerebellu. *Medical Hypotheses*, **76**(4), 514-516 (2011).

Chapman, L.; Chan, H.M. The influence of nutrition on methyl mercury intoxication. *Environ Health Perspect* **108**, 29-56.

Chen, J.; Berry, M.J. 2003. Selenium and selenoproteins in the brain and brain diseases. *J Neurichem* **86**(1), 1-12 (2004).

Chen, C.; Yu, H.; Zhao, J.; Li, B.; Qu, L.; Liu, S.; Zhang, P.; Chai, Z. The roles of serum selenium and selenoproteins on mercury toxicity in environmental and occupational exposure. *Environmental health perspectives*, **114**(2), 297 (2006).

Cherian, M.G.; Hursh, J.G.; Clarkson, T.W.; Allen, J. Radioactive mercury distribution in biological fluids and excretion in human subjects after inhalation of mercury vapor. *Arch Environ Health* **33**, 190-214 (1978).

Cheuk, D.K.; Wong, V. Attention-deficit hyperactivity disorder and blood mercury level: a case control study in Chinase children. *Neuropediatrics* **37**(4), 234-240 (2006).

Choi, A.L.; Budtz-Jørgensen, E.; Jørgensen, P.J.; Steuerwald, U.; Debes, F.; Weihe, P.; Grandjean, P. Selenium as a potential protective factor against mercury developmental neurotoxicity. *Environmental Research* **107**, 45-52 (2008).

Clandinin, M.T.; Chappell, J.E.; Leong, S.; Heim, T.; Swyer, P.R.; Chance, G.W. Intrauterine fatty acid accretion rates in human brain: implications for fatty acid requirements. *Early human development*, **4**(2), 121-129 (1980).

Clark, C.; Combs, G.F.; Turnbull, B.W.; Slate, E.H.; Chalker, D.K.; Chow, J.; Davis, L.S.; Glover, R.A.; Graham, G.F.; Gross, E.G.; Krongard, A.; Lesher, J.L.; Park, H.K.; Sanders, B.B.; Smith, C.L.; Taylor, J.R. Effects of selenium supplementation for cancer prevention in patients with carcinoma of the skin: a randomized controlled trial, *JAMA* **276**(24), 1957-1963 (1996).

Clarkson, T.W. Mercury. *J Am Coll Toxicol* **8**(7), 1291-1296 (1989).

Clarkson, T.W. The three modern faces of mercury. *Environmental Health Perspectives*, 110, 11-22 (2002).

Clarkson, T.W.; Magos, L.; Myers, G.J. The toxicology of mercury-current exposure and clinical manifestations. *N Engl J Med* **349**, 1731-1737 (2003).

Cox, C.; Clarkson, T.W.; Marsh, D.O.; Amin-Zaki, L.; Al-Tikriti, S. Dose-response analysis of infants prenatally exposed to methylmercury poisoning: relationship between concentration in single strands of maternal hair and child effects. *Environ Res* **49**, 318-332 (1987).

Cvetković, J.; Toman, M.J.; Horvat, M. Vsebnosti anorganskega in metiliranega živega srebra v nekaterih organih izbranih vrst rib obremenjenega okolja rek Idrijce in Soče. In: Horvat M, Sakamoto M, Faganeli J. (eds.) *International Workshop Mercury in Contaminated Sites: Characterization, Impacts and Remediation*, Marine Biology Station, Piran, Slovenia, October 2010. Ljubljana: Jožef Stefan Institute, Department of Environmental Sciences, Ljubljana, 1-14 (2010).

Davidson, P.W.; Myers, G.J.; Cox, C.; Axtell, C.; Shamlaye, C.; Sloane-Reeves, J.; Cernichiari, E.; Needham, L.; Choi, A.; Wang, Y.; Berlin, M.; Clarkson, T.W. Effects of prenatal and postnatal methylmercury exposure from fish consumption and neurodevelopment. *JAMA* **280**(8), 701-707 (1998).

Davidson, P.W.; Strain, J.J.; Myers, G.J.; Thurston, S.W.; Bonham, M.P.; Shamlaye, C.F.; Stokes-Riner, A.; Wallace, J.M.W.; Robson, P.J.; Duffy, E.M.; Georger, L.A.; Sloane-Reeves, J.; Cernichiari, E.; Canfield, R.L.; Cox, C.; Huang, L.S.; Janciuras, J.; Clarkson, T.W., 2008. Neurodevelopmental effects of maternal nutritional status and

exposure to methylmercury from eating fish during pregnancy, *Neurotoxicology* **29**(5) 767-775 (2008).

Debes, F.; Budtz-Jorgensen, E.; Weihe, P.; White, R.F.; Grandjean, P. Impact of prenatal methylmercury exposure on neurobehavioral functions at age of 14 years. *Neurotoxicol Teratol* **28**, 536-547 (2006).

Decarli, A.; Franceschi, S.; Ferraroni, M.; Gnagnarella, P.; Parpinel, M.T.; La Vecchia, C.; Negri, E.; Salvini, S.; Falcini, F.; Giacosa, A. Validation of a food-frequency questionnaire to assess dietary intakes in cancer studies in Italy. Results for specific nutrients. *Ann Epidemiol* 6, 110-118 (1996).

Dencker, L.; Danielsson, B.; Khayat, A.; Lindgren, A. (1983). Deposition of metal in the embryo and fetus. In: Clarkson, T.W.; Nordberg, G.F.; Sager, P.R. (eds.) *Reproductive and Developmental Toxicity of Metals* 607–631 *(*Plenum Publishers, New York, 1983).

Dórea, J.G.; Bezerra, V.L.V.A.; Fajon, V.; Horvat, M. Speciation of methyl- and ethyl-mercury in hair of breastfed infants acutely exposed to thimerosal-containing vaccines. *Clinica Chimica Acta* 412, 1563-1566 (2011).

Duffield, A.J.; Thomson, C.D.; Hill, K.E.; Williams, S. An estimation of selenium requirements for New Zealanders. *The American Journal of Clinical Nutrition*, **70**(5), 896-903 (1999).

Dunn, J.D.; Clarkson, T.W. Does mercury exalation signal demethylation of methylmercury? *Health Phys* **38**, 411-414 (1980).

Dyrssen, D.; Wedborg, M. The sulphur-mercury (II) system in natural waters. *Water Air Soil Pollut*, **56**, 507-519 (1991).

EFSA. Opinion of the scientific penal on contaminants in the food chain on a reques from the Commission related to mercury and methylmercury in food. *The EFSA Journal* **34**, 1-14 (2004).

EFSA. Panel on Contaminants in Food Chain. Scientific Opinion on the risk for public health related to the presence of mercury and methylmercury in food. *EFSA Journal* **10**(12), 2985 (2012).

Eide, R.; Wesenberg, G.R. Mercury contents of indicators and target organs in rats after lon-term, low-level, mercury vapor exposure. *Environ Res* 212-222 (1993).

Elinder, C.G.; Gerhardsson, L.; Oberdorster. Biological monitoring of toxic metals-Overview. In: Clarkson, T.W.; Friberg, L.; Nordberg, G.F.; Sager, P.R. *Biological Monitoring of Toxic Metals* 1-71 (Plenum press, New York, 1988).

Endo, T.; Nakaya, S.; Kimura, R. Mechanisms of absorption of inorganic mercury from rat small intestine: III. Comparative absorption studies of inorganic mercury compounds *in vitro*. *Pharmacol Toxicol* 66(5):347-353 (1990).

EPA. Integrated Risk Information System (IRIS) for Mercury, Elemental (CASRN 7439-97-6). Washington, DC: National Center for Environmental Assessment, Office of

Research and Development. Available at: www.epa.gov/iris/subst/0370.htm (1995a).

EPA. Integrated Risk Information System (IRIS) for Mercury Chloride (CASRN 7487-94-7). Washington, DC: National Center for Environmental Assessment, Office of Research and Development. Available at: www.epa.gov/iris/subst/0692.htm (1995b).

EPA. Mercury Study Report to Congress. Volume I: Executive Summary. EPA-452/R-97-007. U.S. Environmental Protection Agency, Office of Air Quality Planning and Office of Reaseach and Development.Washington, DC (1997a).

EPA. Mercury Study Report to Congress, Volume IV: Assessment of Exposure to Mercury in the United States. EPA-452/R-97-007. U.S. Environmental Protection Agency, Office of Air Quality Planning and Office of Reaseach and Development. Washington, DC (1997b).

EPA. Mercury Study Report to Congress, Volume V: Health Effects of Mercury and Mercury Compounds. Washington, DC. Office of Air Quality Planning and Office of Reaseach and Development. EPA-452/R-97-007 (1997c).

EPA. Integrated Risk Information System (IRIS) for Methylmercury (CASRN 222967-92-6). Washington, DC: National Center for Environmental Assessment, Office of Reasech and Development. Available at: www.epa.gov/iris/subst/0073.htm (2001).

EPA. Fact Sheet. Update: National Listing of Fish and Wildlife Advisories. EPA 540-R-01-005. U.S. Environmental Protection Agency, Office of Water. Washington, DC (2003a)

EPA 2003b. Mercury in Marine Life Database. Washington DC: US Environmental Protection Agency, Office of Werlands, Oceans, and Watersheds (2003b).

EU Seafood Industry report. Glitnir Seafood Research (2008)
http://skjol.islandsbanki.is/servlet/file/store156/item49487/20080418_Seafood_EU.pdf

EROSTAT. Fishery statistics (2010)
http://epp.eurostat.ec.europa.eu/statistics_explained/index.php/Fishery_statistics

Falnoga, I.; Jereb, V.; Smrkolj, P. Hg and Se in foodstuffs grown near a Hg mining area. *J Phys* **107**, 447-450 (2003).

Falnoga, I., Kobal, A. B., Stibilj, V., Horvat, M. Selenoprotein P in subjects exposed to mercury and other stress situations such as physical load or metal chelation treatment. *Biological Trace Element Research*, **89**(1), 25-33 (2002).

Falnoga, I.; Tušek-Žnidarič, M. Selenium–mercury interactions in man and animals. *Biological trace element research* **119**(3), 212-220 (2007).

Falnoga, I.; Tusek-Znidaric, M.; Horvat, M.; Stegnar, P. Mercury, selenium, and cadmium in human autopsy samples from Idrija residents and mercury mine workers. *Environmental research* **84**(3), 211-218 (2008).

FAO Fisheries Circular No. 972/4, Part 1: Future prospects for fish and fishery products.

Fish consumption in the European Union in 2015 and 2030 Part 1. European overview (2007).

FDA (U.S. Food and Drug Administration). Mercury Concentrations in Fish: FDA Monitoring Program (1990-2004) (2006).
http//www.cfsan.fda.gov

Fitzgereld, W.F.; Clarkson, T.W. Mercury and monomethylmercury: present and future concerns. *Environ Health Perspect* **96**, 159-166 (1991).

Flora, Swaran JS, Smrati Bhadauria, Satish C. Pant, and Ram K. Dhaked. "Arsenic induced blood and brain oxidative stress and its response to some thiol chelators in rats." *Life sciences (New York, NY: 1973)* 77, no. 18 (2005): 2324-2337

Franceschi, S.; Barbone, F.; Negri, E.; Decarli, A.; Ferraroni, M.; Filiberti, R.; Giacosa, A.; Gnagnarella, P.; Nanni, O.; Salvini, S.; La Vecchio, C. Reproducibility of an Italian food frequency questionnaire for cancer studies. Results for specific nutrients. *Ann Epidemiol* 5, 69-75 (1995).

Franceschi, S.; Negri, E.; Salvini, S.; Decarli, A.; Ferraroni, M.; Filberti, R.; Giacosa, A.; Talamini, R.; Nanni, O.; Panarello, G.; La Vecchia, C. Reproducibility of an Italian food frequency questionnaire for cancer studies: results for specific food items. *Eur J Cancer* **29**A, 2298-2305 (1993).

Freire, C.; Ramos, R.; Lopez-Espinosa, M-J.; Díez, S.; Vioque, J.; Ballester, F.; Fernández, M-F. Hair mercury levels, fish consumption, and cognitive development in preschool children from Granada, Spain. *Environ Res* **110**, 96-104 (2010).

Gailer, J. Arsenic–selenium and mercury–selenium bonds in biology. *Coordination Chemistry Reviews* **251**(1), 234-254 (2007).

Ganther, H.E.; Goudie, C.; Sunde, M.L.; Kopecky, M.J.; Wagner, P.; Oh, S-H.; Hoekstra, W.G. Selenium: relation to decreased toxicity of methylmercury added to diets
399 containing tuna. *Science* **175**(4026), 1122-1124 (1972).

George, N.G.; Singh, S.P.; Prince, R.C.; Pickering I.J. Chemical forms of mercury and selenium in fish following digestion with simulated gastric fluid. Chemical Research *Toxicology* 21, 2106-2110 (2008).

Gibičar, D.; Horvat, M.; Logar, M.; Fajon, V.; Falnoga, I.; Ferrara, R.; Lanzillotta, E.; Ceccarini, C.; Mazzolai, B.; Denby, B.; Pacyna, J. Human exposure to mercury in the vicinity of chlor-alkali plant. *Environ Res* **109,** 355–367 (2009).

Gibičar, D.; Horvat, M.; Nakou, S.; Sarafidou, J.; Yager, J. Pilot study of intrauterine exposure to methylmercury in Eastern Aegean islands, Greece**.** *Sci Tot Environ* **367,** 586-595 (2006).

Gil, F., Pla, A. Biomarkers as biological indicators of xenobiotic exposure. *J Appl Toxicol*

21, 245-255 (2001).

Gnamuš, A.; Byrne, A.R.; Horvat, M. Mercury in the Soil-Plant-Predator Food Chain of a Temperate Forest in Slovenia. *Environ Sci Tehnol* 34, 3337-3345 (2000).

Gosar, M.; Šajn, R. Geochemical soil and attic dust survey in Idrija, Slovenia. *J Phys IV France* 107, 561-564 (2003).

Gradjean, P.; Budtz-Jørgensen, E.; Jørgensen, P.J.; Weihe, P. Umbilical cord mercury concentration as biomarker of prenatal exposure to methylmercury. *Environ Health Perspect* 113, 905-908 (2005).

Grandjean, P.; Budtz-Jorgensen, E. Total imprecision of exposure biomarkers: implications for calculating exposure limits. *Am J Ind Med* **50**(10), 712-719 (2007).

Gradjean, P.; Jørgensen, P.J.; Weihe, P. Validity of mercury exposure biomarkers. In: Wilson, S.H.; Suk, W.A. (eds.) *Biomarkers of Environmentally associated disease*. 235-247 (CRC Press, Boca Raton, 2002).

Grandjean, P.; Weihe, P.; White, R.F.; Debes, F.; Araki, S.; Yokayama, K.; Murata, K.; Sørensen, N.; Dahl, R.; Jørgensen, P.J. Cognitive deficit in 7-year-old children with prenatal exposure to methylmercury. *Neurotoxicology and Teratology* **19**(6), 417-428 (1997).

Grandjean, P.; Weihe, P.; White, R.F.; Debes, F. Cognitive performance of children prenatally exposed to »safe« levels of methylmercury. *Environmental research* **77**, 165-172 (1998).

Greener, Y.; Kochen, J.A. In vitro studies on methyl mercury distribution in human blood. *Teratology* 28, 375-387 (1983).

Greim, H., Snyder, R. *Toxicology and Risk Assessment: A Comprehensive introduction* (John Wiley, West Sussex, 2008).

Groenlund, R.; Edner, H.; Svanberg, S.; Kotnik, J.; Horvat, M. Mercury emissions from the Idrija mercury mine measured by differential absorption Lidar techniques and a point monitoring absorption spectrometer. *Atmos Env* **39**, 4067–4074 (2005).

Grum, D. K.; Kobal, A.B.; Arnerič, N.; Horvat, M.; Ženko, B.; Džeroski, S.; Osredkar, J. Personality traits in miners with past occupational elemental mercury exposure. *Environmental health perspectives* **114**(2), 290 (2006).

Gundacker, C.; Wittmann, K.J.; Kukuckova, M.; Komarnicki, G.; Hikkel, I.; Martin, G. Genetic background of lead and mercury metabolism in a group of medical students in Austria. *Environmental research* **109**(6), 786-796 (2009).

Gundacker, C.; Komarnicki, G.; Jagiello, P.; Gencikova, A.; Dahmen, N.; Wittmann, K. J.; Gencik, M. Glutathione- S-transferase polymorphism, metallothionein expression, and mercury levels among students in Austria. *Science of the Total Environment* **385**(1), 37-47 (2007).

Halbach, S.; Clarkson, T.W. Enzymatic oxidation of mercury vapor by erythrocytes. Enzymatic oxidation of mercury vapor by erythrocytes. *Biochem Biophys Acta* **523**, 522-531 (1978).

Hines, M.E.; Faganeli, J.; Adatto, I.; Horvat, M. Microbial mercury transformations in marine, estuarine and freshwater sediment downstream of the Idrija Mercury Mine, Slovenija. *Appl Geochem* **21**, 1924-1939 (2006).

Hopenhayn, C. Arsenic in drinking water: impact on human health. *Elements* **2**(2), 103-107 (2006).

Hughes, M.F. Biomarkers of exposure: A case study with inorganic arsenic. *Environ Health Perspect* **114**(11), 1790-1769 (2002).

Horvat, M.; Coveli, S.; Faganeli, J.; Logar, M.; Fajon, V.; Rajar, R.; Širca, A.; Žagar, D. Mercury in contaminated coastal environments; a case study: the Gulf of Trieste. *Sci total environ* **238**(237), 43-56 (1999).

Horvat, M.; Gibičar, D. Exposure to methylmercury due to consumption of fish in the Mediterranean. *Toxicology Letters* 164, 10–11 (2006).

Horvat, M.; Jereb, V.; Fajon, V.; Logar, M.; Kotnik, J.; Faganeli, J.; Hines, M.E.; Bonzongo, J-C. Mercury distribution in water, sediment and soil in the Idrijca and Soča river systems. *Geochem Explor Environ Anal* **2**, 287-296 (2002).

Horvat, M.; Nolde, N.; Fajon, V.; Jereb, V.; Logar, M.; Lojen, S.; Jacimovic, R.; Falnoga, I.; Liya, Q.; Faganeli, J.; Drobne, D. Total mercury and selenium in merury polluted areas in the province Guizhou China. *Sci Total Env* **304**, 231-256 (2003).

Horvat, M.; Prosenc, A.; Smrke, J.; Konda, D.; Byrne, A.R.; Stegnar, P.; Begič, I. Trace elements in the hair, blood, cord blood and placenta of pregnant women from Central Slovenia. In: Aitio, A.; Aro, A.; Järvisalo, J.; Vainio, H. (eds.). *Trace Elements in Health and Disease* 85-91 (The Royal Society of Chemistry, Cambridge, 1988b).

Horvat, M.; Snoj Tratnik, J.; Miklavčič, A. Mercury: Biomarkers of exposure and human biomonitoring. In: Knudsen, L.E.; Merlo D.F. (eds.). *Biomarkers and Human Biomonitoring* 381-417 (Royal Society of Chemistry, Cambridge, 2011a) 381-417.

Horvat, M.; Šlejkovec, Z.; Falnoga, I. Arsenic: Biomarkers of exposure and human biomonitoring. In: Knudsen, L.E.; Merlo D.F. (eds.). *Biomarkers and Human Biomonitoring* 381-417 (Royal Society of Chemistry, Cambridge, 2011b) 418-446.

Horvat, M.; Stegnar, P.; Byrne, A.R.; Dermelj, M. A study of trace elements in human placenta, blood and hair from Yugoslav Central Adriatic. In: Brätter, P., Schramel, P. (eds.) *Trace element analytical chemistry in medicine and biology*. 5. proceedings of the Fifth International Workshop Neuherberg, Federal Republic of Germany (Walter de Gruyter, Berlin, New York, 1988a).

Horvat, M.; Toman, M.J.; Stergaršek, J.; Kotnik, J.; Fajon, V.; Gibičar, D. Mercury and selenium in fish species in the Idrijca river polluted due to past mercury mining. In:

Horvat M, Ogrinc N, Faganeli J, Kotnik J. (eds). *Mercury as a global pollutant* 27 June - 2 July, 2004 1-3 (RMZ - Materials and geoenvironment, Ljubljana, 2004).

Hughes, M.F.; Thomas, D.J.; Kenyon, E.M. Toxicology and epidemiology of arsenic and its compounds. In: Henke, E.K. (ed.). *Arsenic. Environmental Chemistry, Health Threats and Waste Treatmnet* 237-277 (John Wiley & Sons Ltd, West Sussex, 2009).

Hursh, J.B.; Clarkson, T.W.; Cherian, M.G. Clearance of mercury (Hg-197, Hg-203) vapor inhaled by human subject. *Arch Environ Health* **31**, 302-309 (1976).

IARC. *Monographs on the Evaluation of Carcinogenic Risks to Humans: Institute of Medicine, Food and Nutrition Board. Dietary Reference Intakes: Vitamin C, Vitamin E, Selenium, and Carotenoids* (DC: National Academy Press, Washington, 2000).

Ishihara, N. Extraction of methylmercury in human feces. *Archives of Environmental Health* **55**, 44-47 (2000).

James, R.C.; Warren, D.A.; Helmes, N.C.; Roberts, S.M. Risk Assessment. In: Williams, P.L.; James, R.C.; Roberts S.M. (eds.). *Principles of Toxicology Environmental and Industrial Applications* 437-477 (John Wiley & Sons, New York, 2000)

Jarosińska, D.; Horvat, M.; Sällsten, G.; Mazzolai, B.; Dąbkowska, B.; Prokopowicz, A.; Biesiada, M.; Barregård, L. Urinary mercury and biomarkers of early renal dysfunction in environmentally and occupationally exposed adults: A three-country study. *Environmental research* **108**(2), 224-232(2008).

Jedrychowski, W.; Perera, F.; Jankowski, J.; Rauh, V.; Flak, E.; Caldwell, K.L., Jones R.L.; Pac A.; Lisowska-Miszczyk I. Fish consumption in pregnancy, cord blood mercury level and cognitive and psychomotor development of infants followed over the first three years of life Krakow epidemiologic study. *Environment International* **33**, 1057-1062 (2007).

Jereb, V.; Falnoga, I.; Milačič, R.; Stibilj, V.; Horvat, M. Hg and Se, Cd, Pb, in fudstuffs and soil selected individual gardens near Hg mining area. *RMZ-mater geoenviron* **51**, 114 (2004).

Joas, R.; Casteleyn, L.; Biot, P.; Kolossa-Gehring, M.; Castano, A.; Angerer, J.; Schoeters, G. et al. Harmonised human biomonitoring in Europe: Activities towards an EU HBM framework. *International journal of hygiene and environmental health* (2011).

Kaur, P.; Evje, L.; Aschner, M.; Syversen, T. The in vitro effects of selenomethionine on methylmercury-induced neurotoxicity. *Toxicology in Vitro*, **23**(3), 378-385 (2009).

Kaise, T.; Fukuit, S. The chemical form and acute toxicity of arsenic compounds in marine organisms. *Appl Org Chem* **6**, 155-160 (1992).

Karagas, M.R.; Choi, A.L.; Oken, E.; Horvat, M.; Schoeny, R.; Kamai, E.; Cowell, W.;

Grandjean, P.; Korrick, S. Evidance on the human health effects of low-level methylmercury exposure. *Environ Health Perspect* 120, 799-806 (2012).

Kelly, B.C.; Ikonomou, M.G.; Higgs, D.A.; Oakes, J.; Dubetz, C. Mercury and other trace elements in farmed and wild salmon from British Columbia, Canada. *Environ Toxicol Chem* **27**, 1361-1370 (2008).

Kershaw, T.G.; Clarkson, T.W.; Dhahir, P.H. The relationship between blood levels and dose of methylmercury in man. *Arch Environ Health* 56, 565-575 (1980).

Khan, M.A.; Wang, F. Chemical demethylation of methylmercury by selenoamino acids. *Chemical research in toxicology*, *23*(7), 1202-1206 (2010).

Kingman, A.; Albertini, T.; Brown, L.J. Mercury concentrations in urine and whole blood associated with amalgam exposure in a US military populations. *J Dent Res* **77**, 461-467 (1996).

Klautau-Guimarães, M.N.; Ascenção, R.D.; Caldart, F.A.; Grisolia, C.K.; de Souza, J.R.; Barbosa, A.C.; Cordeiro, C.M.T; Ferrari, I. Analysis of genetic susceptibility to mercury contamination evaluated through molecular biomarkers in at-risk Amazon Ameridian populations. *Gen Molecul Biol* **28**, 827-832 (2005).

Kobal, A.B.; Barbone, F.; Horvat, M.; Osredkar, J.; Tamburlini, J.; Križaj, B. Final Report of Research Project: Exposure of Women in the Fertile Period to Inorganic and Methyl-Mercury and Potential DNA damage from Polluted Areas in the Town of Idrija in Slovenia and in the Coastal Friuli-Venezia-Giulia Region of Italy. 11 (2005).

Kobal, A.; Dizdarevič, T. The health safety programme for workers exposed to elemental mercury at the mercury mine in Idrija. *Water, Air, & Soil Pollution* **97**(1), 169-184 (1997).

Kobal, A.B.; Flisar, Ž.; Miklavčič, V.; Dizdarevič, T.; Sešek-Briški, A. Renal function in miners intermittently exposed to elemental mercury vapour. *Arhiv za higijenu rada i toksikologiju* **51**(4), 369-380 (2001).

Kobal, A. B.; Grum, D.K. Scopoli's work in the field of mercurialism in light of today's knowledge: Past and present perspectives. *American journal of industrial medicine* **53**(5), 535-547 (2010).

Kobal, A.B.; Horvat, M.; Prezelj, M.; Briški, A.S.; Krsnik, M.; Dizdarevič, T.; Mazej, D.; Falnoga, I.; Stibilj, V.; Arnerić N.; Kobal Grum D.; Osredkar, J. The impact of long-term past exposure to elemental mercury on antioxidative capacity and lipid peroxidation in mercury miners. *Journal of Trace Elements in Medicine and Biology* 17(4), 261-274 (2004).

Kobal, A.B.; Prezelj, M.; Horvat, M.; Krsnik, M.; Gibicar, D.; Osredkar, J. Glutathione level after long-term occupational elemental mercury exposure. *Environmental research*, **107**(1), 115 (2008).

Kocman, D.; Horvat, M.; Kotnik, J. Mercury fractionation in contaminated soils from the Idrija mine region. *J Environ Monit* **6**, 696-703 (2004).

Kocman, D.; Kanduč, T.; Ogrinc, N.; Horvat, M. Distribution and partitioning of mercury in a river catchment impacted by former mercury mining activity. *Biogeochem* **104**, 183-201 (2011b).

Kocman, D.; Vreča, P.; Fajon, V.; Horvat, M. Atmospheric distribution and deposition of mercury in the Idrija Hg mine region, Slovenia. *Environ Res* **111**(1), 1-9(2011a).

Koeman, J.H.; Peeters, H.M.; Koudstaal-Hol, C.H.M.; Tjioe, P.S.; De Goeij, J.J.M. Mercury-selenium correlations in marine mammals. *Nature*, 385-386(1973).

Kohrle, J.; Jakob, F.; Contempré, B.; Dumont, J.E. Selenium, the thyroid, and the endocrine system. *Endocr Rev* **26**(7), 944-984 (2005).

Kosta, L.; Byrne, A.R.; Zelenko, V. Correlation between selenium and mercury in man following exposure to inorganic mercury. *Nature*, 238-239 (1975).

Kosta, L.; Byrne, A.R.; Zelenko, V.; Stegnar, P.; Dermelj, M.; Ravnik, V. Studies on uptake, distribution and transformations of mercury in living organisms in Idrija region and comparative areas. *Vestn Slov Kem Drus* **21**, 49-76 (1974).

Kotnik, J.; Horvat, M.; Dizdarović, T. Current and past mercury distribution in air over the Idrija Hg mine region, Slovenia. *Atmos Environ* **39**, 7570-7579 (2005).

Kris-Etherton, P.M.; Harris, W.S.; Appel, L.J. Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. *Circulation* **106**(21), 2747-2757 (2002).

Kristan, U.; Arribére, M.A.; Stibilj, V. Selenium species and their distribution in freshwater fish from Argentina. *Biological trace element research*, **151**(2), 240-246 (2013).

Kristan, U.; Miklavčič, A.; Horvat, M.; Pohar, J.; Stibilj, V. The concentration of chemical elements in the selected fish on the Slovenian market [abstract]. *Trendi in izzivi v živilstvu, prehrani, gostinstvu in turizmu* 2012.

Langworth, S.; Almkvist, O.; Söderman, E.; Wikström, B-O. Effects of occupational exposure to mercury vapour on the central nervous system. *British J Ind Med* **47**, 545-555 (1992).

Levander, O.A. Metabolic interrelationships between arsenic and selenium. Environ Health Perspect **19**, 159-164 (1977).

Lesky, E. *Arbeitsmedizin im 18. Jahrhundert: Werksarzt und Arbeiter im Quecksilbergwerk Idria.* (Verlag des Notringes der wissenschaftlichen Verbände Österreichs, Wien, 1956).

Lindström, K. Selenium as a growth factor for plankton algae in laboratory experiments and in some Swedish lakes. *Hydrobiology* 101, 35-48 (1948).

Lioy, P.J. Assessing total human exposure to contaminants. *Environ Sci Techol* **24**, 7 (1990).

Lourdes, M.A.; Culvin-Aralar, A.; Furness, R.W. Mercury and selenium interaction: a review. *Ecotoxicol Environ Safety* **21**, 348–364 (1991) .

Magos, L.; Butler, W.H. Cumulative effects of methylmercury dicyandiamide given orally to rats. *Food Cosmet Toxicol* **10**, 513-517 (1972).

Magos, L.; Brown, A.W.; Sparrow, S.; Bailey, E.; Snowden, R.E., Skipp, W.R. The comparative toxicology of ethyl- and methylmercury. *Arch Toxicol* **57**, 260-267 (1985).

Magos, L.; Clarkson, T. W. Overview of the clinical toxicity of mercury. *Annals of Clinical Biochemistry* **43**(4), 257-268 (2006).

Magos, L.; Halbach, S.; Clarkson, T.W. Role of catalase in the oxidation of mercury vapor. *Biochem Pharmacol* **27**, 1373-1377 (1978).

Magos, L.; Webb, M.; Hudson, A.R. Complex formation between selenium and methylmercury. *Chemico-biological interactions* **28**(2), 359-362 (1979).

Mandal, B.K.; Suzuki, K.T. Arsenic round the world: a review. *Talanta* **58**(1), 201-235 (2002).

Mason, H.J.; Hindell, P.; Williams, N.R. Biological monitoring and exposure to mercury. *Occup Med* **51**, 2-11 (2001).

Mazej, D.; Falnoga, I.; Stibilj, V. Selenium determination in whole blood, plasma and selenoprotein P by hydride generation atomic fluorescence spectrometry. *Acta chimica slovenica* **50**(2), 185-198 (2003).

Mergler, D.; Anderson, H.A.; Chan, L.H.M.; Mahaffey, K.R.; Murray, M.; Sakamoto, M.; Stern, A.H. Methylmercury exposure and health effects in humans: a worldwide concern. *A Journal of the Human Environment* **36**(1), 3-11 (2007).

Miklavčič, V. Mercury in the town of Idrija (Slovenia) after 500 years of mining and smelting. In: Ebinghaus, R.; Turner, R.R.; de Lacerda, L.D.; Vasiliev, O.; Salomons W.(*eds.*) *Mercury Contaminated Sites.* 259-269 (Springer-Verlag, Berlin, 1999).

Miklavčič, A.; Casetta, A.; Snoj Tratnik, J.; Mazej, D.; Krsnik, M.; Mariuz, M.; Sofianou, K.; Špirić, Z.; Barbone, F.; Horvat, M. Mercury, arsenic and selenium exposure levels in relation to fish consumption in the Mediterranean area. *Environmental Research* **120**, 7-17 (2013a).

Miklavčič, A.; Cuderman, P.; Mazej, D.; Snoj Tratnik, J.; Krsnik, M.; Planinšek, P.; Osredkar, J.; Horvat, M. Biomarkers of low-level mercury exposure through fish consumption in pregnant and lactating Slovenian women. *Environmental research* **111**(8),

1201-1207 (2011).

Miklavčič, A.; Mazej, D.; Jaćimović, R.; Dizdarović, T.; Horvat, M. Mercury levels in food items from the Idrija mercury mine area. *Environ Res* In press (2013b).

Miklavčič, A.; Stibilj, V.; Heath, E.; Polak, T.; Snoj, Tratnik, J.; Klavž, J.; Mazej, D.; Horvat, M. Mercury, selenium, PCBs and fatty acids in fresh and canned fish available on the Slovenian market. *Food Chem* **124**, 711-72 (2011).

Miura, K.; Clarkson, T.W. Reduced methylmercury accumulation in a methylmercury-resistant rat pheochromocytoma PC12 cell line. *Toxicol Appl Pharmacol* **118**, 39-45 (1993).

Miura, K.; Ikeda, K.; Naganuma, A.; Imura, N. Important rule of glutathione in susceptibility of mammalian cells to methylmercury. *In Vitro Technol* **7**, 59-64 (1994).

Miura, K.; Imura, N. Mechanism of methylmercury cytotoxicity.*Crit Rev Toxicol* **18**(3), 161-168 (1987).

Morcillo, M.A.; Santamaria, J. Whole-body retention, and urinary and fecal extraction of mercury after subchronic oral exposure to mercuric chloride in rats. *BioMetals* **4**(8), 301-308 (1995).

Moreno, P.; Quijano, M.A.; Gutierrez, A.M.; Pérez-Conde, M.C.; Cámara, C. Fractionation studies of selenium compounds from oysters, and their determination by high-performance liquid chromatography coupled to inductively coupled plasma mass spectrometry. *J Anal At Spectrom* **16**(9), 1044-1050 (2001).

MPP and NRDC. Mercury in soaps and creams-presentation and draft fact sheet provided by Michael Bender of the Mercury Polycy Project (MPP) in coordination with Natural Resources Defence Council (NRDC), at the UNEP Regional Awareness Raisin Workshop on Mercury Pollution in Porto of Spain, Trinided and Tobago, 18-21 Jnuary 2005.

Murata, K.; Dakeishi, M.; Shimada, M.; Satoh, H. Assessment of intrauterine methyl mercury exposure affecting child development: messages from the newborn. *The Tohoku Journal Experim Med* **213**, 187-202 (2007).

Nakamura, I.; Hosokawa, K.; Tamura, H.; Miura, T. Reduced mercury excretion with feces in germfree mice after oral administration of methyl mercury chloride. *Bulletin of environmental contamination and toxicology* **17**(5), 528-533 (1977).

Nelson, M.; Atkinson, M.; Darbyshire, S. Food photography: use of food photographs for estimating portion size and the nutrient content of meals. *British Journal of Nutrition* **76**, 31-49 (1996).

Nielsen, J.B.; Andersen, O. Disposition and retention of mercuric chloride in mice after oral and parenteral administration. *J Toxicol Environ Health* **68**(3), 167-180 (1990).

Nielsen-Kudsk, F. The influence of ethyl alcohol on the absorption of methylmercury vapor from the lungs of man. *Acta Pharmacol Toxicol* **23**, 263-274 (1965).

Norseth. T.; Clarkson, T.W. Intestinal transport of Hg-203 labeled metylmercury chloride: Role of biotransformation in rats. *Arch Environ Health* **22**, 568-577 (1971).

NRC (National Research Council). *Arsenic in Drinking Water.* (National Academy Press, Washington, 1999).

NRC (National Research Council). *Toxicological Effects of Methylmercury*. (National Academy Press, Washington, 2000)

NRC (National Research Coucil). Subcommitte on biological markers in urinary toxicology. Biological markers in urinary toxicology. Board on environmental studies and toxicology commission on life sciences. (National Academy Press, Washington, 1995).

Nuttall, K.L. A model for metal selenide formation under biological conditions. *Med Hypoth* **24**, 217-221 (1987).

Parks, J.M.; Johs, A.; Podar, M.; Bridou, R.; Hurt R.A.; Smith, S.D.; Tomanicek, S.J.; Qian, Y.; Brown, S.D.; Brandt, C.C.; Palumbo, A.V.; Smith, J.C.; Wall, J.D.; Dwayne, A. E.; Liang, L. *Science* In press (2013).

Pedersen, E.B.; Jørgensen, M.E.; Pedersen, M.B.; Siggaard, C.; Sørensen, T.B.; Mulvad, G.; Hensen, J.C.; Asmud, G.; Skjoldborg H. Relationship between mercury in blood and 24h ambulatory blood pressure in Greenlanders and Danes. *AJH* **18**, 612-618 (2005).

Perelló, G.; Martí-Cid, R.; Llobet, J.M.; Domingo, J.L. Effects of various cooking processes on the concentrations of arsenic, cadmium, mercury, and lead in foods. *Journal of agricultural and food chemistry* **56**(23), 11262-11269 (2008).

Philbert, M.A.; Billingsley, M.L.; Reuhl, K.R. Mechanisms of injury in the central nervous system. *Toxicol Pathol* **28**(1), 43-53 (2000).

Piotrowski, J.K.; Szymańska, J.A.; Skrzypińska-Gawrysiak, M.; Kotelo, J.; Sporny, S. Intestinal absorption of inorganic mercury in rat. *Pharmacology & toxicology* **70**(1), 53-55 (1992).

Pirrone, N.; Wichmann-Fiebig, M.; Ahrens, R.; Pacyna, J.M.; Borowiak, A.; Boffetta, P.; Hansen, J.C.; Grandjean, P.; Ehrlich, H.C.; Petersen, G.; Horvat, M. Exposure to mercury in Europe. *Ambient Air Pollution by Mercury (Hg)*. Position Paper. Luxembourg, Belgium 235 (2002).

Plessi, M.; Bertelli, D.; Monzani, A. Mercury and selenium content in selected seafood. *Journal of Food Composition and Analysis* **14**(5), 461-467 (2001).

Pokorny, B.; Ribarič-Lasnik, C. Seasonal variability of mercury and heavy metals in roe deer (Capreolus capreolus) kidney. *Environmental Pollution* **117**(1), 35-46 (2002).

Rabenstein, D.L; Evens C.A. The mobility of methylmercury in biological sistems. *Bioinorg Chem* **8**(2), 107-114 (1978).

Ralston, N.V.; Blackwell, J.L.; Raymond, L.J. Importance of molar ratios in selenium-

dependent protection against methylmercury toxicity. *Biological trace element research* **119**(3), 255-268 (2007).

Ralston, N.V.; Ralston, C.R.; Blackwell, J.L.; Raymond, L.J. Dietary and tissue selenium in relation to methylmercury toxicity. *Neurotoxicology* **29**(5), 802-811 (2008).

Ralston, N.V.; Raymond, L.J. Dietary selenium's protective effects against methylmercury toxicity. *Toxicology* **278**(1), 112-123 (2010).

Rayman, M. The importance of selenium to human health. *Lancet* **356**, 233-241 (2010).

Raymond, L.; Ralston, V.C.N. Mercury: selenium interactions and health implications. *Seychelles Medical and Dental Journal* **7**(1), 72-75 (2004).

Referenčne vrednosti za vnos hranil. 215 (Littera picta d.o.o., Ljubljana, 2004).

Reilly, C. *Selenium in food and health*. 338 (Blackie Academic & Professional, 1. edition, London, 1996).

Renzoni, A.; Zino, F.; Franchi E. Mercury levels along the food chain and risk for exposed populations. *Environ Res* **77**, 68-72 (1997).

Rice, D.C. Blood mercury concentrations following methylmercury exposure in adult and infant monkeys. *Environ Res* **49**(1), 115-126 (1989).

Rice, D., Barone J.S. Critical periods of vulnerability for the developing nervous system: evidence from humans and animal models. *Environmental health perspectives* **108**, 511 (2000).

Rodier, P.M.; Aschner, M.; Sager, PR. Mitotic arest in the developing CNS after prenatal exposure to methyl mercury. *Neurobehav Toxicol Teratol* **6**, 379-385(1984).

Rodrigues, J.L.; Serpeloni, J.M.; Batista, B.L.; Souza, S.S.; Barbosa, F. Identification and distribution of mercury species in rat tissues following administration of thimerosal or methylmercury. *Archives of toxicology* **84**(11), 891-896 (2010).

Rothstein, A.; Hayas, A.L. The turnover of mercury in rats exposed repeatedly to inhalation of vapor. *Health Phys* **10**, 1099-1113 (1964).

Rowland, I.; Davies, M.; Evans, J. Tissue content of mercury in rats given methylmercury chloride orally: Influence of intestinal flora. *Arch Environ Heath* **35**, 155-160 (1980).

Rutishauser, I.H.E. Dietary intake measurements. *Public Health Nutrition* **8**, 1100-1107 (2005).

Ryan, P.B.; Burke, T.A.; Cohen Hubal, E.A.; Cura, J.J.; McKone, T.E. Using biomarkers to inform cumulative risk assessment. *Environ Health Perspect* **115**(5), 833-840.

Schrauzer, G.N. Anticarcenogenc effects of selenium. *Cellular and Molecular Life Science.* **57**, 1864-1873 (2000).

Schweizer, U.; Braüer, A.U.; Kohrle, J.; Nitsch, R.; Savaskan, N.E. Selenium and brain function: a poorly recognized liaison. *Brain Res Rev* **45**(3), 167-178 (2004).

Sakamoto, M.; Kubota, M.; Liu, X.J.; Murata, K.; Nakai, K.; Satoh, H. Maternal and fetal mercury and n-3 polyunsaturated fatty acids as a risk and benefit of fish consumption to fetus. *Environmental science & technology* **38**(14), 3860-3863 (2004).

Sakamoto, M.; Kubota, M.; Murata, K.; Nakai, K.; Sonoda, I.; Satoh, H. Changes in mercury concentrations of segmental maternal hair during gestation and their correlations with other biomarkers of fetal exposure to methylmercury in the Japanese population. *Environmental research* **106**(2), 270 (2008).

Sakamoto, M.; Murata, K.; Kubota, M.; Nakai, K.; Satoh, H. Mercury and heavy metal profiles of maternal and umbilical cord RBCs in Japanese population. *Ecotoxicology and environmental safety* **73**(1), 1-6 (2010).

Sakurai, T. ; Kaise, T. ; Ochi, T. ; Saitoh, T. ; Matsubara, C. Study of in vitro cytotoxicity of a water soluble organic arsenic compound, arsenosugars, in seaweed. *Toxicology* **122**, 205-212 (1997).

Sallsten, G.; Barregard, L.; Schutz, A. Clearance half life of mercury in urine after the cessation of long term occupational exposure: influence of a chelating agent (DMPS) on eycretion of mercury in urine. *Occup Environ Med* **51**(5), 337-342 (1995).

Sallsten, G.; Thoren, J.; Barregard, L.; Schutz, A.; Skarping G. Long term use of nicotine chewing gum and mercury exposure from dental amalgam fillings. *J Dent Res* **75**, 594-598 (1996).

Salonen, J.T.; Seppänen, K.; Lakka T.A.; Salonen R.; Kaplan G.A. Mercury accumulation and accelerated progression of carotid atherosclerosis: a population-based prospective 4-year follow-up study in men in eastern Finland. *Atherosclerosis* **148**, 265-273 (2000).

Sandborgh-Englund, G.; Elinder, C.G.; Johanson, G.; Lind, B.; Skare, I.; Ekstrand, J. The absorption, blood levels, and excretion of mercury after a single dose of mercury vapor in humans. *Toxicology and applied pharmacology* **150**(1), 146-153 (1998).

Sarafian, T.A.; Bredesen, D.E.; Verity, M.A. Cellular resistance to methylmercury. *Neurotoxicology* **17**(1), 27 (1996).

Schoeman, K.; Bend, J.R.; Hill, J.; Nash, K.; Koren G. Defining a lowest observable adverse effect hair concentrations of mercury for neurodevelopmental effects of prenatal methylmercury exposure through maternal fish consumption: A systematic review. *Ther Drug Monit* **31**, 670-682 (2009).

Schoof, R.A.; Yost L.J.; Eickhoff, J.; Crecelius, E.A.; Cragin, D.W.; Meacher, D.M.; Menzel, D.B. A market basket survey of inorganic arsenic in food. *Food Chem Toxicol* **37**, 839-846 (1999).

Schulte, P.A.; Waters, M. Using molecular epidemiology in assessing exposure for risk assessment. *Annals of the New York Academy of Sciences* **895**, 101–111 (1999).

Selin, NE. Global biogeochemical cycling of mercury: A review. *Annual Review of Environment and Resources.* 43-63 (2009).

Sin, Y.M.; Lim, Y.F.; Wong, M.K. Uptake and distribution of mercury in mice from ingesting soluble and insoluble mercury compounds. *Bull Environ Contam Toxicol* **31**(5), 605-612. (1983)

Smrkolj, P.; Pograjc, L.; Hlastan-Ribič, C.; Stibilj, V. Selenium content in selected Slovenian foodstuffs and estimated daily intakes of selenium. *Food chemistry* ***90***(4), 691-697 (2005).

Smrkolj, P.; Stibilj, V. Determination of selenium in vegetables by hydride generation atomic fluorescence spectrometry. *Analytica chimica acta* **512**(1), 11-17 (2004).

Soleo, L.; Elia, G.; Russo, A.; Schiavulli, N.; Lasorsa, G.; Mangili, A.; Gilberti, E.; Ronchi, A.; Balducci, C.; Minoia, C.; Aprea, C.; Sciarra, G.F.; Valente, T.; Fenga, C. Reference values of urinary mercury in the Italian population. *Ital Med Lav Ergon*, **25**, 107-113 (2003).

Stern, A.H.; Smith, A.E. An assessment of the cord blood: maternal blood methyl mercury ratio: implications for risk assessment. *Environ Health Perspect* **111**, 1465-1470 (2003).

Steuerwald, U.; Weihe, P.; Jøgensen, P.J.; Bjerve, K.; Brock, J.; Heinzow, B.; Budtz-Jørgensen, E.; Grandjean, P. Maternal seafood diet, methylmercury exposure, and neonatal neurologic function. *The Journal of Pediatrics* **136**, 599-605 (2000).

Storelli, M.M.; Barone, G.; Cuttone, G.; Giungato, D.; Garofalo, R. Occurance of toxic metals (Hg, Cd and Pb) in fresh and canned tuna: Public health implications. *Food Chem Toxicol* **48**, 3167-3170 (2010).

Suda, I.; Hirayama, K. Degradation of methyl and ethyl mercury into inorganic mercury by hydroxyl radical produced from rat liver microsomes. *Archives of toxicology* **66**(6), 398-402 (1992).

Sue, Y.J. Mercury. In: Goldfrank, L.R.; Flomenbaum, N.E.; Lewin, N.A. (eds.) *Goldfrank Toxicologic Emergencies, Fifth Edition* 1051-1062 (Appleton and lange, Norwalk, 1992).

Sundberg, J.; Oskarsson, A. Placental and lactational transfer of mercury from rats exposed to methylmercury in their diet: Speciation of mercury in the offspring. *J Trace Elem Exp Med* **5**(1), 47-56 (1992).

Sunderland, E.M. Mercury exposure from domestic and imported estuarine and marine fish in the U.S. Seafood Market. *Environ Health Perspect* **115**, 235-242 (2007).

Suzuki, T.; Imura, N.; Clarkson, T.W. Overview. In: Suzuki, T.; Imura, N.; Clarkson, T.W. (eds.) *Advances in mercury toxicology*. 1-32 (Plenum Press, New York, 1991).

Suzuki, T.K.; Sasakura, C.; Yoneda S. Binding sites for the (Hg-Se) complex on selenoprotein. *Biochimica et Biophysica Acta* **1429**, 102-112 (1998).

Syversen, T.; Kaur, P. The toxicology of mercury and its compounds. *J Trace Elem Med Biol* **26**, 215-226 (2012).

Šlejkovec, Z.; Bajc, Z.; Doganoc, D.Z. Arsenic speciation patterns in freshwater fish. *Talanta* **62**, 931-936 (2004).

Tao, S.S.H.; Bolger, P.M. Dietary arsenic intakes in the United States: FDA total diet study, September 1991-December 1996. *Food Additives & Contaminants* **16**(11), 465-472 (1999).

Teisinger, J.; Fiserova-Bergerova, V. Pulmonary retention and excretion of mercury vapors in man. Pulmonary retention and excretion of mercury vapors in man. *Ind Med Surg* **34**, 580 (1965).

Tomiyasu, T.; Mutsuyama, A.; Imura, R.; Kondamatani, H.; Miyamoto, Y.; Kono, Y.; Kocman, D.; Kotnik, J.; Fajon, V.; Horvat, M. The distribution of total and methylmercury concentrations in soils near the Idrija mercury mine, Slovenia, and the dependence of the mercury concentrations on the chemical composition and organic carbon levels of the soil. *Environ Earth Sci* **65**, 1309-1322 (2012).

Tsutomu, U.; Akiko, I.; Sei-ichiro, H.; Akira, N.; Nobumasa, I. Absorption of methylmercury compounds from rat intestine. *Toxicology letters* ***50***(2), 159-164 (1990).

Turner, M.D.; Smith, J.C.; Kilpper, R.W.; Forbes, G.B.; Clarkson, T.W. Absorption of natural methylmercury (MeHg) from fish. *Clinical Research* **23**(2), 1975.

UNEP. Global Mercury Assessment. UNEP Chemicals Mercury Programme (2002). (www.chem.unep.ch/mercury/report/Final_Assessment_report.htm).

UNEP. Reducing Mercury Use in Artisanal and Small-scale Gold Mining (2012). (http://www.unep.org/hazardoussubstances/Portals/9/Mercury/Documents/ASGM)

UNEP. Global Mercury Assessment 2013: Sources, emissions, releases, and environmental transport (2013).
(http://www.unep.org/publications/contents/pub_details_search.asp?ID=6282).

UNEP and WHO. Guidance for identifying populations at risk from mercury exposure. United Nations Environment Programme and World Health Organization, Geneva, 2008.

Usydus, Z.; Szlinder-Richert, J.; Polak-Juszczak, L.; Kanderska, J.; Adamczyk, M.; Malesa-Ciecwierz, M.; Ruczynska, W. Food of marine origin: Between benefits and potential risks. Part I. Canned fish on the Polish market. *Food Chem* **11**, 556-563 (2008).

Vahter, M. Species difference in the metabolism of arsenic compounds. *Appl Organomet Chem* **8**, 175−182 (1994).

Vahter, M.; Åkesson, A.; Lind, B.; Björs, U.; Schütz, A.; Berglund, M. Longitudinal Study of Methylmercury and Inorganic Mercury in Blood and Urine of Pregnant and Lactating Women, as Well as in Umbilical Cord Blood. *Environ Res* **84**, 186-194 (2000).

Valent, F.; Horvat, M.; Sofianou-Katsoulis, A.; Spirić, Z.; Mazej, D.; Little, D. A.;

Prasouli, A.; Mariuz, M.; Tamburlini, G.; Nakou, S.; Barbone, F. Neurodevelopmental Effects of Low-level Prenatal Mercury Exposure From Maternal Fish Consumption in a Mediterranean Cohort: Study Rationale and Design. *Journal of Epidemiology* In press. (2013).

van Elteren, J.T.; Stibilj, V.; Šlejkovec, *Z.* Speciation of inorganic arsenic in some bottled Slovene mineral waters using HPLC–HGAFS and selective coprecipitation combined with FI-HGAFS. *Water research* ***36***(12), 2967-2974 (2002).

Weihe, P.; Hansen, J.C.; Murata, K.; Debes, F.; Jørgensen, P.; Steuerwald, U.; White, R.F.; Grandjean P. Neurobehaviour performance of Inuit children with increased prenatal exposure to methylmercury. *Int J Circumpolar Health* **61**, 41-49 (2002).

Weihe, P.; Grandjean, P.; Jørgensen, P.J. Application of hair-mercury analysis to determine the impact of a seafood advisory. *Environ Res* **97**, 201-208 (2005).

Whanger, P.D. Selenium in the treatment of heavy metal poisoning and chemical carcinogenesis. *J Trace Elem Electrolytes – Heath Dis* **6**(4), 209-221 (1992).

WHO. Environmental Health Criteria 70, Principles of the safety assessment of food additives and contaminants in food (1987).
(www.inchem.org/documents/ehc/ehc/ehc70.htm)

WHO. Methyl mercury. Vol. 101. Geneva, Switzerland: Word Health Organization 1990; International Programe on Chemistry Safety (1990).

WHO. Inorganic Mercury. In: Environmental Health Criteria 118. IPCS (ed.). Geneva, World Health Organization (1991).

WHO. Trace elements in human nutrition and health. WHO, Geneva (1996).

WHO. Concise International Chemical Assessment Document 50. Elemental mercury and Inorganic mercury compounds: Human health aspects. World Health Organization Geneva (2003).

WHO. Techical Report Series 922. Sixty-first report of the Joint FAO/WHO Expert Commitee on Food Additives (JEFCA) (2004).

WHO. Evaluation of certain food additives and contaminants (Sixty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 940 (2007)
(whqlibdoc.who.int/trs/WHO_TRS_940_eng.pdf)

WHO. Children's exposure to mercury compounds. Geneva, World Health Organization (2011).

WHO, UNEP. Guidance for identifying populations at risk from mercury exposure. IOMC (ed.). Geneva, World Health Organization (2008).

Wright, N.; Yeoman, W.B; Carter, G.F. Massive oral igestion of elemental mercury without poisoning. *Lancet* **1**(8161), 206 (1980).

Yoshida, S.; Haratake, M.; Fuchigami, T.; Nakayama, M. Selenium in Seafood Materials. *Journal of Health Science* **57**(3), 215-224 (2011).

Zartarian, V.; Bahadori, T.; McKone, T. Adoption of an official ISEA glossary. *Journal of exposure analysis and environmental epidemiology* **15**(1), 1-5 (2005).

Zeng, H. Arsenic suppresses necrosis induced by selenite in human leukemia HL-60 Cells. *Biol Trace Elem Res* **83**, 1-14 (2001).

Zeng, H.; Uthus, E.O.; Combs, G.F. Mechanistic aspect of the interaction between selenium and arsenic. *J Inorg Biochem* **99**, 1269-1274 (2005).

Žižek, S.; Horvat, M.; Gibičar, D.; Fajon, V.; Toman, M.J. Bioaccumulation of mercury in benthic communities of a river ecosystem affected by mercury mining. *Sci total environ* **377**, 407-415 (2007).

Žižek, S.; Milačič, R.; Kovač, N.; Jaćimović, R.; Toman, M.J.; Horvat, M. Periphyton as a bioindicator of mercury pollution in a temperate torrential river ecosystem. *Chemosphere* **85**(5), 883-891.

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Appendix

Personal bibliography for 2007-2013

ANA MIKLAVČIČ [30883]

ARTICLES AND OTHER COMPONENT PARTS

1.01 Izvirni znanstveni članek

1. BELLANGER, Martine, PICHERY, C., AERTS, D., BERGLUND, M., CASTAÑO, A., CEJCHANOVÁ, M., CRETTAZ, P., DAVIDSON, F., ESTEBAN, Marta, FISCHER, M. E., GURZAU, A. E., HALZLOVA, K., KATSONOURI, A., KNUDSEN, Lisbeth E., KOLOSSA-GEHRING, M., KOPPEN, G., LIGOCKA, D., MIKLAVČIČ, Ana, REIS, F., SNOJ TRATNIK, Janja, WEIHE, Pal, GRANDJEAN, Phillipe. Economic benefits of methylmercury exposure control in Europe: : Monetary value of neurotoxicity prevention. *Environmental health*. [Online ed.], 2013, no. 3, vol. 12, 20 str. [COBISS.SI-ID 26469927]

2. MIKLAVČIČ, Ana, CASETTA, Anica, SNOJ TRATNIK, Janja, MAZEJ, Darja, KRSNIK, Mladen, MARIUZ, Marika, SOFIANOU, Katia, ŠPIRIĆ, Zdravko, BARBONE, Fabio, HORVAT, Milena. Mercury, arsenic and selenium exposure levels in relation to fish consumption in the Mediterranean area. *Environ. res. (N.Y. N.Y.)*, 2013, vol. 120, str. 7-17, doi: 10.1016/j.envres.2012.08.010. [COBISS.SI-ID 26110247]

3. MIKLAVČIČ, Ana, CUDERMAN, Petra, MAZEJ, Darja, SNOJ TRATNIK, Janja, KRSNIK, Mladen, PLANINŠEK, Petra, OSREDKAR, Joško, HORVAT, Milena. Biomarkers of low-level mercury exposure through fish consumption in pregnant and lactating Slovenian women. *Environ. res. (N.Y. N.Y.)*, 2011, vol. 111, no. 8, str. 1201-1207. http://www.sciencedirect.com/science/journal/00139351, doi: 10.1016/j.envres.2011.07.006. [COBISS.SI-ID 24972583]

4. MIKLAVČIČ, Ana, STIBILJ, Vekoslava, HEATH, Ester, POLAK, Tomaž, SNOJ TRATNIK, Janja, KLAVŽ, Janez, MAZEJ, Darja, HORVAT, Milena. Mercury, selenium, PCBs and fatty acids in fresh and canned fish available on the Slovenian market. *Food chem.*. [Print ed.], 2011, vol. 124, issue 3, str. 711-720, doi: 10.1016/j.foodchem.2010.06.040. [COBISS.SI-ID 23731239]

1.06 Objavljeni znanstveni prispevek na konferenci (vabljeno predavanje)

5. SNOJ TRATNIK, Janja, MIKLAVČIČ, Ana, JURKOVIĆ MLAKAR, Simona, MAZEJ, Darja, KRSNIK, Mladen, OSREDKAR, Joško, BARBONE, Fabio, MARIUZ, Marika, VALENT, Francesca, SOFIANOU, Katia, SPIRIĆ, Zdravko, MARC, Janja, HORVAT, Milena. Biomarkers of low level mercury exposure : exposure, impact and genetic susceptibility. V: *Current topics of mercury impact to human and environment : NIMD forum 2012, 26 January, 2012, Minamata, Japan*. Minamata: National Institute for Minamata Disease, 2012, str. 18-19. [COBISS.SI-ID 25590567]

1.08 Objavljeni znanstveni prispevek na konferenci

6. KRISTAN, Urška, MIKLAVČIČ, Ana, HORVAT, Milena, POHAR, Jurij, STIBILJ, Vekoslava. Vsebnost kemijskih elementov v izbranih ribah na slovenskem trgu. V: KRŽIN STEPIŠNIK, Jasna (ur.), LOBOREC, Vesna (ur.), VULIĆ, Gordana (ur.), KOSTADINOV, Marija (ur.), VIDRIH, Tjaša (ur.), OZIMEK, Boštjan (ur.), CVITKOVIČ, Dejan (ur.), SUWA-STANOJEVIĆ, Milena (ur.). *Trendi in izzivi v živilstvu, prehrani, gostinstvu in turizmu : zbornik prispevkov 2.*

mednarodne strokovne konference, 16.-17. november 2012, Ljubljana, Slovenija : 2nd International Professional Conference proceedings, November 16th-17th 2012, Ljubljana, Slovenia. Ljubljana: Biotehniški izobraževalni center, Višja strokovna šola: = Biotehnical Educational Centre, Vocational College, 2012, str. 111-119. [COBISS.SI-ID 26285607]

7. MIKLAVČIČ, Ana, MAZEJ, Darja, SNOJ TRATNIK, Janja, KOBAL, Alfred Bogomir, KRSNIK, Mladen, PLANINŠEK, Petra, OSREDKAR, Joško, HORVAT, Milena. Mercury, selenium and arsenic exposure assessment trough fish consumption. V: 13th International Neutrotoxicology Association Meeting & 11th International Symposium on Neurobehavioral Methods and Effects in Occupational and Environmental Health, June 5-10, 2011, Xi'an. *Program and proceedings*. 2011, 2011, 168-169. [COBISS.SI-ID 25361959]

8. MIKLAVČIČ, Ana, MAZEJ, Darja, SNOJ TRATNIK, Janja, KOBAL, Alfred Bogomir, KRSNIK, Mladen, PLANINŠEK, Petra, OSREDKAR, Joško, HORVAT, Milena. Relation of fish consumption to mercury, selenium and arsenic levels in cord blood and breast milk from Slovenian pregnant women. V: 3rd International Symposium on Trace Elements & Health, TRACEL 2011, Murcia, 24-27 May, 2011. *TRACEL 2011*. Murcia, 2011, str. 75-76. [COBISS.SI-ID 25780007]

9. MIKLAVČIČ, Ana, CUDERMAN, Petra, MAZEJ, Darja, SNOJ TRATNIK, Janja, KOBAL, Alfred Bogomir, KRSNIK, Mladen, PLANINŠEK, Petra, OSREDKAR, Joško, HORVAT, Milena. Biomarkers of low-level mercury exposure in pregnant and lactating Slovenian women. V: BARGAŃSKA, Żaneta (ur.). *Proceedings of the 15th International Conference on Heavy Metals in the Environmental, September 19-23, 2010, Gdańsk, Poland*. Gdańsk: Chemical Faculty, Gdańsk University of Technology, 2010, str. 881-885. [COBISS.SI-ID 23957799]

10. CIGIĆ, Blaž, MIKLAVČIČ, Ana, KRALJ CIGIĆ, Irena, KOŠMERL, Tatjana. Preparation and characterisation of lyophilised wine extracts = Préparation et caractérisation des extraits lyophilisés de vin. V: *32nd World Congress of Vine and Wine [and] 7th General Assembly of the OIV, June 28th - July 3rd, 2009, Zagreb, Croatia : final papers*. [Zagreb: Ministarstvo poljoprivrede, ribarstva i ruralnog razvoja, 2009], str. [1-10]. [COBISS.SI-ID 3653240]

1.12 Objavljeni povzetek znanstvenega prispevka na konferenci

11. MIKLAVČIČ, Ana, HORVAT, Milena. Mercury exposure assessment in Slovenian population. V: LISJAK, Darja (ur.), DUŠAK, Peter (ur.), KRALJ, Slavko (ur.). 7th Young Researchers' Day, 19 February, 2013, Ljubljana, Slovenia. *[Program and abstract book]*. Ljubljana: Institut Jožef Stefan, 2013, str. 2. [COBISS.SI-ID 26568743]

12. SNOJ TRATNIK, Janja, MIKLAVČIČ, Ana, JURKOVIĆ MLAKAR, Simona, MAZEJ, Darja, KRSNIK, Mladen, OSREDKAR, Joško, BARBONE, Fabio, MARIUZ, Marika, VALENT, Francesca, SOFIANOU, Katia, SPIRIĆ, Zdravko, MARC, Janja, HORVAT, Milena. Mercury exposure in relation in selenium and glutathione S-transferase gene deletion variants in pregnant women from Mediterranean. V: 6th SETAC World Congress/SETAC Europe [and] 22nd Annual Meeting, 20-24 May 2012, Berlin, Germany. *Abstract book*. Brussels: SETAC, 2012, str. 126. [COBISS.SI-ID 25837351]

13. MIKLAVČIČ, Ana, HORVAT, Milena. Mercury levels in food from the Idrija mercury mine area. V: 6th SETAC World Congress/SETAC Europe [and] 22nd Annual Meeting, 20-24 May 2012, Berlin, Germany. *Abstract book*. Brussels: SETAC, 2012, str. 465. [COBISS.SI-ID 25836071]

14. MIKLAVČIČ, Ana, CASETTA, Anica, SNOJ TRATNIK, Janja, MAZEJ, Darja, KRSNIK, Mladen, MARIUZ, Marika, SOFIANOU, Katia, SPIRIĆ, Zdravko, BARBONE, Fabio, HORVAT, Milena. Relation of fish consumption to biomarkers of mercury, selenium and arsenic exposure from Mediterranean women : [presented at ISSE 2012, 35th International Spring Seminar on Electronics Technology, May 9-13, 2012, Bad Aussee, Austria]. *Epidemiology (Camb. Mass. Print)*, 2012, vol. 23, no. 5, str. O-168. [COBISS.SI-ID 26046759]

15. MIKLAVČIČ, Ana, CASETTA, Anica, SNOJ TRATNIK, Janja, MAZEJ, Darja, KRSNIK, Mladen, MARIUZ, Marika, SOFINAU, Katia, SPIRIĆ, Zdravko, BARBONE, Fabio, HORVAT, Milena. Mercury, arsenic and selenium exposure levels in relation of fish consumption in Mediterranean. V: ŽAGAR, Kristina (ur.), LENART, Alenka (ur.), PEČKO, Darja (ur.). 6th Young Researchers' Day 2012, 27 and 28 Febraury, 2012, Ljubljana. *[Program and abstract book]*. Ljubljana: Jožef Stefan Institute, Department of Nanostructured Materials, 2012. [COBISS.SI-ID 25780263]

16. BARBONE, Fabio, MARIUZ, Marika, VALENT, Francesca, SOFIANOU, Katia, ŠPIRIĆ, Zdravko, KOTNIK, Jože, HORVAT, Milena, SNOJ TRATNIK, Janja, MIKLAVČIČ, Ana, MAZEJ, Darja, KRSNIK, Mladen. Relation between mercury and selenium in umbilical cord blood, maternal blood and breast milk in pregnant women from Mediterranean. V: *10th International Conference on Mercury as a Global Pollutant : Abstract Volume : July 24-29, 2011, Halifax, Nova Scotia, Canada*. Halifax: ICMGP, 2011, str. 20. [COBISS.SI-ID 24942631]

17. SNOJ TRATNIK, Janja, MRAK, Irena, BEGIĆ, Irena, MIKLAVČIČ, Ana, PLANINŠEK, Petra, CUDERMAN, Petra, KRSNIK, Mladen, BARBONE, Fabio, MARIUZ, Marika, SOFIANOU, Katia, ŠPIRIĆ, Zdravko, HORVAT, Milena. Suitability of biomarkers of low-to-medium mercury exposure in pregnant women. V: *10th International Conference on*

Mercury as a Global Pollutant : Abstract Volume : July 24-29, 2011, Halifax, Nova Scotia, Canada. Halifax: ICMGP, 2011, str. 315. [COBISS.SI-ID 24962087]

18. KRISTAN, Urška, STIBILJ, Vekoslava, HORVAT, Milena, MIKLAVČIČ, Ana, ARRIBÉRE, Maria. Distribution of selenium, mercury and theirs species in fish muscle from fresh water lakes in Argentina. V: 13th Workshop on Progress in Trace Metal Speciation for Environmental Analytical Chemistry, Pau, May 18 to 20, 2011. *Book of abstracts and conference program*. Pau: Université de Pau, 2011, 2011, str. 72. [COBISS.SI-ID 24917543]

19. MIKLAVČIČ, Ana, CUDERMAN, Petra, MAZEJ, Darja, SNOJ TRATNIK, Janja, KOBAL, Alfred Bogomir, KRSNIK, Mladen, PLANINŠEK, Petra, OSREDKAR, Joško, HORVAT, Milena. Comparison of biomarkers of low-level mercury exposure. V: Environmental Health 2011, Resetting our Priorities, 6-9 February 2011, Salvador. *Proceedings : Resetting our priorities*. Salvador: Elsevier, 2011. [COBISS.SI-ID 24751655]

20. MIKLAVČIČ, Ana, CUDERMAN, Petra, MAZEJ, Darja, SNOJ TRATNIK, Janja, KOBAL, Alfred Bogomir, KRSNIK, Mladen, PLANINŠEK, Petra, OSREDKAR, Joško, HORVAT, Milena. Biološki kazalci izpostavljenosti nizkim koncentracijam živega srebra preko uživanja rib. V: PRIBOŠIČ, Irena (ur.), KRNEL, Kristoffer (ur.). 5. dan mladih raziskovalcev, Ljubljana, Februar 2011. *[Program in povzetki]*. Ljubljana: Institut "Jožef Stefan", 2011. [COBISS.SI-ID 25762855]

21. MIKLAVČIČ, Ana, HORVAT, Milena. Živo srebro iz kontaminiranega območja Idrije. V: KUŠČER, Danjela (ur.), PERC, Branka (ur.). 4. Dan Mladih Raziskovalcev KMBO, Ljubljana, Slovenija, 11.2.2010. *[Program in povzetki]*. Ljubljana: Institut "Jožef Stefan", 2010, 1 str. [COBISS.SI-ID 25762599]

22. KOŠMERL, Tatjana, MIKLAVČIČ, Ana, KRALJ CIGIĆ, Irena, CIGIĆ, Blaž. Preparation and characterisation of lyophilised wine extracts = Préparation et caractérisation des extraits lyophilisés de vin = Preparazione e descrizione degli estratti liofilizzati del vino. V: KUBANOVIĆ, Veronika (ur.). *32nd World Congress of Vine and Wine : 7th General Assembly of the OIV, June 28th - July 3rd 2009 - Zagreb - Croatia : [presentation summaries] : 7. Generalna skupština OIV-a, 28. lipnja - 3. srpnja 2009 - Zagreb - Hrvatska : [Knjiga sažetaka] : 7ème Assemblée générale de l' OIV, 28 juin - 3 juillet 2009 - Zagreb - Croatie : [Résumés des communications] : 7. Generalversammlung der OIV, 28 Juni - 3 Juli 2009 - Zagreb - Kroatien : [Zusammenfassungen der Beiträge] : 7a Asamblea general de la OIV, 28 junio - 3 julio 2009 - Zagreb - Croacia : [Resúmenes de comunicaciones] : 7a Assemblea generale dell' OIV, 28 giugno - 3 luglio 2009 - Zagabria - Croazia : [Riassunti delle comunicazioni]*. [Zagreb]: Ministarstvo poljoprivrede, ribarstva i ruralnog razvoja, [2009], str. 110, II.C.14. [COBISS.SI-ID 3652728]

23. MIKLAVČIČ, Ana, HORVAT, Milena. Koristi in tveganja uživanja rib prisotnih na slovenskem tržišču. V: ISKRA, Jernej (ur.), MILOŠEV, Ingrid (ur.). *Dan mladih raziskovalcev 2009*. Ljubljana: Institut "Jožef Stefan", 2009, 1 str. [COBISS.SI-ID 22478119]

24. MIKLAVČIČ, Ana, SNOJ TRATNIK, Janja, ZAJC, Antonija, VOLK, Saša, KLAVŽ, Janez, KURE, Viktor, ZUPANC, Mojca, STIBILJ, Vekoslava, HEATH, Ester, POLAK, Tomaž, ŽLENDER, Božidar, GOLOB, Terezija, HORVAT, Milena. Mercury, selenium, PCBs and fatty acids in fresh and canned fish on the Slovenian market. V: *ICMGP2009, 9th International Conference on Mercury as a Global Pollutant, June 7-12, 2009, Guiyang, China*. [S. l.: s. n.], 2009. [COBISS.SI-ID 22732327]

25. KRALJ CIGIĆ, Irena, MIKLAVČIČ, Ana, KOŠMERL, Tatjana, CIGIĆ, Blaž. Influence of freeze drying on the wine aroma = Vpliv liofilizacije na aromo vina. V: BAVCON KRALJ, Mojca (ur.), TREBŠE, Polonca (ur.). 15th International Symposium Spectroscopy in Theory and Practice = 15. mednarodni simpozij Spektroskopija v teoriji in praksi, Nova Gorica, Slovenija, 18.-21. april 2007. *Book of abstracts*. Nova Gorica: Univerza, 2007, str. 90. [COBISS.SI-ID 28568581]

1.16 Samostojni znanstveni sestavek ali poglavje v monografski publikaciji

26. HORVAT, Milena, SNOJ TRATNIK, Janja, MIKLAVČIČ, Ana. Mercury : biomarkers of exposure and human biomonitoring. V: KNUDSEN, Lisbet E. (ur.), MERLO, Domenico Franco (ur.). *Biomarkers and human biomonitoring. Volume 1, Ongoing programs and exposures*, (Issues in toxicology). Cambridge: Royal Society of Chemistry, 2011, str. 381-417. [COBISS.SI-ID 25408807]

MONOGRAFIJE IN DRUGA ZAKLJUČENA DELA

2.11 Diplomsko delo

27. MIKLAVČIČ, Ana. *Priprava in karakterizacija ekstraktov liofiliziranih vin : diplomsko delo, univerzitetni študij = Preparation and characterization of liophylizated wine extracts : graduation thesis, university studies*, (Biotehniška

fakulteta, Oddelek za živilstvo, Ljubljana, Diplomske naloge, 1280). Ljubljana: [A. Miklavčič], 2008. XIV, 92 f., graf. prikazi, tabele. http://www.digitalna-knjiznica.bf.uni-lj.si/dn_miklavcic_ana.pdf. [COBISS.SI-ID 3456632]

2.13 Elaborat, predštudija, študija

28. HORVAT, Milena, SNOJ TRATNIK, Janja, ŽIGON, Stojan, MAZEJ, Darja, PAVLIN, Majda, MIKLAVČIČ, Ana. *Stable isotope technique to assess human milk intake in infants living in areas contaminated with mercury, lead and cadmium - Slovenia : progress report for the period 2011/2012*, (IJS delovno poročilo, 11074). 2012. [COBISS.SI-ID 26009383]

29. HORVAT, Milena, MAZEJ, Darja, MILAČIČ, Radmila, SNOJ TRATNIK, Janja, MIKLAVČIČ, Ana, PLANINŠEK, Petra. *Stable isotope technique to assess human milk intake in infants living in areas contaminated with mercury, lead and cadmium - Slovenia : VA-6 varnostna analitika*, (IJS delovno poročilo, 10522). 2010. [COBISS.SI-ID 23841319]

30. HORVAT, Milena, PLANINŠEK, Petra, FAJON, Vesna, SNOJ TRATNIK, Janja, MIKLAVČIČ, Ana, MAZEJ, Darja. *Vsebnost živega srebra v krvi, laseh, urinu in materinem mleku pri dojecih materah na območju Ljubljane*, (IJS delovno poročilo, 10589). 2010. [COBISS.SI-ID 24187175]

31. MAZEJ, Darja, MIKLAVČIČ, Ana, JAĆIMOVIĆ, Radojko, HORVAT, Milena. *Determination of As, Cd, Pb and Hg in milk powder (FAPAS (PT 07120)) using CVAAS, ICP-MS and k0-INAA : results of the determination of trace elements and isotopic composition of [sup](13)C and [sup](15)N in four plant samples using k[sub]0-INAA, DMA and IRMS, provided by JSI*, (IJS delovno poročilo, 10282). 2009. [COBISS.SI-ID 23010087]

32. HORVAT, Milena, SNOJ TRATNIK, Janja, GIBIČAR, Darija, MIKLAVČIČ, Ana, ŽIGON, Stojan. *IAEA - CRP : Exposure to toxic and potentially toxic elements in women of childbearing age in developing countries : final report*, (IJS delovno poročilo, 10258). 2009. [COBISS.SI-ID 22754343]

33. BURNIK ŠTURM, Martina, JAĆIMOVIĆ, Radojko, MIKLAVČIČ, Ana, HORVAT, Milena. *International Plant-analytical Exchange Program, IPE 2009.2 : results of the determination of trace elements and isotopic composition of [sup](13)C and [sup](15)N in four plant samples using k[sub]0-INAA, DMA and IRMS, provided by JSI*, (IJS delovno poročilo, 10311). 2009. [COBISS.SI-ID 23009831]

34. ZULIANI, Tea, MILAČIČ, Radmila, ŠČANČAR, Janez, SNOJ TRATNIK, Janja, MIKLAVČIČ, Ana, HORVAT, Milena. *Analiza kadmija, bakra, celotnega kroma, svinca, niklja, cinka, vanadija in živega srebra v vzorcu površinske vode : Aquacheck EQS metals*, (IJS delovno poročilo, 9962). 2008. [COBISS.SI-ID 21759015]

35. KOTNIK, Jože, HORVAT, Milena, SNOJ TRATNIK, Janja, MIKLAVČIČ, Ana, FAJON, Vesna. *Testiranje primernosti EIGENBRODT "wet-only" vzorčevalnika za meritve živega srebra v padavinah*, (IJS delovno poročilo, 9936). 2008. [COBISS.SI-ID 21748775]

IZVEDENA DELA (DOGODKI)

3.16 Vabljeno predavanje na konferenci brez natisa

36. HORVAT, Milena, KOTNIK, Jože, KOCMAN, David, MIKLAVČIČ, Ana, ŽAGAR, Dušan, FAGANELI, Jadran, FAJON, Vesna, BEGU, Ermira, BRATKIČ, Arne, KORON, Neža. *Mercury in the Mediterranean : presented at Symposium of the Institute of oeanography and Fisheries, 26-27 February, 2013, Split, Croatia*. 2013. [COBISS.SI-ID 26572839]

PHIME Project

Brief questionnaire for mothers

Code No
|_|_|_|_|

Q1 Mother's name (First name and Last name)
……..…… ……..
Q2 Mother's date of birth |__|__| |__|__| |__|__|
mm dd yy
Q3 Mother's place of birth (Town and Country)
.....
Q4 Address……..……..……
Q5 Telephone number ……..…

Q6 Since what year have you been resident in Friuli-Venezia Giulia? |__|__|__|__|

Q7 Do you plan to move out of the region within the next 2 years? 1.YES 2. NO

Q8 Mother's usual occupation held in the past year ……..….
Q9 Years of schooling |__|__|

Q10 Number of children |__|__|
Q11 Birth date of youngest child |__|__| |__|__| |__|__|
mm dd yy

Q12 Father's name ..……..…….
Q13 Father's date of birth |__|__| |__|__| |__|__|
mm dd yy
Q14 Father's current occupation …….……..……..……
Q15 Years of schooling |__|__|

Q16 Due date |__|__| |__|__| |__|__|
mm dd yy
Q17 Pregnancy problems 1.YES 2. NO

Q18 What problems? ……..……..……...

Q19 Are you being treated for any illnesses? 1.YES 2. NO

Q20 What illnesses? ……..…….

Q21 Weight before pregnancy (kg) |__|__|__|
Q22 Height (cm) |__|__|__|

Q23 Smoking status:
Before pregnancy 1.YES 2. NO
Currently 1.YES 2. NO

(only for current smoker)
Q24 How many cigarettes do you smoke per day? |_|_|

Q25 How many times do you eat the following foods and beverages? (fill circle)

FOODS	FREQUENCY									
	Never	**Less than once per month**	**1-3 per month**	**1 per week**	**2 per week**	**3 per week**	**4 per week**	**5 per week**	**6 per week**	**At least once per day**
Vegetables* *(1 serving)*	1	2	3	4	5	6	7	8	9	10
Milk products° *(1 serving)*	1	2	3	4	5	6	7	8	9	10
Egg *(one)*	1	2	3	4	5	6	7	8	9	10
Meat *(1 serving, 150g)*	1	2	3	4	5	6	7	8	9	10
Fresh fish *(1 serving, 150g)*	1	2	3	4	5	6	7	8	9	10
Frozen fish *(1 serving, 150g)*	1	2	3	4	5	6	7	8	9	10
Tinned fish *(1 can, 80g)*	1	2	3	4	5	6	7	8	9	10
Alcoholic beverages^ *(1 serving)*	1	2	3	4	5	6	7	8	9	10

**For Vegetables: all vegetables except potatoes*
°For Milk products: 1 serving = 1 yogurt or 1 slice of cheese (100g) or 1 cup/glass of milk (200g)
^For Alcoholic beverages: 1 serving = 1 glass of wine (125ml) or 1 can of beer or 1small glass of spirits (30ml)

Q26 Intake of alcoholic beverages:
Before pregnancy 1.YES 2. NO
Currently 1.YES 2. NO

Q27 Is your alcohol intake concentrated during certain days of the week ? 1.YES 2. NO

Q28 If YES, how many day(s) per week? 1 2 3 4 5

Q29 Were you away from your current home during the past year? 1.YES 2. NO

Q30 How long? 1. number of weeks |__|__|
2. number of months |__|__|

Q31 Where? ……..……

Q32 Date of Hair Sampling |__|__| |__|__| |__|__|
mm dd yy

Q33 Place ……..……

Q34 Measurements
Hgtot |__|__|__|__|

Q35 Date of measurement |__|__| |__|__| |__|__|
mm dd yy

INSTRUCTIONS FOR COMPLETION

The objective of this study is to obtain information on the dietary habits of women during pregnancy.

The first part of this questionnaire consists of a series of general questions and following this section, there will be a series of questions that refer to the frequency with which you consume a portino of the foods listed. We ask that you compile these sections as clearly and completely as possibile using short answers when specifically requested and leaving fields blank where marked with an asterisk (*). All data will be used for scientific purposes only and according to the laws of privacy and confidentiality (D.Lgs n.196, 30 giugno 2003)

To respond you must place a single 'X in the box corresponding to your habits, if possible using only red, green, or blue ink. With the exception of some questions (indicated clearly on the questionnaire) there should be only one 'X' per line.

We request that you respond to all questions including for those food items you do not consume, by putting an 'X' in the box corresponding to 'never' . Before beginning we ask that you read attentively the various categories of foods indicated in the questionnaire and keep track of the time required to complete the questionnaire.

SOME EXAMPLES

General Questions

Indicate your response to each question by marking an 'X' or circling the corresponding number

Example:

Sex:

1. *Male*	*or*	**1**. *Male*
~~**2**. *Female*~~		(**2**. *Female*)

University of Udine - Chair of Hygiene and Epidemiology

EPIDEMIOLOGIC STUDY OF MATERNAL AND NEONATAL HEALTH

Questionnaire ID Number /_/_/_/_/*

Start Date dd /_/_/ **mm** /_/_/ **yy** /_/_/_/_/
Start time hh/_/_/:mm/_/_/

GENERAL DATA -- MOTHER

Family Name & Name _____
Home Address: Street _____ **n°** _____
City _____ /_/_/_/_/_/_/_/*
Province _____ /_/_/_/_/_/_/_/*
Postal Code /_/_/_/_/_/

Date of birth: dd_/_/ **mm** /_/_/ **yyyy** /_/_/_/_/

Place of Birth:
City _____/_/_/_/_/_/_/_/*
Province_____/_/_/_/_/_/_/_/*
Postal Code /_/_/_/_/_/_/*

Country of Birth _____/_/_/_/_/*

Marital Status:

1. married/living together
2. widow
3. separated/divorced
4. single/never married

HEIGHT AND WEIGHT

Height (in cm) /_/_/_/
Weight (in kg) **before pregnancy** /_/_/_/ **at delivery** /_/_/_/

EDUCATIONAL TITLES

1. none
2. elementary school diploma
3. middle school diploma
4. high school diploma
5. university degree

OCCUPATION

Currently you are:

1. employed on maternity leave/leave of absence
2. employed worker
3. housewife
4. in search of employment
5. student
6. resigned from work
7. other conditions (please specify)_____/_/_/_/_/

If you have worked during this pregnancy, please indicate your occupation
_____/_/_/_/_/*

and the sector_____/_/_/_/_/*

To which of the following employment categories do you belong?

1. manager/entrepreneur
2. professional/specialist
3. technician/associate professional
4. clerk
5. service worker or shop and market sales worker
6. craft worker/skilled and related trade worker
7. plant and machine operators and assemblers
8. elementary occupations
9. armed forces
10. farmer/animal farmer
11. fisherman

Until which month did you work ? mm /_/_/

HUSBAND/LIVE-IN MATE

Nazione di nascita _____/_/_/_/_/*

Highest educational title

1. none
2. elementary school diploma
3. middle school diploma
4. high school diploma
5. university degree

Currently he is:

1. employed worker/leave of absence
2. househusband
3. in search of employment
4. student
5. resigned from work
6. other conditions (please specify)_____/_/_/_/_/

If he works/worked, please indicate his occupation

_____/_/_/_/_/*

and the sector_____/_/_/_/_/*

To which of the following employment categories does he belong?

1. manager/entrepreneur
2. professional/specialist
3. technician/associate professional
4. clerk
5. service worker or shop and market sales worker
6. craft worker/skilled and related trade worker
7. plant and machine operators and assemblers
8. elementary occupations
9. armed forces
10. farmer/animal farmer
11. fisherman

Height (in cm) /_/_/_/
Weight (in kg) /_/_/_/

LIVING CONDITIONS

The house in which you live is:

1. owned
2. rent/lease
3. guest
4. live with parents/in-laws

How many bedrooms are there in your home? /_/

What is the surface area of your home?

1. less than 50 m^2
2. 50-100 m^2
3. more than 100 m^2

How many persons live in your home (including you and your newborn)?
Adults (of age) **/_/**
Children (under age) **/_/**

In the family, how many cars do you have? (if none, write 0) /_/

INFORMATION ON YOUR CHILD

Family Name & Name _____

Date of birth: **dd /_/_/** **mm /_/_/** **yyyy /_/_/_/_/**
Sesso

1. Male
2. Female

Birthweight **gm** /_/_/_/_/

Length at Birth **cm** /_/_/

The delivery was:

1. Spontaneous vaginal delivery
2. Induced with medications
3. Planned Cesarean Section
4. Emergency Cesarean Section
5. Vacuum
6. Rotation of breech presentation
7. Forceps

After delivery did your newborn have any of the following conditions?

	yes	**no**
jaundice	θ	θ
infection	θ	θ
respiratory difficulty	θ	θ
convulsions	θ	θ

Since birth has he/she been readmitted to the hospital?

1. Yes
2. No

If yes, please indicate the details relative to each admission:

Age in weeks	Reason for admission	Ward and hospital	Treatments

Excluding any hospital admissions, has your newborn required medical treatment?

1. Yes
2. No

If yes, please indicate the details relative to each episode:

Problem	Treatment

HEALTH STATUS AND MEDICAL HISTORY

Was the recent pregnancy your first?

1. Yes
2. No

If no, how many pregnancies have you had previously? /_/

Please indicate the year and outcome of each previous pregnancy:

Pregnancy	Year	Outcome
1	/_/_/_/_/	**1.**live full term birth **2.live pre term birth** **3.**live underweight birth **4.**live birth with malformations/genetic syndromes **5. miscarriage** **6.still birth**
2	/_/_/_/_/	**1.**live full term birth **2.live pre term birth** **3.**live underweight birth **4.**live birth with malformations/genetic syndromes **5. miscarriage** **6.still birth**
3	/_/_/_/_/	**1.**live full term birth **2.live pre term birth** **3.**live underweight birth **4.**live birth with malformations/genetic syndromes **5. miscarriage** **6.still birth**
4	/_/_/_/_/	**1.**live full term birth **2.live pre term birth** **3.**live underweight birth **4.**live birth with malformations/genetic syndromes **5. miscarriage** **6.still birth**
5	/_/_/_/_/	**1.**live full term birth **2.live pre term birth** **3.**live underweight birth **4.**live birth with malformations/genetic syndromes **5. miscarriage** **6.still birth**

What is the actual health status of:

Father of the child	1. living in good health	
	2. living with illness: _____	
	3. deceased: cause _____	
		age at death: years |__|__|
Other Chidren (excluding the newborn)		
Child 1	1. living in good health	
	2. living with illness: _____	
	3. deceased: cause _____	
		age at death: years |__|__|
Child 2	1. living in good health	
	2. living with illness: _____	
	3. deceased: cause _____	
		age at death: years |__|__|
Child 3	1. living in good health	
	2. living with illness: _____	
	3. deceased: cause _____	
		age at death: years |__|__|
Child 4	1. living in good health	
	2. living with illness: _____	
	3. deceased: cause _____	
		age at death: years |__|__|

Has anyone in your family suffered, or died from:

Cardiovascular Disease	**1**.no one	**2**.father of the child	**3**.other child	**4**.grandparent of child	**5**.aunt/uncle of child
Neurologic Disease	**1**.no one	**2**.father of the child	**3**.other child	**4**.grandparent of child	**5**.aunt/uncle of child
Genetic Disease	**1**.no one	**2**.father of the child	**3**.other child	**4**.grandparent of child	**5**.aunt/uncle of child
Tumours	**1**.no one	**2**.father of the child	**3**.other child	**4**.grandparent of child	**5**.aunt/uncle of child
Diabetes mellitus	**1**.no one	**2**.father of the child	**3**.other child	**4**.grandparent of child	**5**.aunt/uncle of child
Hypertension	**1**.no one	**2**.father of the child	**3**.other child	**4**.grandparent of child	**5**.aunt/uncle of child

Prima della gravidanza soffriva o in gravidanza ha sofferto di:

Diabetes	**1**.no, never	**2**.yes, only in pregnancy	**3**.yes, only before the pregnancy	**4**.yes, both before and during pregnancy
Asthma	**1**.no, never	**2**.yes, only in pregnancy	**3**.yes, only before the pregnancy	**4**.yes, both before and during pregnancy
Allergy	**1**.no, never	**2**.yes, only in pregnancy	**3**.yes, only before the pregnancy	**4**.yes, both before and during pregnancy
Epilepsy	**1**.no, never	**2**.yes, only in pregnancy	**3**.yes, only before the pregnancy	**4**.yes, both before and during pregnancy
Hypertension (high blood pressare)	**1**.no, never	**2**.yes, only in pregnancy	**3**.yes, only before the pregnancy	**4**.yes, both before and during pregnancy
Vomiting	**1**.no, never	**2**.yes, only in pregnancy	**3**.yes, only before the pregnancy	**4**.yes, both before and during pregnancy
Hypothyroidism	**1**.no, never	**2**.yes, only in pregnancy	**3**.yes, only before the pregnancy	**4**.yes, both before and during pregnancy
Hyperthyroidism	**1**.no, never	**2**.yes, only in pregnancy	**3**.yes, only before the pregnancy	**4**.yes, both before and during pregnancy
Lupus	**1**.no, never	**2**.yes, only in pregnancy	**3**.yes, only before the pregnancy	**4**.yes, both before and during pregnancy
Rheumatologic Disease	**1**.no, never	**2**.yes, only in pregnancy	**3**.yes, only before the pregnancy	**4**.yes, both before and during pregnancy
Urinary Infections	**1**.no, never	**2**.yes, only in pregnancy	**3**.yes, only before the pregnancy	**4**.yes, both before and during pregnancy
Infections	**1**.no, never	**2**.yes, only in pregnancy	**3**.yes, only before the pregnancy	**4**.yes, both before and during pregnancy
Fever	**1**.no, never	**2**.yes, only in pregnancy	**3**.yes, only before the pregnancy	**4**.yes, both before and during pregnancy
Convulsions/Seizures	**1**.no, never	**2**.yes, only in pregnancy	**3**.yes, only before the pregnancy	**4**.yes, both before and during pregnancy
Anemia	**1**.no, never	**2**.yes, only in pregnancy	**3**.yes, only before the pregnancy	**4**.yes, both before and during pregnancy
Cardiovascular Disease	**1**.no, never	**2**.yes, only in pregnancy	**3**.yes, only before the pregnancy	**4**.yes, both before and during pregnancy
Neurologic Disease	**1**.no, never	**2**.yes, only in pregnancy	**3**.yes, only before the pregnancy	**4**.yes, both before and during pregnancy
Genetic Disease	**1**.no, never	**2**.yes, only in pregnancy	**3**.yes, only before the pregnancy	**4**.yes, both before and during pregnancy
Tumours	**1**.no, never	**2**.yes, only in pregnancy	**3**.yes, only before the pregnancy	**4**.yes, both before and during pregnancy

Have you taken any medications in pregnancy?

1. Yes
2. No

If yes, which?

Brand/Commercial Name	Reason for taking?	From (month)	To (month)	Times per day

How many prenatal visits did you have during the pregnancy? /_/_/

How many sonograms did you have during the pregnancy? /_/_/

During pregnancy, did you have any dental visits?

1. Yes
2. No

If yes, did you have any work on dental fillings?

1. Yes
2. No

How many fillings do you have?

1. less than 3
2. from 3 to 5
3. from 5 to 9
4. 10 or more
5. I don't know

SMOKING

Are you a smoker?

1. No, I have never smoked
2. Yes, I smoke
3. Ex-smoker: I smoked but I quit before the last pregnancy
4. Ex-smoker: I smoked but I quit after the last pregnancy

If you smoke or smoked, at what age did you start? /_/_/

How much do you smoke/smoked on average daily?

	Before pregnancy	During Pregnancy	Now
Cigarette	/_/_/	/_/_/	/_/_/
Cigars	/_/_/	/_/_/	/_/_/
Pipe	/_/_/	/_/_/	/_/_/

If you smoke or smoked, did you quit during the pregnancy?

1. Yes, in the /_/ month
2. No

If you are an ex-smoker, at what age did you quit smoking? /_/_/

Do you identify yourself with one or more of the following situations?

	Before pregnancy	During Pregnancy	Now
I live with a smoker(s)	θ	θ	θ
I work in an environment in which many people smoke	θ	θ	θ

Breastfeeding

Until now, how has your baby been breastfed in each week?

Type of Breastfeeding	**Week**					
EXCLUSIVE BREASTFEEDING Maternal milk sucked or expressed manually, drops or medicinal syrups (vitamins, minerals and medications) and nothing else	1	2	3	4	5	6
PREDOMINANT BREASTFEEDING Maternal milk sucked or expressed manually, drops or medicinal syrups (vitamins, minerals and medications) with the addition of non nutritive liquids (plain or sweetened water, water based drinks like tea, cammomile, herbal infusions, oral rehydration solutions)	1	2	3	4	5	6
PARTIAL BREASTFEEDING Maternal milk sucked or expressed manually, drops or medicinal syrups (vitamins, minerals and medications) with the addition of nutritive liquid foods (liquid orpowdered milk)	1	2	3	4	5	6
ARTIFICIAL FEEDING Nutrition without maternal milk	1	2	3	4	5	6

GENERAL CHARACTERISTICS OF DIETARY HABITS

During the pregnancy did you happen to eat differently than use for any period of time?

1. Yes
2. No

If yes, for which reasons (external to the pregnancy) ?

1. I changed jobs
2. I was following a particular diet (please specify the reason for the diet.....)
3. I was ill
4. other, please specify.....

If yes, for which reasons (linked to the pregnancy) ?

1. nausea
2. vomiting
3. abdominal pain/reflux/heartburn
4. other, please specify.....

	never	Less than once per week	Once per week	2-4 times per week	5-6 times per week.	7-8 times per week.	9-10 times per week	11-12 times per week .	More than 12 times per week
With what frequency did you consume **lunch or dinner** away from home? (e.g. at work, in a bar, restaurant, home of friends, etc.)?	θ	θ	θ	θ	θ	θ	θ	θ	θ

During the pregnancy did you use any **vitamins, minerals or other supplements**?

1. Yes
1. No

If yes, please indicate the exact name of the supplement	Irregular use	Regular use (please indicate the daily dose in number of tablets, drops or mL)	For how many months did you take the supplement?
	θ	θ	**1**.<1 month **2**.1-3 months **3**.4-6 months **4**.7-9 months **5**.I don't know
	θ	θ	**1**.<1 month **2**.1-3 months **3**.4-6 months **4**.7-9 months **5**.I don't know
	θ	θ	**1**.<1 month **2**.1-3 months **3**.4-6 months **4**.7-9 months **5**.I don't know
	θ	θ	**1**.<1 month **2**.1-3 months **3**.4-6 months **4**.7-9 months **5**.I don't know

In the 3 months preceding the pregnancy, did you consume folic acid (folate)?

1. Yes
2. No

Period	If yes, please indicate the exact name of the supplement	Irregular use	Regular use (please indicate the daily dose in number of tablets, drops or mL)
3 months before the pregnancy		θ	θ
2 months before the pregnancy		θ	θ
1 month before the pregnancy		θ	θ

What fats/oils di you use normally *(even more than 1 type):*	**6. one**	**Olive oil**	**Virgin or extra virgin olive oil**	**Oil of sansa**	**Sunflower, corn or soy oil**	**Peanut oil**	**Mixed seed oil**	**7. utter**	**8. argarine spreadable (tub)**	**9. argarine hard (stick)**	**White sauce**	**Shortening**	**10. don't know**
On bread	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
To season raw vegetables	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
To season or prepare cooked vegetables	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
To cook meat	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
To cook fish	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
To fry foods	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
To season pasta (noodles) or rice	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
To cook the sauce for the pasta	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
For baking	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ

Could you tell us how much olive oil <u>virgin or extra virgin</u> you ate each day, either cooked or uncooked ? *Consider all cooked dishes, vegetables, salads, oil added at table (e.g. in soups, vegetables, on bread, etc.)*	**I did not eat olive oil**	**Less than 1 tablespoon**	**1-3 tablespoons**	**4-6 tablespoons**	**More than 6 tablespoons**	**I don't know**	**Also home made or produced?**
	θ	θ	θ	θ	θ	θ	θ

Some types of fruit can be consumed with or without the skin. Which of the following fruits did you consume usually with the skin and which without?

With the skin	θ apple	θ pear	θ peach	θ nectarine, plum	θ apricot	θ grape	θ fig
Without the skin	θ apple	θ pear	θ peach	θ nectarine, plum	θ apricot	θ grape	θ fig

DIETARY HABITS

HOT BEVERAGES – MILK – SUGAR		FREQUENCY										
		never	Less than once per month	1-3 times per month	Once per week	2-4 times per week.	5-6 times per week	1 time per day	2-3 times per day	4-5 times per day	More than 5 times per day	Also home made or produced?
Tea	(1 coffee cup/mug)	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Herbal tea, decaffeinated tea, etc.	(1 coffee cup/mug)	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Coffee	(1 espresso cup)	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Decaffeinated coffee	(1 espresso cup)	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Milk in coffee or tea	(1 tablespoon)	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Cappuccino or Coffee Latte	(1 coffee cup/mug)	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Hot chocolate	(1 coffee cup/mug)	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Other milk, whole	(1 coffee cup/mug, glass)	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Other milk, skim or low fat	(1 coffee cup/mug, glass))	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Plain or fruit yogurt, whole	(1 jar)	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Plain or fruit yogurt, low fat	(1 jar)	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Sugar, added to drinks or other foods	(1 teaspoon)	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Artificial sweeteners, saccharin, aspartame, etc.	(1 packet)	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Powdered cocoa	(1 tablespoon)	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ

BREAD – CEREALS - FIRST COURSE	never	Less than once per month	1-3 times per month	Once per week	2-4 times per week.	5-6 times per week	1 time per day	2-3 times per day	More than 3 times per day	Also home made or produced ?
Bread (1 roll, 50g)	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Whole wheat bread (1 roll, 50g)	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Crackers (1 pacchetto), breadsticks (n. 5), melba toast (n. 3)	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Whole wheat crackers (1 packet), Whole wheat breadsticks (n. 5), Whole wheat melba toast (n. 3)	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Breakfast cereals: e.g. cornflakes, rice crispies, muesli, etc. (1 portion, about 30 g)	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Pizza bread (1 piece)	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Pizza (1 plate or 1 slice)	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Polenta (1 slice)	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Pasta or rice with oil or butter, risotto (1 plate)	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Pasta or rice with tomato sauce (1 plate)	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Pasta or rice with meat sauce (1 plate)	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Pasta or rice with pesto sauce (1 plate)	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Pasta or rice with vegetables (broccoli, zucchini, etc.) (1 plate)	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Lasagna, cannelloni, tortellini, etc. with meat sauce (1 plate)	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Lasagna, cannelloni, tortellini, etc. di magro (1 plate)	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Broth – also with con pasta or rice (1 plate)	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Vegetable soup, stew, pasta with beans (1 plate)	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Soups or other first courses with cereals like: barley, spelt, pearl millet (1 plate)	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Grated cheese, on first course pasta and soups (1 teaspoon)	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ

MAIN COURSE		never	Less than once per month	1-3 times per month	Once per week	2-4 times per week.	5-6 times per week	1 time per day	2-3 times per day	More than 3 times per day	Also home made or produced?
Egg: hard-boiled, soft-boiled, poached o raw	(n. 1)	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Egg: fried, scrambled, omelette	(n. 1)	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Chicken or turkey with little added seasoning or boiled	(200 g)	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Chicken or turkey roasted, fried or stewed	(200 g)	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Rabbit, prepared in any manner	(200 g)	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Lean meats: veal, beef, or horse *(roast beef, steak, etc)*	(120g)	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Meat stew (gulasch), pot roast, meatballs, cutlet, roast etc. veal, beef, or horse	(150 g	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Pork, prepared in any manner	(150 g)	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Lamb, prepared in any manner	(150 g)	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Liver meats any animal	(150 g)	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Other organ meats: heart, brain, kidneys, thymus, etc.	(150 g)	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Raw ham, smoked ham, dried beef	(4 fette)	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Cooked ham	(4 fette)	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Salami, mortadella, bacon, etc.	(50 g)	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Sausage, frankfurters, cotechino, (*Cevapcici, musetto, etc)*	(120 g)	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Wild game meats *(hare, pheasant, venison, etc)*	(150g)	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Fish: boiled, grilled, baked	(150 g)	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Shellfish: boiled, grilled, baked	(150 g)	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Fried Fish	(150 g)	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Fried shellfish	(150 g)	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Tuna, mackerel, sardines in oil	(1 small can, single portion)	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Ricotta or mozzarella, as a main course	(100 g)	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Other cheeses, as a main course	(80 g)	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Cheese as a last course or snack	(25 g)	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ

VEGETABLES	never	Less than once per month	1-3 times per month	Once per week	2-4 times per week	5-6 times per week	1 time per day	2-3 times per day	More than 3 times per day	Also home made or produced?
Beetroot (1 portion)	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Carrots (1 portion)	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Artichokes (n. 1), cardoon (1 portion)	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Cauliflower (1 portion)	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Cabbage, savoy cabbage, broccoli, turnip greens, brussel sprouts (1 portion)	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Onions, raw or cooked, scallions, leeks (excluding onions used in seasoning) (n. 1)	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Green beans (1 portion)	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Fennel, raw or cooked (1 portion)	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Mushrooms (1 portion)	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Green or red salad (1 portion)	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Mixed salad with carrots, tomatoes, cucumbers, etc. (1 portion)	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Legumes: beans, chick peas, lentils, peas, etc. (1 portion)	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Eggplant, aubergine (1 portion)	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Potatoes: mashed or boiled (1 portion)	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Potatoes : fried or roasted (1 portion)	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Peppers, sweet (n. 1)	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Tomatoes (1 medium)	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Spinach, swiss chard, chicory (1 portion)	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Zucchini (1 portion)	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ

FRUIT		never	Less than once per month	1-3 times per month	Once per week	2-4 times per week.	5-6 times per week	1 time per day	2-3 times per day	More than 3 times per day	Also home grown?
Apples	(n. 1)	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Pears	(n. 1)	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Oranges	(n. 1)	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Mandarin oranges (n. 2), tangerines (n. 3), other citrus fruits (mapo, etc)		θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Grapefruit	(half)	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Lemons: e.g. on vegetables, in tea or other	(1 squeeze)	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Fresh squeezed citrus fruits: orange, g.fruit, lemon, etc.	(1 glass)	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Watermelon	(1 slice)	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Banana	(n. 1)	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Strawberries	(1 cup, 150 gr)	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Cherries	(150 gr)	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Kiwi	(n. 1)	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Melon	(2 slices)	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Peaches (n. 1), nectarines (n. 1), apricots	(n. 2)	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Plums	(n. 2)	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Figs	(n.2, medium)	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Grapes	(1 bunch)	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Pineapple	(2 slice)	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Other exotic fruits _____	(1 piece, 1 slice)	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Cooked fruits, sugared fruits, mixed fruits	(1 small cup)	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Dried fruits: walnuts, hazelnuts, almonds, etc.	(1 handful)	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Raisins, dried prunes, dried figs, etc.	(1 handful)	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ

SWEETS – VARIOUS FOODS	never	Less than once per month	1-3 times per month	Once per week	2-4 times per week.	5-6 times per week	1 time per day	2-3 times per day	More than 3 times per day
Cookies (all types) (n. 7)	θ	θ	θ	θ	θ	θ	θ	θ	θ
Whole wheat cookies (n. 7)	θ	θ	θ	θ	θ	θ	θ	θ	θ
Croissant, donuts, commercially prepared cakes, sponge cakes or other leavened cakes, without cream (n. 1 or 1 slice)	θ	θ	θ	θ	θ	θ	θ	θ	θ
Pastries with cream (1 large or 3 small), cream filled donuts (n. 1)	θ	θ	θ	θ	θ	θ	θ	θ	θ
Fruit or jam tart (1 slice) Pastries with fruit (1 large or 3 small)	θ	θ	θ	θ	θ	θ	θ	θ	θ
Puddings, cream caramel, spoon desserts (1 small cup)	θ	θ	θ	θ	θ	θ	θ	θ	θ
Chocolate (10 gr), chocolate candy (n. 1)	θ	θ	θ	θ	θ	θ	θ	θ	θ
Chocolate bar: like Mars, Kit-Kat, Bounty, etc. (n. 1)	θ	θ	θ	θ	θ	θ	θ	θ	θ
Candies "normal" (n. 1)	θ	θ	θ	θ	θ	θ	θ	θ	θ
Candies "light" (without sugar) (n. 1)	θ	θ	θ	θ	θ	θ	θ	θ	θ
Chewing gum "normal" (n. 1)	θ	θ	θ	θ	θ	θ	θ	θ	θ
Chewing gum "light" (without sugar) (n. 1)	θ	θ	θ	θ	θ	θ	θ	θ	θ
Ice cream (n. 1)	θ	θ	θ	θ	θ	θ	θ	θ	θ
Jam or honey (1 teaspoon)	θ	θ	θ	θ	θ	θ	θ	θ	θ
Butter on bread (5 g)	θ	θ	θ	θ	θ	θ	θ	θ	θ
Olive oil on bread (10 g)	θ	θ	θ	θ	θ	θ	θ	θ	θ
Green or black olives (natural or salted) (5 g)									
Mayonnaise, pink sauce, tuna sauce, etc. (1 tablespoon)	θ	θ	θ	θ	θ	θ	θ	θ	θ
Pickled vegetables (pickles, spring onions, mixed, etc) (50 g)	θ	θ	θ	θ	θ	θ	θ	θ	θ
Potato chips, salted snacks, etc. (1 handful)	θ	θ	θ	θ	θ	θ	θ	θ	θ
Pop corn	θ	θ	θ	θ	θ	θ	θ	θ	θ

NON ALCOHOLIC BEVERAGES	never	Less than once per month	1-3 times per month	Once per week	2-4 times per week.	5-6 times per week	1 time per day	2-3 times per day	4-5 times per day	More than 5 times per day
Coca-Cola, Pepsi-Cola, Chinotto (1 glass)	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Other carbonated drinks: orange soda, club soda, aperitifs Non alcoholic, etc. (1 glass)	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
"Light" drinks (without sugar) (1 glass)	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Commercial fruit juices (1 glass)	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Mineral water (with gas or natural) (1 glass)	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Tap or Faucet water (1 glass)	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Well or tank water (1 glass)	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ

ALCOHOLIC BEVERAGES	never	Less than once per month	1-3 times per month	Once per week	2-4 times per week.	5-6 times per week	1 time per day	2-3 times per day	4-5 times per day	More than 5 times per day
White wine, sparkling wine, champagne (1 glass)	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Vino rosso o rosato (1 glass)	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Beer (1 can)	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Alcoholic aperitifs: e.g. vermouth, Campari, Marsala, (n. 1)	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Spirits, digestifs, sweet liqueurs (1 small glass)	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Whisky, cognac, brandy, grappa, vodka, acquavite, tequila etc. (1 small glass)	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ

SPECIAL SECTION. These questions refer to your consumption of some particular types of FISH and their origin.

Dishes based on	FREQUENCY								Type					
	never	Less than once per month	1-3 times per month	Once per week	2-4 times per week.	5-6 times per week	1 time per day	More than 2 times per day	Fresh from the region	Fresh from other origin	Fresh, I don't know the origin	Frozen	Canned	I don't know
Fish broth	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Eel	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Sea Bass	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Gilt head bream	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Grey Mullet	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Riboni	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Dover Sole	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Tuna	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Blue Whiting	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Piked dogfish (Asià)	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Mackerel	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Smooth dogfish	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Plaice	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Monkfish	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Shark	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
John Dory	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Anchovy	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Sardine	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Mediterranean shad	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Trout/salmon	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
European Hake/Cod	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ
Other fish (specify)														
	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ	θ

During the pregnancy, how often on average did you eat fish in a restaurant?

1. never
2. less than once per month
3. 1-3 times per month
4. 1 time per week
5. 2-4 times per week
6. more than 4 times per week

If you are breastfeeding, how often do you eat fish now?

		never	less than once per month	1-3 times per month	1 time per week	2-4 times per week	5-6 times per week	1 time per day	2-3 times per day	More than 3 times per day
Fish: boiled, grilled, baked	(150 g)	θ	θ	θ	θ	θ	θ	θ	θ	θ
Shellfish: boiled, grilled, baked	(150 g)	θ	θ	θ	θ	θ	θ	θ	θ	θ
Fried Fish	(150 g)	θ	θ	θ	θ	θ	θ	θ	θ	θ
Fried shellfish	(150 g)	θ	θ	θ	θ	θ	θ	θ	θ	θ
Tuna, mackerel, sardines in oil	(1 small can, single portion)	θ	θ	θ	θ	θ	θ	θ	θ	θ

Where do you usually obtain <u>fresh fish</u> during the <u>winter</u> (2 primary) ?

1. fish shop or stand in supermarket
2. local fish market
3. supermarket
4. direct from fisherman
5. fished for myself
6. other:.....
7. I don't buy fresh fish

Can you indicate the location (municipality, division, province) of your usual supplier of <u>fresh fish</u> in the <u>winter</u>? (2 primary)

1. Municipality/subdivision _____**/_/_/_/_/_/_/***

Province _____**/_/_/_/_/_/_/***

2. Municipality/subdivision _____**/_/_/_/_/_/_/***

Province _____**/_/_/_/_/_/_/***

Where do you usually obtain <u>fresh fish</u> during the <u>summer</u> (2 primary) ?

1. fish shop or stand in supermarket
2. local fish market
3. supermarket
4. direct from fisherman
5. fished for myself
6. other:.....
7. I don't buy fresh fish

Can you indicate the location (municipality, division, province) of your usual supplier of <u>fresh fish</u> in the <u>summer</u>? (2 primary)

1. Municipality/subdivision _____**/_/_/_/_/_/_/***

Province_____**/_/_/_/_/_/_/***

2. Municipality/subdivision_____**/_/_/_/_/_/_/***

Province _____**/_/_/_/_/_/_/***

OTHER FOODS consumed <u>at least once per week</u>. Please specify the foods:	**F R E Q U E N CY**						
	once per week	**2-4 times per week**	**5-6 times per week**	**1 time per day**	**2-3 times per day**	**4-5 times per day**	**More than 5 times per day**
	θ	θ	θ	θ	θ	θ	θ
	θ	θ	θ	θ	θ	θ	θ
	θ	θ	θ	θ	θ	θ	θ
	θ	θ	θ	θ	θ	θ	θ
	θ	θ	θ	θ	θ	θ	θ
	θ	θ	θ	θ	θ	θ	θ

In general, with what frequency did you consume a portion of the following foods?	**F R E Q U E N C Y**								
	never	**Less than once per week**	**1 time per week**	**2-4 times per week**	**5-6 times per week**	**1 time per day**	**2-3 times per day**	**4-5 times per day**	**More than 5 times per day**
First courses pastas (e.g. pasta with sauce, rice, etc.)	θ	θ	θ	θ	θ	θ	θ	θ	θ
First course soups/broth (e.g. vegetable soups, purees, etc.)	θ	θ	θ	θ	θ	θ	θ	θ	θ
Main courses with chicken/turkey (prepared in any manner)	θ	θ	θ	θ	θ	θ	θ	θ	θ
Main courses with other types of meats (prepared in any manner)	θ	θ	θ	θ	θ	θ	θ	θ	θ
Main courses with fish (prepared in any manner)	θ	θ	θ	θ	θ	θ	θ	θ	θ
A portion of deli meats/cold cuts	θ	θ	θ	θ	θ	θ	θ	θ	θ
A portion of cheese	θ	θ	θ	θ	θ	θ	θ	θ	θ
Cooked or raw vegetables, including salads	θ	θ	θ	θ	θ	θ	θ	θ	θ
Fresh fruits	θ	θ	θ	θ	θ	θ	θ	θ	θ

RESIDENTIAL HISTORY IN THE LAST 5 YEARS

How long have you lived in your present home?

from: mm/_/_/ yy /_/_/

In which part of the city do you live?

1. Center
2. Periphery (suburb)
3. Rural area

Your home is located less than 1 km from:

Industry

1. Yes. Please specify names _____
2. No

Highway

1. Yes
2. No

State road

1. Yes
2. No

Provincial road

1. Yes
2. No

Other high traffic roads

1. Yes
2. No

Train station

1. Yes
2. No

Airport

1. Yes
2. No

From your house, have you ever noticed odors originating from the following sources?

	Never	Sometimes	Often
Agriculture	θ	θ	θ
Industry	θ	θ	θ

<u>During the course of the last 5 years</u>, have you ever changed homes?

1. Yes
2. No

If "yes", please describe your previous residences starting with the most recent. Consider only the residences in which you lived for <u>at least 6 months</u>.

<u>1st PREVIOUS RESIDENCE</u>

How long have you lived in your present home?

from: mm/_/_/ yy /_/_/ to: mm/_/_/ yy /_/_/

In which part of the city do you live?

4. Center
5. Periphery (suburb)
6. Rural area

Your home is located less than 1 km from:

Industry

3. Yes. Please specify names _____
4. No

Highway

3. Yes
4. No

State road

3. Yes
4. No

Provincial road

3. Yes
4. No

Other high traffic roads

3. Yes
4. No

Train station

3. Yes
4. No

Airport

3. Yes
4. No

From your house, have you ever noticed odors originating from the following sources?

	Never	Sometimes	Often
Agriculture	θ	θ	θ
Industry	θ	θ	θ

2nd PREVIOUS RESIDENCE

How long have you lived in your present home?

from: mm/_/_/ yy /_/_/ to: mm/_/_/ yy /_/_/

In which part of the city do you live?

1. Center
2. Periphery (suburb)
3. Rural area

Your home is located less than 1 km from:

Industry

1. Yes. Please specify names _____
2. No

Highway

1. Yes
2. No

State road

1. Yes
2. No

Provincial road

1. Yes
2. No

Other high traffic roads

1. Yes
2. No

Train station

1. Yes
2. No

Airport

1. Yes
2. No

From your house, have you ever noticed odors originating from the following sources?

	Never	Sometimes	Often
Agriculture	θ	θ	θ
Industry	θ	θ	θ

3rd PREVIOUS RESIDENCE

How long have you lived in your present home?

from: mm/_/_/ yy /_/_/ to: mm/_/_/ yy /_/_/

In which part of the city do you live?

4. Center
5. Periphery (suburb)
6. Rural area

Your home is located less than 1 km from:

Industry

3. Yes. Please specify names _____
4. No

Highway

3. Yes
4. No

State road

3. Yes
4. No

Provincial road

3. Yes
4. No

Other high traffic roads

3. Yes
4. No

Train station

3. Yes
4. No

Airport

3. Yes
4. No

From your house, have you ever noticed odors originating from the following sources?

	Never	Sometimes	Often
Agriculture	θ	θ	θ
Industry	θ	θ	θ

OCCUPATIONAL HISTORY IN THE LAST 5 YEARS

In the last 5 years, have you been employed at least once?

1. Yes
2. No

If yes, please describe your work if performed for <u>at least 6 months.</u>

<u>MOST RECENT WORK</u>

For how long have you been working (or worked) for this company, office, etc. ?
from: mm/_/_/ yy /_/_/ to: mm/_/_/ yy /_/_/

What are (were) your principal activities?

_____
_____ **/_/_/_/_/_/***

What was the principal activity of the company, office, etc.?
Activity: …… **/_/_/_/_/_/***
Name: …… **/_/_/_/_/_/***
Municipality: …… **/_/_/_/_/_/***
Province/Country: …… **/_/_/_/_/_/***

Do you hold a second job?

1. Yes. Which: _____ **/_/_/_/_/_/***
2. No

<u>1st PREVIOUS JOB</u>

For how long did you work for this company, office, etc. ?
from: mm/_/_/ yy /_/_/ to: mm/_/_/ yy /_/_/

What were your principal activities?

_____
_____ **/_/_/_/_/_/***

What was the principal activity of the company, office, etc.?
Activity: …… **/_/_/_/_/_/***
Name: …… **/_/_/_/_/_/***
Municipality: …… **/_/_/_/_/_/***
Province/Country: …… **/_/_/_/_/_/***

2nd PREVIOUS JOB

For how long did you work for this company, office, etc. ?
from: mm/_/_/ yy /_/_/ to: mm/_/_/ yy /_/_/

What were your principal activities?

_____

_____ **/_/_/_/_/_/***

What was the principal activity of the company, office, etc.?
Activity: …… **/_/_/_/_/_/***
Name: …… **/_/_/_/_/_/***
Municipality: …… **/_/_/_/_/_/***
Province/Country: …… **/_/_/_/_/_/***

3rd PREVIOUS JOB

For how long did you work for this company, office, etc. ?
from: mm/_/_/ yy /_/_/ to: mm/_/_/ yy /_/_/

What are were your principal activities?

_____

_____ **/_/_/_/_/_/***

What was the principal activity of the company, office, etc.?
Activity: …… **/_/_/_/_/_/***
Name: …… **/_/_/_/_/_/***
Municipality: …… **/_/_/_/_/_/***
Province/Country: …… **/_/_/_/_/_/***

4th PREVIOUS JOB

For how long did you work for this company, office, etc. ?
from: mm/_/_/ yy /_/_/ to: mm/_/_/ yy /_/_/

What were your principal activities?

_____

_____ **/_/_/_/_/_/***

What was the principal activity of the company, office, etc.?
Activity: …… **/_/_/_/_/_/***
Name: …… **/_/_/_/_/_/***
Municipality: …… **/_/_/_/_/_/***
Province/Country: …… **/_/_/_/_/_/***

You have now completed the questionnaire.

Date of completion: dd /_/_/ mm /_/_/ yy /_/_/_/_/
Time at completion: hh/_/_/:mm/_/_/
Please estimate the actual time required to complete the questionnaire:
hh/_/_/ mm/_/_/

WE THANK YOU VERY MUCH FOR YOUR PRECIOUS COLLABORATION AND FOR THE TIME YOU DEDICATED TO THIS RESEARCH.

Table 1: THg and MeHg in fish from different supermarkets in Slovenia.

species of fsih	origin/ FAO region	THg (ng/g)	MeHg (ng/g)
trout 1	Italia	16.4	6.1
trout 2	Italia	14.7	13.7
trout 3	Italia	12.3	10.4
trout 4	Italia	14.1	9.4
trout 5	Italia	14.6	8.3
trout 6	Italia	15.7	8.5
trout 7	Italia	14.6	5.6
trout 8	Slovenia	27.1	
trout 9	Slovenia	17.1	
trout 10	Slovenia	12.1	
trout 11	Slovenia	14.4	
European Hake/Cod 1	JZ Atlantic FAO 41	11.0	9.0
European Hake/Cod 2	JZ Atlantic FAO 41	19.4	9.4
European Hake/Cod 3	Atlantic	11.0	9.3
European Hake/Cod 4	Atlantic	23.3	16.9
European Hake/Cod 5	FAO 41	11.0	8.7
European Hake/Cod 6	FAO 41	11.8	8.7
European Hake/Cod 7	FAO 41	14.1	14.0
European Hake/Cod 8	FAO 41	12.8	12.3
European Hake/Cod 9	JZ Atlantic FAO 41	29.0	16.4
European Hake/Cod 10	JZ Atlantic FAO 41	17.2	12.0
gilt head bream 1	Greece	14.4	8.8
gilt head bream 2	Greece	17.2	17.2
gilt head bream 3	Greece	14.7	13.8
gilt head bream 4	Greece	18.8	19.1
gilt head bream 5	Greece	15.1	10.3
gilt head bream 6	Greece	15.0	12.0
gilt head bream 7	Greece	19.9	
gilt head bream 8	Greece	44.2	
gilt head bream 9	Greece	20.6	
gilt head bream 10	Greece	50.3	
gilt head bream 11	Greece	31.3	
sea bass 1	Greece	17.0	11.9
sea bass 2	Greece	17.9	6.3
sea bass 3	Greece	19.8	22.7
sea bass 4	Greece	18.0	19.8
sea bass 5	Greece	16.9	18.2
sea bass 6	Greece	16.1	17.3
sea bass 7	Greece	14.1	5.8
sea bass 8	Greece	17.0	5.8
sea bass 9	Greece	32.6	
sea bass 10	Greece	42.1	
sea bass 11	Greece	43.9	
sea bass 12	Greece	38.9	
sea bass 13	Greece	61.9	

Table 2**.** Biological markers of Hg exposure in pregnant women and infants from Idrija town-exposed group (N-11) and control group (N-7): T-Hg in urine (1st - 3rd trimester of pregnancy), in blood (3rd trimester - cord blood), and in hair (1st trimester - infants) (Kobal et al., in review in Chemosphere).

	I. trimester Mean/SD/(range)	III. trimester Mean/SD/(range)	Infants Mean/SD/(range)
Exposed group			
T-Hg in urine (μg/g crea.)	3.7/1.9/(1.7-6.3)		
T-Hg in hair (μg/g)	0.28/0.12/(0.06-0.41)		0.12/0.06/(0.03-0.20)
Hair Methyl-Hg (%)	77/14/(55-100)		
T-Hg in blood (μg/L)	1.42/1.30/(0.21-3.43)	2.61/1.31/(0.92-4.78)*	
Control group			
T-Hg in urine (μg/g crea.)	2.1/0.9/(1.0-3.4)		
T-Hg in hair (μg/g)	0.36/0.17/(0.19-0.60)		0.12/0.06/(0.06-0.19)
Hair Methyl-Hg (%)	73/17/(55-95)		
T-Hg in blood (μg/L)	NA		

NA – not available; *p<0,05

Table 3: THg, MeHg in hair, cord blood and breast milk of in this study involved Slovenian women.

ID	Hair THg (ng/g)	Hair MeHg (ng/g)	Cord blood THg (ng/g)	Cord blood MeHg (ng/g)	Milk THg (ng/g)	Milk MeHg (ng/g)
400001	434					
400002	789					
400003	768					
400004	34					
400005	124					
400006	799					
400007	578					
400008	364					
400009	266					
400010	391					
400011	389					
400012	1523	1523				
400013	1445	1359				
400014	770					
400015	793					
400016	32					
400017	614					
400018	474					
400019	916					
400020	690					
400021	544					
400022	649					
400023	720					
400024	487					
400025	789					
400026	1155	1155				
400027	721					
400028	479					
400029	378					
400030	464					
400031	310					
400032	1708	1352				
400033	283					
400034	674		4.9			
400035	241					
400036	312					
400037	482					
400038	348					
400039	36					
400040	159					
400041	152					
400042	229					
400043	197					
400044	148					
400045	1135	1135				
400046	74					
400047	294					
400048	495					
400049	191					
400050	107					

ID	Hair THg (ng/g)	Hair MeHg (ng/g)	Cord blood THg (ng/g)	Cord blood MeHg (ng/g)	Milk THg (ng/g)	Milk MeHg (ng/g)
400051	154					
400052	1343	624				
400053	134					
400054	1021	1021				
400055	866					
400056	15					
400057	966					
400058	715					
400059	193					
400060	275					
400061	1020	1020				
400062	131					
400063	31					
400064	305					
400070	167		1.08			
400071	392					
400072	192				0.12	
400073			1.92			
400074			0.57			
400075			1.68			
400076			3.61		0.25	
400077	73					
400078			0.38		0.10	
400079	343		1.61		0.37	
400080	202					
400081	202					
400082	301					
400083	49					
400084	98					
400085	75		0.66			
400086	275					
400087	545					
400088	586					
400089	54		0.68			
400090	354		1.91		0.70	
400091	85				0.48	
400092	687		2.40			
400093	397		2.94		2.86	
400094	389					
400095			0.52			
400096	277		1.83		0.48	
400097	374		2.07			
400098	632		5.03		0.27	
400099	724		3.39		0.47	
400100	65		0.76		0.10	
400101	185		0.80		0.12	
400102	661					
400103	356		1.58		0.44	
400104	837		2.41		0.42	
400105	83		0.42		0.10	
400106	528		2.50		0.21	
400107	227		1.75		0.29	
400108	57		0.53			

ID	Hair THg (ng/g)	Hair MeHg (ng/g)	Cord blood THg (ng/g)	Cord blood MeHg (ng/g)	Milk THg (ng/g)	Milk MeHg (ng/g)
400109	491		3.14		0.27	
400110	700		2.63		0.21	
400111	251		1.99		0.46	
400112	896		5.39		0.41	
400113	166		1.15		0.41	
400114	35		0.45			
400115	210		1.50		0.25	
400116	33		0.30			
400117	812		4.3			
400118	289					
400119	121					
400120	302					
400121	762		2.13		0.23	
400122	190		0.75			
400123	376		5.11		0.10	
400124	166		1.48		0.33	
400125	54					
400126	41		0.34			
400127	329					
400128	339					
400129	403		2.90		0.42	
400130	347		2.42		0.35	
400131	528		2.54		0.14	
400132	348		1.87		0.40	
400133	685		2.36			
400134	319				0.46	
400135	549		2.52		0.39	
400136	71		0.39		0.20	
400137	79		0.42		0.11	
400138	848		7.28		0.24	
400139	264					
400140	427		2.75		0.85	
400141	221		1.09		0.23	
400142	506				0.26	
400143	396		1.73		0.17	
400144	535		2.55		0.30	
400145	525		2.99			
400146	90		0.40		0.05	
400147	341		4.20		0.47	
400148	64		0.29		0.03	
400149	68		0.50		0.12	
400150	25		0.73		0.68	
400151			1.96			
400152	54					
400153	767					
400154	143					
400155	348		2.53			
400156	1420	1406	6.96	6.96		
400157	209		1.55		0.26	
400158	84		0.55		0.51	
400159	78		0.53		0.22	

ID	Hair THg (ng/g)	Hair MeHg (ng/g)	Cord blood THg (ng/g)	Cord blood MeHg (ng/g)	Milk THg (ng/g)	Milk MeHg (ng/g)
400160	739		4.20		0.36	
400161	292		1.50			
400162	460		5.29			
400163	72		0.73			
400164			2.21			
400165	384		1.94			
400166	798		4.62		0.28	
400167	778		3.95		0.40	
400168	591		2.97		0.82	
400169	355					
400170	100					
400171	473		3.99		0.19	
400172	253		1.65		0.50	
400173	304				0.18	
400174	563					
400175	1065	1065	7.33	6.60		
400176	327					
400177	107		0.70		0.06	
400178	773				0.24	
400179			1.19		0.83	
400180	118		1.21		0.38	
400181	292		1.32		0.09	
400182	453		2.29		0.96	
400183	273					
400184	139					
400185	200		0.87			
400186	185		0.97			
400187			1.60			
400188	73		0.70		0.44	
400189	956		5.51		0.22	
400190	832		5.10			
400191			2.22		0.19	
400192	870		2.61		0.13	
400193	73		0.52			
400194	2075	2075	9.54	9.54		0.03
400195	42		0.59		0.30	
400196	52		0.73			
400197	1719	1719				
400198	243		1.75		0.31	
400199	1159	1159	9.95	9.95		
400200	120		1.08			
400201	313		3.40		0.33	
400202	374					
400203	129					
400204	434					
400205	674					
400206	73		1.01		0.34	
400207	129		0.19			
400208	257		1.26			
400209	528		1.84			
400210			5.97			
400211	171		1.84		1.57	
400212	370		1.76		0.23	

ID	Hair THg (ng/g)	Hair MeHg (ng/g)	Cord blood THg (ng/g)	Cord blood MeHg (ng/g)	Milk THg (ng/g)	Milk MeHg (ng/g)
400213	912		3.90			
400214	47		0.50		0.14	
400215	126		0.54		0.14	
400216	160					
400217	284					
400218	180					
400219	485					
400220			0.19			
400221	116		0.65		0.18	
400222	306		1.72		0.13	
400223	269					
400224	467					
400225	761					
400226	199		2.29		0.59	
400227	302					
400228	765		2.79		0.24	
400229	85		0.82		0.15	
400230	414		2.69		0.30	
400231	314		2.62			
400232	371		3.78			
400233	376		1.72		0.25	
400234	1201	1201			0.17	0.09
400235	144		0.87		0.47	
400236	443				0.90	
400237	501		4.16		0.22	
400238	670		2.96			
400239	262		1.24		0.24	
400240	209		1.17			
400241	675		3.94		0.38	
400242	350		2.05		0.02	
400243	54		0.42		0.17	
400244	144		1.20		0.30	
400245	872					
400246	486					
400247	298					
400248	1475	1475	4.98	4.98	0.44	0.15
400249	321					
400250	759		5.60		0.33	
400251	257		1.91		0.36	
400252	358		1.67		0.61	
400253	89		0.64		0.09	
400254	511		2.23			
400255	281		0.75			
400256	206		1.00			
400257	585					
400258	45		0.20			
400259	54		0.72			
400260	239		0.90		0.15	
400261	813		4.16			
400262	89		1.23			
400263	426		1.92			

ID	Hair THg (ng/g)	Hair MeHg (ng/g)	Cord blood THg (ng/g)	Cord blood MeHg (ng/g)	Milk THg (ng/g)	Milk MeHg (ng/g)
400264	356					
400265	75					
400266	442					
400267	144		0.47		0.10	
400268	386		2.34			
400269	31		0.40		0.26	
400270	276		0.50			
400271	431		1.52			
400272	344				0.22	
400273	497		2.00		0.38	
400274	224		0.74		0.19	
400275			2.57			
400276	192		0.46			
400277			0.52			
400278	576					
400279	90.6					
400280	298		1.39			
400281			1.77			
400282	109		0.59			
400283	192		1.88			
400284	650		4.16			
400285	89.3		0.86		0.46	
400286	628		3.90			
400287	399		1.71		0.18	
400288	476				0.36	
400289	180		1.17		0.28	
400290	327		2.57			
400291			1.43			
400292	116		1.03			
400293	216		1.84		0.73	
400294	426		2.40		0.24	
400295	270		1.58		0.50	
400296	463		3.00		0.23	
400297	488		3.31			
400298	562		2.85		0.23	
400299			0.30			
400300	323		2.09		0.36	
400301	75.9		0.52		0.09	
400302	176		1.05		0.07	
400303	604		2.72		0.59	
400304	349		2.28		0.15	
400305	447		2.28		0.36	
400306	114		0.81			
400307	44		0.44			
400308	197		1.47			
400309	380		1.40		0.04	
400310	296		1.67		0.24	
400311	274		1.22		0.09	
400312	602		3.65		1.68	
400313	212		0.97		0.20	
400314	319		2.55		0.61	
400315	284		1.85			
400316	413		1.87		0.18	

ID	Hair THg (ng/g)	Hair MeHg (ng/g)	Cord blood THg (ng/g)	Cord blood MeHg (ng/g)	Milk THg (ng/g)	Milk MeHg (ng/g)
400317	226		1.17		0.10	
400318	36		0.25			
400319	668		4.78		0.22	
400320	241		1.28		0.22	
400321	48		0.16		0.15	
400322	264		3.24		0.75	
400323	41		0.24		0.05	
400324	439		2.74		0.19	
400325	344		2.41			
400326	81		0.28		0.19	
400327			1.14			
400328	34		0.35			
400329	24		0.30			
400330	149		1.07		0.04	
400331	129					
400332			1.26			
400333	489		2.52		0.16	
400334	816		4.74		0.42	
400335	146		0.77		0.18	
400336	150		1.56		0.16	
400337			0.53			
400338	306		1.31		0.14	
400339	292		2.08		0.19	
400340	508		3.42			
400341	252					
400342	80					
400343	482					
400344	104				1.28	
400345	1270	1270				
400346						
400347	253					
400348	350				0.62	
400349	320				0.28	
400350					0.49	
400351					0.45	
400382						
400383						
400384						
400385						
400386						
400387						
400388						
400389						
400390						
400391	194		0.48			
400392	24		0.46			
400393						
400394	161					
400395	123		0.47		0.21	
400396	178		0.85		0.19	
400397	530		1.63			

ID	Hair THg (ng/g)	Hair MeHg (ng/g)	Cord blood THg (ng/g)	Cord blood MeHg (ng/g)	Milk THg (ng/g)	Milk MeHg (ng/g)
400398	121				0.27	
400399	74		0.25			
400400	57		1.00		0.11	
400401	337		0.25		0.01	
400402	346		2.30		0.16	
400403	405		2.20			
400404	822		4.44		0.04	
400405	565		4.11			
400406	217		1.31		0.31	
400407	273		1.28			
400408	111		0.61			
400409	381		1.53		0.06	
400410	32		0.39			
400411	399		1.44			
400412	526		3.09		0.09	
400413	64		0.49		0.20	
400414	77		0.63		0.08	
400415	299		1.53			
400416	1030	841	6.44	5.57	0.14	0.10
400417	265		1.27			
400418	165		0.81			
400419	1880	1531	6.44	3.32	1.40	0.04
400420	167		1.02		0.21	
400421	177		1.06		0.20	
400422	110		0.64		0.15	
400423	80		0.60		0.21	
400424	296		1.33		0.10	
400425	432		2.59		0.33	
400426	1760	1495				
400427	952		3.98		0.27	
400428	246		2.23			
400429	205		0.92			
400430	296		1.74		0.51	
400431	781		5.73		0.39	
400432	164		1.31		0.07	
400433	41		0.17		0.05	
400434	192		1.10			
400435	263		1.10			
400436	151		1.10		0.26	
400437	353		2.14		0.07	
400438	26		0.50		0.24	
400439	277		2.06			
400440	414		0.78		<LOD	
400441	327		1.53			
400442	35		0.36			
400443	316		1.37		0.14	
400444	100		0.80		0.13	
400445	642		3.74			
400446	470		3.52		0.34	
400447	431		1.44			
400448	137		0.79		0.14	
400449	542		3.58		1.59	
400450	406		1.28		0.38	

ID	Hair THg (ng/g)	Hair MeHg (ng/g)	Cord blood THg (ng/g)	Cord blood MeHg (ng/g)	Milk THg (ng/g)	Milk MeHg (ng/g)
400451	693		4.10		0.19	
400452	596		2.08			
400453	56		0.23		0.06	
400454	1730	1616	6.14	6.14		
400455	144		0.70			
400456	555		2.79			
400457	394		1.61		0.20	
400458	725		5.10			
400459	199		0.97		0.17	
400460	105		1.31		0.07	
400461	628		5.28		0.24	
400462	397		2.83			
400463	377		2.67		0.09	
400464	437					
400465	1220	1220			0.59	
400466	306		2.22			
400467			0.83			
400468	232		1.81		0.47	
400469	187					
400470			2.35		0.08	
400471	232				0.09	
400472	247		1.88		0.16	
400473	701		5.95		0.22	
400474			3.23		0.17	
400475	180		0.84		0.14	
400476	423		1.38			
400477			1.65		0.24	
400478	138		0.67		0.03	
400479	1110	1110	8.12	7.17	0.15	0.07
400480	485		3.01			
400481	194		0.89		0.20	
400482	272		2.23		0.53	
400483	190		0.58			
400484			0.76			
400485	384		2.70		0.14	
400486	692		3.46		0.63	
400487	596		2.73		0.29	
400488	48		0.48		0.21	
400489	287		1.10		0.08	
400490	238		1.36		1.50	
400491	334		1.72			
400492	205		0.94			
400493	391		1.62		0.24	
400494	40		0.26			
400495	115		0.53			
400496	199		0.82			
400497	438		2.19		0.60	
400498	95		0.53			
400499	553		4.56		0.21	
400500	322		1.06		0.17	
400501	138		0.56		0.34	

ID	Hair THg (ng/g)	Hair MeHg (ng/g)	Cord blood THg (ng/g)	Cord blood MeHg (ng/g)	Milk THg (ng/g)	Milk MeHg (ng/g)
400502	307		1.01		0.17	
400503	178		0.67		0.09	
400504	183		1.12		0.15	
400505	202		2.08			
400506	253		1.68		0.90	
400507	64		0.53			
400508	463		3.08			
400509	1460	1119				
400510	235				0.12	
400511	430				0.14	
400512					0.16	
400513	180		0.61			
400514	357		2.10		0.38	
400515	107		0.95			
400516	501		4.57			
400517	907		2.56		0.49	
400518	785		7.47		0.24	
400519	810					
400520	435					
400521	1050	576	4.27	4.27		
400522	36		0.26			
400523	759		6.48			
400524	29		0.64			
400525	688					
400526	127		0.45			
400527	345		1.47		0.27	
400528	155		0.52		0.13	
400529	174		0.96			
400530	126		0.50		0.13	
400531	749				0.56	
400532	863		4.33		0.12	
400533	315		1.38		0.30	
400534	759		4.31			
400535	520		1.52			
400536	349					
400537	110					
400538	318					
400539	329					
400540	220					
400541	349					
400542	135					
400543	119					
400544	50					
400545	127		0.55			
400546	23					
400547	335		4.14			
400548			2.16			
400549	194					
400550	181		0.69			
400551	170		0.80			
400552	256		1.45		0.22	
400553	340		1.50		0.04	
400554	151		1.72		0.05	

ID	Hair THg (ng/g)	Hair MeHg (ng/g)	Cord blood THg (ng/g)	Cord blood MeHg (ng/g)	Milk THg (ng/g)	Milk MeHg (ng/g)
400555	470		0.93		<LOD	
400556			1.20			
400557	250		4.20			
400558	275		1.46			
400559	278		1.84		0.20	
400560	63		0.24			
400561	197		0.91		0.09	
400562	299		1.02			
400563	115		0.22		0.03	
400564	179		0.64			
400565	222		1.76		0.21	
400566	173		1.27			
400567			1.94		0.13	
400568	196		0.68			
400569	369		0.48			
400570	925		3.27		0.19	
400571			1.92		0.31	
400572	121		0.46			
400573	113		3.65		0.06	
400574	279		2.09			
400575			2.49		0.17	
400576	28		0.40			
400577	217		1.55			
400578	660		4.82		0.08	
400579	212		0.91			
400580	77		1.05			
400581	50		0.24		0.03	
400582	182		0.88		0.09	
400583	235		1.43		0.13	
400584	182		2.00		0.07	
400585	144		1.01		<LOD	
400586	330		1.35		0.14	
400587	231		0.99		0.03	
400588	1590	1590	5.92	5.13	1.01	0.05
400589	140		1.00		0.26	
400590	205		1.15			
400591	144		1.25			
400592	68		0.39			
400593	147		0.56			
400594			2.84			
400595	107		0.41			
400596	1541	1541	8.67	8.10		
400597	469		4.17			
400598	381		2.00			
400599	252		1.12			
400600	450		2.62			
400601	138		0.94		0.07	
400602	1266	1266	3.49	3.04	0.10	0.07
400603	37		0.93		0.22	
400604	368		1.86		0.07	
400605	374		4.25			

ID	Hair THg (ng/g)	Hair MeHg (ng/g)	Cord blood THg (ng/g)	Cord blood MeHg (ng/g)	Milk THg (ng/g)	Milk MeHg (ng/g)
400606	630		1.75			
400607	914		7.81		0.28	
400608	408		3.40			
400609	569		1.74		0.19	
400610	417		1.71		0.25	
400611	519		1.80			
400612	262		1.39			
400613	407		2.17		0.05	
400614	560		2.52		0.18	
400615	421		0.99		0.05	
400616	437		2.51		0.12	
400617	2439	2439	14.05	14.05	1.07	
400618	374		0.89			
400619	284		1.17		0.02	
400620	33		0.44		0.26	
400621	302		2.87		0.15	
400622	173		0.82		0.03	
400623	107		0.69			
400624	144		1.09		0.23	
400625	55		0.33			
400626	478		2.41			
400627	97		0.70		0.72	
400628	128		1.22		0.19	
400629	622		2.85			
400630	236		1.03		0.02	
400631	90		0.82		0.06	
400632	655		2.03		0.03	
400633	180		2.11		0.06	
400634	228		1.58			
400635	848				0.06	
400636	317		1.67		0.04	
400637	627		5.00		0.03	
400638	66		0.55			
400639	128		1.09		0.03	
400640	189		1.14		0.09	
400641	271		0.85			
400642	349		2.50		0.09	
400643					0.30	
400644	36		0.26		0.06	
400645	517		4.55		0.13	
400646	250		1.68		0.09	
400647	233		1.71			
400648	358		0.89		<LOD	
400649	356		2.30		0.28	
400650	802		4.16		0.24	
400651	52		0.33			
400652	74		0.51			
400653	96		0.46			
400654	302		1.75		0.08	
400655	496					
400656	103		0.52			