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

A. Miklavčič [a], P. Cuderman [a,1], D. Mazej [a], J. Snoj Tratnik [a], M. Krsnik [b], P. Planinšek [a], J. Osredkar [b], M. Horvat [a,*]

[a] Department of Environmental Sciences, "Jozef Stefan" Institute, Jamova 39, 1000 Ljubljana, Slovenia
[b] Institute of Clinical Chemistry and Biochemistry, Ljubljana University Medical Centre, Njegoseva 4, 1525 Ljubljana, Slovenia

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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.

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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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* Corresponding author. Fax: +386 588 5346.
E-mail addresses: ana.miklavcic@ijs.si (A. Miklavčič), petra.cuderman@ijs.si (P. Cuderman), darja.mazej@ijs.si (D. Mazej), janja.tratnik@ijs.si (J. Snoj Tratnik), mladen.krsnik@kclj.si (M. Krsnik), petra.planinsek@ijs.si (P. Planinšek), josko.osredkar@kclj.si (J. Osredkar), milena.horvat@ijs.si (M. Horvat).
[1] Present address: Lek Pharmaceuticals d.d., Verovškova 57, 1526 Ljubljana, Slovenia.

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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.

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