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Aufgrund des von mir gewählten Doppelstudiums Anthropologie und Ökologie in der Studienrichtung Biologie, entstanden zwei von mir verfasste Diplomarbeiten. Beide Diplomarbeiten befassen sich mit der pränatalen Quecksilberbelastung (mit unterschiedlichen Schwerpunkten), daher kann es sowohl zu inhaltlichen als auch zu sprachlichen Überschneidungen kommen.

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Abstract

Mercury (Hg), a ubiquitous heavy metal and well-known neurotoxin, is able to cross the blood-brain barrier as well as the placenta barrier. The main exposure sources of humans are dental amalgam fillings (Hg^0) and fish consumption (Methyl-Hg). Fish is an excellent source of minerals, vitamins and polyunsaturated fatty acids, especially EPA and DHA. During pregnancy, these nutrients, especially DHA, are required for regular development of placenta and fetal brain, for fetal growth and sufficient gestational length. However the fetus is very sensitive to the neurotoxin Methyl-Hg. Consequently, it is discussed whether fish consumption during pregnancy is a risk or a benefit to the developing infant.

The aim of this study was to determine Hg exposure in Vienna and Bratislava and to assess the role of fish consumption in prenatal Hg exposure.

Study design: Overall 200 pregnant women from Ruzinov clinic in Bratislava, Slovakia, and Semmelweis Clinic in Vienna, Austria, were recruited. Hg concentrations were measured in the erythrocyte fraction of maternal blood (MatBl-Ery-Hg) and cord blood (CordBl-Ery-Hg) by CV-AFS. Fish consumption was surveyed with a food-frequency questionnaire. The determinants of Hg exposure were evaluated using bivariate statistical analysis as well as categorical regression (CATREG).

Results: The mean MatBl-Ery-Hg level amounts to 1.7±0.8 µg/kg (Bratislava) and 1.9±1.4 µg/kg (Vienna) while the mean CordBl-Ery-Hg level is higher, i.e., 2.3 ± 1 µg/kg (Bratislava) and 2.9 ±1.9 µg/kg (Vienna). CordBl-Ery-Hg concentrations are well correlating with MatBl-Ery-Hg concentrations ($r=0.717$, $P<0.001$). The mean weekly fish consumption of pregnant women was 190 ±220 g in a range between 0-1050 g. The fish and sea food consumption habits are significantly related to MatBl-Ery-Hg levels ($p<0.01$) and CordBl-Ery-Hg ($p<0.001$). A high education level is associated with elevated fish consumption ($p<0.001$). In addition the number of dental amalgam fillings contributes to MatBl-Ery-Hg exposure ($p<0.01$). The newborn anthropometry (birth weight, birth length, head circumference) was neither influenced by Hg exposure nor fish consumption.

Conclusion: The mean Hg exposure of our study participants is not of concern, 98% of Matbl-Ery-Hg and CordBbl-Ery-Hg levels are below the alert level of 5 µg/L(i.e. the HBM1-value of the German Biomonitoring Commission) Fish consumption is essential for a regular development of the fetus. Therefore it is recommended that

pregnant women or women of child-bearing age should consume two to three times per week fish species such as carp, trout, anchovies or salmon, which are rich in PUFAs and poor in Methly-Hg. Furthermore the women should avoid consumption of tuna, shark or swordfish, which are highly contaminated in MeHg.

Keywords: mercury exposure, fish consumption, pregnancy, PUFAs

Zusammenfassung

Das weit verbreitete Schwermetall Quecksilber (Hg) ist ein Neurotoxin, welches sowohl die Blut-Hirnschranke als auch die Plazenta Schranke ungehindert passieren kann. Neben den amalgamhaltigen Zahnfüllungen (Hg^0) gilt der Fischverzehr (Methyl-Hg) als wohl größte Hg Expositionsquelle. Fisch enthält wichtige Mineralstoffe, Vitamine und mehrfach ungesättigten Fettsäuren – im besonderen EPA und DHA. Der Bedarf an Nährstoffen, besonders an DHA, erhöht sich nachweislich während einer Schwangerschaft. Um eine gesunde Entwicklung der Plazenta und des fetalen Gehirns, intrauterinen Wachstum und um eine ausreichend lange Schwangerschaftsdauer zu garantieren, wird Schwangeren daher zum Fischverzehr geraten. Allerdings reagiert der Fötus sehr empfindlich auf das Neurotoxin Methyl-Hg (MeHg). Das Nutzen/Risiko –Verhältnis (positive Wirkung von essentiellen Nährstoffen und gleichzeitig negative Wirkung von MeHg) führte zu einer Diskussion über adäquate Ernährungsempfehlungen für schwangere Frauen. Das Ziel dieser Studie war es, herauszufinden wie stark die Hg-Belastung in Wien und Bratislava ist und den Einfluss des Fischverzehrs auf die pränatale Hg-Belastung einzuschätzen.

Studiendesign: 200 schwangere Probandinnen aus der Semmelweis Klinik in Wien (Österreich) und aus der Ruzinov Klinik in Bratislava (Slowakei) wurden rekrutiert. Der Hg-Gehalt in der Erythrozytenfraktion des maternalen Blutes (MatBl-Ery-Hg) sowie des Nabelschnurblutes (NAB-Ery-Hg) wurde mittels CV-AFS gemessen. Zudem wurde der durchschnittliche Fischkonsum der Probandinnen anhand von Fragebögen ermittelt. Die Einflussfaktoren auf den MatBl-Ery-Hg Gehalt sowie im NAB-Ery-Hg Gehalt wurden mittels bivariate statistischer Analyse und der kategorialen Regression (CATREG) ermittelt.

Ergebnisse: Der Mittelwert des Hg-Gehaltes im MatBl-Ery-Hg lag bei 1.7±0.8 µg/kg (Bratislava) und 1.9±1.4 µg/kg (Wien), der des NAB-Ery-Hg bei 2.3 ±1 µg/kg (Bratislava) bzw. 2.9 ±1.9 µg/kg (Wien). Die Hg-Gehalte im maternalen Blut korrelierten mit den Werten im Nabelschnurblut ($r=0.717$, $p<0.001$). In der gesamten Gruppe lag der durchschnittliche wöchentliche Fischverzehr bei 190 ±220 g in einem Bereich von 0-1050 g. Der Fischkonsum der Probandinnen hatte einen signifikanten Einfluss auf MatBl-Ery-Hg ($p<0.01$) und NAB-Ery-Hg ($p<0.001$). Zudem zeigte sich auch, dass bei höherem Ausbildungsgrad der Probandinnen der Fischkonsum stieg ($p<0.001$). Ebenso konnte ein statistischer Zusammenhang zwischen der Anzahl der

Amalgamfüllungen der Probandinnen und den MatBl-Ery-Hg-Werten nachgewiesen werden ($p < 0.01$). Die Auswertung zeigte allerdings auch, dass die Anthropometrie der Neugeborenen weder durch die Hg-Belastung noch durch den Fischkonsum beeinflusst wurde.

Conclusio: Die durchschnittliche Hg-Belastung der Mutter-Kind-Paare aus Wien und Bratislava ist nicht alarmierend, da 98% der MatBl-Ery-Hg- und NAB-Ery-Hg-Werte unter dem Kontrollwert von 5 µg/L (entspricht dem HBM1-Wert der deutschen Komission für Human- Biomonitoring) liegen. Der Verzehr von Fisch ist nach wie vor eine der wichtigsten Nährstoffquellen für Schwangere, so dass zum Konsum von zwei bis drei Portionen Fisch/Meeresfrüchte pro Woche geraten wird. Wobei der Konsum von z.B. Sardellen, Karpfen, Forelle oder Lachs, die einen hohen Anteil an ungesättigten Fettsäuren und einen niedrigen MeHg-Gehalt aufweise, empfohlen wird. Weiters sollten Schwangere Frauen und Frauen im gebärfähigen Alter den Verzehr von Fischarten, die einen hohen MeHg-Gehalt ausweisen, wie beispielsweise Tunfisch, Haifisch oder Schwertfisch, vermeiden.

Schlüsselwörter: Quecksilberbelastung, Fischkonsum, Schwangerschaft, mehrfach ungesättigte Fettsäuren

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Abbrevation

CATREG	Categorical regression analysis
CNS	Central nervous system
CordBl-Ery-Hg	Cord blood erythrocyte mercury
NAB-Ery-Hg	Nabelschnurblut Erythrozyten Quecksilber
CV-AFS	Cold vapor atomic fluorescence spectroscopy
dH_20	Millipore water
DHA	Docosahexaeonic acid
EPA	Eicosapentaenic acid
Ery	Erythrocytes
EtHg	Ethyl mercury
GSH	Glutathione
HBM	Human BioMonitoring
Hg	Mercury
Hg^0	Elemental mercury (vapor or liquid)
Hg^{2+}	Mercuric mercury
MatBl-Ery-Hg	Maternal blood erythrocyte mercury
MatHair-Hg	Maternal hair mercury
MeHg	Methyl mercury
n3-PUFAs	Omega 3 poly unsaturated fatty acids
PTWI	Provisional tolerable weekly intake
PUFAs	Polyunsaturated fatty acids
ROS	Reactive oxygen species
Se	Selenium
WHO	World Health Organization

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1I Introduction

Mercury (Hg) is a ubiquitous heavy metal with a silver-whitish shiny appearance. Hg is highly toxic for the human body, because it is able to cross the blood-brain barrier as well as the placenta barrier. There are three forms of Hg, inorganic elemental Hg (Hg^0) and two organic compounds methylmercury (MeHg) and ethylmercury (EtHg) which can adversely affect human health. These three forms of Hg differ in toxicological properties. Inorganic Hg targets other organs than the organic compounds and thus cause different severities of damage to the human body.[5] The main sources of Hg exposure for the general population are consumption of fish and seafood (MeHg), dental amalgam fillings (Hg^0) and vaccines or medical preparations (EtHg).[3]

1I1 Inorganic Hg

The inorganic Hg compounds include elemental mercury (Hg^0) as well as compounds of mercurous ($Hg\text{-}Hg^{2+}$) and mercuric mercury (Hg^{2+}) e.g. Hg salts like calomel or Hg (II) chloride. In comparison with other inorganic Hg compounds, only Hg^0 is liquid at room temperature and the higher the temperature raises, the more is Hg^0 likely to evaporate into the gas phase and forming Hg^0 vapor. Hg^0 is used in dental amalgam fillings.[3] Furthermore Hg^0 is present in fluorescents light bulbs and generally does not pose a risk, unless the fluorescents light bulb is broken or damaged, when Hg^0 vapor is released.

In the past, Hg^0 was also used in thermometers, barometers, batteries and medical instruments, but this use is forbidden in the European Union since 1995.[6] In other parts of the World (Africa, Asia and South America), elemental Hg is an essential requisite in gold mining, where extraction of gold by amalgamation is a very common method. By burning the amalgam, Hg^0 is evaporated and only the gold is left over. During this procedure a high volume of Hg^0 vapor is released, which is then inhaled by the gold miners or gold merchants.[7] Amalgam fillings also can continuously release some Hg^0 vapor, which is then either inhaled or absorbed through the oral mucosa.

Upon Hg^0 inhalation, almost 80 % of the Hg^0 is absorbed through the lungs. There it can easily cross the cell membranes to enter the cardiovascular system per diffusion, where it binds on the erythrocytes to reach target organs like brain and kidneys. Hg^0

mainly accumulates in the kidneys where it can cause dysfunction in primary urine resorption in the proximal tubule, which can lead to proteinuria. Hg^0 has a limited capacity to cross the blood-brain barrier and to accumulate in brain.[6] Basically, absorbed Hg^0 is excreted by urine. Hg^0 has a half-life time ranging between 35-90 days in the human body.[3]

1I2 organic Hg

1I2I1 Ethylmercury (EtHg)

EtHg also called thiomersal is widely used as a preservative in vaccines or cosmetic products. The content of thiomersal in medicine products is 0.001%-0.01%. Until the 1970's EtHg was also used as a fungicide. The brain and the kidneys are target organs of EtHg. Compared to other Hg forms, EtHg has a very short half-life time of 2-8 days. Based on this short half-life time and the low doses used in medical preparations, it has been concluded that thiomersal does not substantially contribute to Hg body burden.[3]

1I2I2 Methylmercury (MeHg)

MeHg is the most common form of organic Hg in the environment. In humans, fish consumption is the main source of MeHg exposure. The target organ is the central nervous system (CNS). MeHg can lead to neuronal degeneration by affecting the microtubule integrity during development of the CNS or by depolymerization of existing microtubules.[8] Furthermore it induces glial proliferation, demyelination and a loss of granule cells and motor neurons.[6] Although MeHg mainly accumulates in glia cells in the brain, the neuronal cells seem to be more sensitive to its toxicity.[9] In case of the developing brain, MeHg can also cause a disarrangement of neuronal migration, which leads to a disturbance in nervous conduction of impulses and/or the cytoarchitecture of the brain. [10] MeHg has a half-life time of about 50-90 days.[3]

1I3 Hg in the environment: global cycling

In the environment, because of a biogeochemical cycle, Hg is continually cycled and recycled. (Fig. 1) The natural emission of Hg is caused by volcanic activity, forest fire and erosion of rocks or soil. The natural emissions have a low impact on global Hg pollution. Most of the Hg pollution is caused by anthropogenic activities including

coal-fired power plants, fossil fuels burning, gold mining or use of Hg containing products such as light bulbs.[5] In the Northern hemisphere the Hg concentration in air ranges between 1.5–1.7 ng/m³ and in the Southern hemisphere it ranges between 1.1-1.3 ng/m³.[11] In the past 150 years the Hg exposure has been tripled. Two thirds of the cycling Hg can be attributed to anthropogenic sources.

Once Hg^0 is emitted to the atmosphere, it can remain there for years and cover huge distances. The Hg^0 is slowly converted to Hg^{2+}, which returns to the earth surface by rainwater. If it reaches the water surface, most of Hg^{2+} is reduced to Hg^0 and vaporized back to the atmosphere, the remaining small proportion is down welling to the aquatic sediment as Hg^{2+}.[12] The European Food Safety Authority (EFSA) [9] has reviewed that the total Hg concentration in fresh water (1.0-20.1 ng/L) is higher compared to marine water (0.2-0.5 ng/L).

In the aquatic sediment, microorganisms, especially sulfate-reducing bacteria in the sediment convert Hg^{2+} to MeHg. This process is called biomethylation and happens in the uppermost five cm of the sediments, where the rate of sulfate-reducing bacteria is highest.[13] Biomethylation is influenced by pH, temperature and salinity of the water. The biomethylation of Hg^{2+} could be interpreted as a protective measure for microorganisms, because Hg^{2+} is more toxic to them than MeHg. The dissolved MeHg is released to the water column and absorbed by phytoplankton.[3]

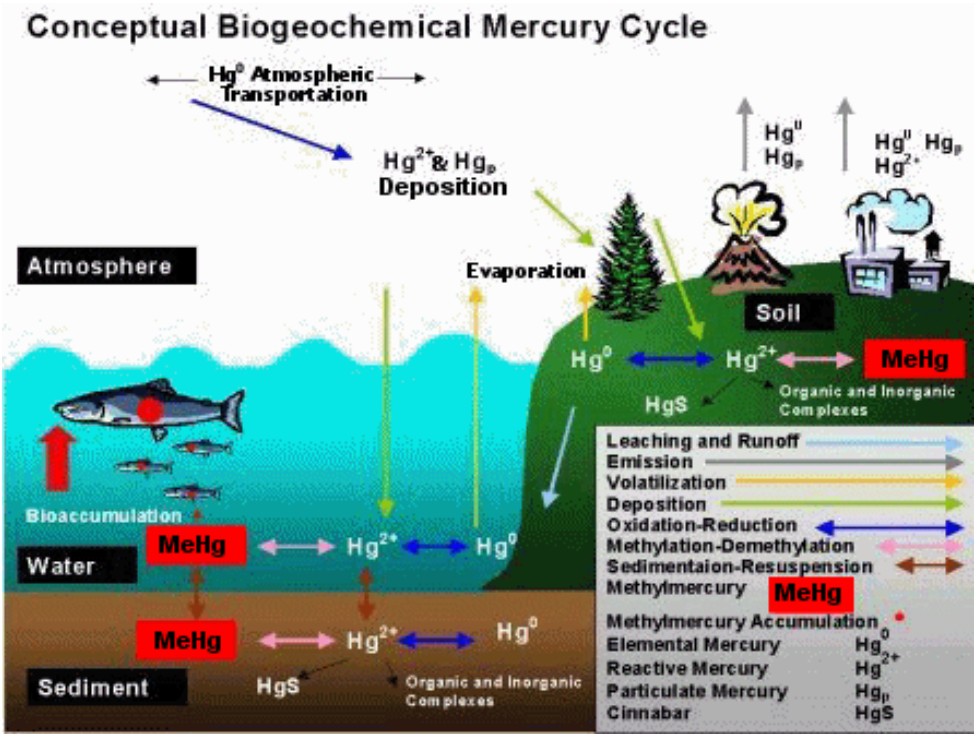

Figure 1. Hg cycle [1]

MeHg enters the aquatic food chain by crossing biological membranes. It undergoes biomagnification at each trophic level of the food chain. Despite the different types of aquatic food chains and pollution degrees, the patterns of MeHg biomagnification are always similar. Compared to other trace elements, MeHg has a high biomagnification rate. The MeHg concentration in fish is by factor 10^6 to 10^7 higher than in the ambient surface water.[14, 15] The MeHg concentration rises in the food web in following order phytoplankton <zooplankton and benthic primary producers consumers <detritivorous and opportunistic benthic invertebrates <epipelagic fish <demersal fish.[16] Therefore MeHg exposure of fish depends on the position in the food web and on the age of fish (Fig. 2).

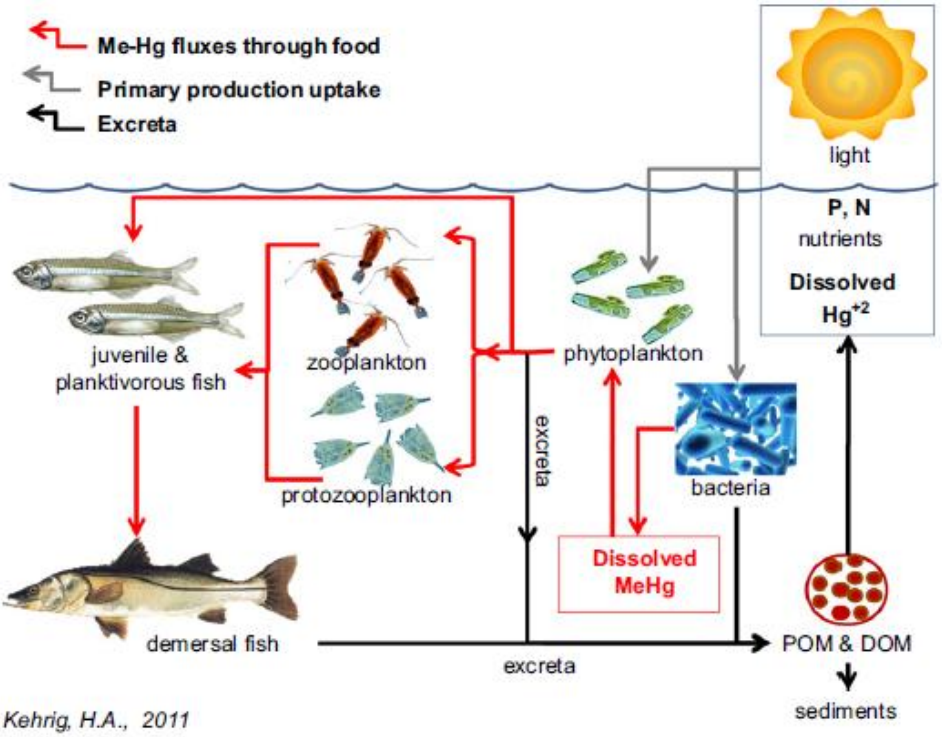

Figure 2. Food web including the trophic transfer of MeHg. [2]

1I4 Fish: a source of MeHg and healthy nutrients

Nearly all Hg in fish is MeHg, because the uptake of Hg^0 is less efficient and if it is absorbed, Hg^0 can be more rapidly eliminated than MeHg.[17] Almost 90% of MeHg content in fish stems from dietary uptake. 80% of the total food-derived MeHg in fish is incorporated by fish of the next higher trophic level.[18] Another way of MeHg uptake is via the gills, but compared to the dietary uptake it is subsidiary.[19] After passing the fish gut, MeHg binds to the erythrocytes, where it is transported to organs or tissues. A high concentration of MeHg is relocated to the skeletal muscle, where MeHg accumulates bound to thiol-groups (i.e., cysteine) in proteins.[18, 20] This accumulation pattern may help to reduce MeHg accumulation in the CNS. No relation was found between MeHg exposure and fat content of fish.[9] Wiener and Spry [21] concluded that long-term exposure of MeHg causes incoordination, disorder in swimming activity, reduced appetite or even mortality. MeHg concentrations in fish increase by age and size because the elimination rate is very low in relation to the uptake rate.[19] Exposure to high levels of MeHg affects reproduction because it can cause dysfunction in the gonadal system or can reduce the success of spawning. Especially, fish embryos and juvenile fishes react very sensitive to MeHg.[22]

Moreover fish is an important source of vitamins (A,B,D,E), minerals (e.g. selenium and iodine) and polyunsaturated fatty acids (PUFAs) including eicosapentaenic acid (EPA) and docosahexaeonic acid (DHA). Selenium (Se) is an essential trace element and a component of many enzymes e.g. Glutathione peroxidase or other selenoenzymes of the thyroid. Se appears to be the most important antagonist of Hg. It is binding Hg in a 1:1 ratio in that way preventing that Hg becomes further metabolized. The World Health Organization (WHO) recommends for pregnant women a daily uptake of 28-30 µg Se to support fetal growth and development during the second and third trimester.[23, 24]

During the second half of pregnancy, the fetal brain increases in volume. In this period the need of PUFAs, especially of DHA, is very high. DHA, an important n3-PUFA, is a component of the cell membrane of neurons and the retina. In the brain grey tissue, it can represent 50% of the fatty acids. DHA influences the thickness of membranes, membrane alignment, permeability of membranes, and the activity of membrane associated proteins and ion channels.[25] DHA is also essential for placenta development. EPA, the precursor of DHA, reduces the production of proinflammatory eicosanoids and supports the production of prostacyclin (a vasodilator and an inhibitor of platelet aggregation). Additionally EPA has beneficial effects in preventing cardiovascular diseases. In general, the PUFAs reduce expression of prostaglandins which may prevent preterm birth. Especially DHA intake during pregnancy is associated with improved mental development and cognitive abilities of infants and children. For this reason, experts recommend a daily uptake of 200 mg DHA during pregnancy.[23, 26, 27]

1I5 Disposition of MeHg in the human body

Approximately 95% of MeHg in contaminated food is absorbed in the gastrointestinal tract. It takes MeHg about 30 hours to disperse in the whole body. 10% of the absorbed MeHg is found in the brain. The concentration of MeHg in erythrocytes (ery), where it binds to the cysteinyl residues of the hemoglobin, is 20 times higher than in the plasma.[28] The absorbed MeHg is transported to the liver, where it attaches to the thiol-group of cysteine (see Fig. 3). Attached to cysteine, MeHg is able to enter a cell, while it is effluxed conjugated to glutathione (GSH). This is the reason why MeHg can be easily transported through the body. MeHg is excreted from liver cells to the bile by forming a complex with reduced GSH and finally

transported out of the cell by carriers transporting GSH and GSH conjugates. In the bile the GSH complex is hydrolyzed by enzymes into glutamic acid, glycine and cysteine (the latter as a MeHg-cysteine-complex). Partly this complex is reabsorbed in the bloodstream, where it can be transported to the brain, the kidneys or to the fetus, or it is secreted into the intestinal tract. One part of the secreted MeHg-cysteine-complex is also reabsorbed and can enter again the bloodstream, whereas the other part is slowly demethylated to Hg^{2+} by microorganism of the intestinal tract. Most of Hg^{2+} is excreted by feces, contributing to 1% loss of body burden per day. (Fig.4) [3]

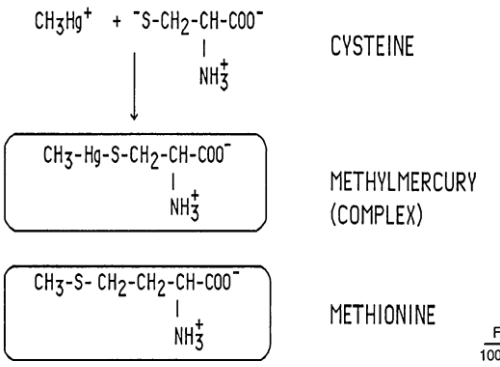

Figure 3. Similarity of the MeHg-complex with the amino acid methionine [3]

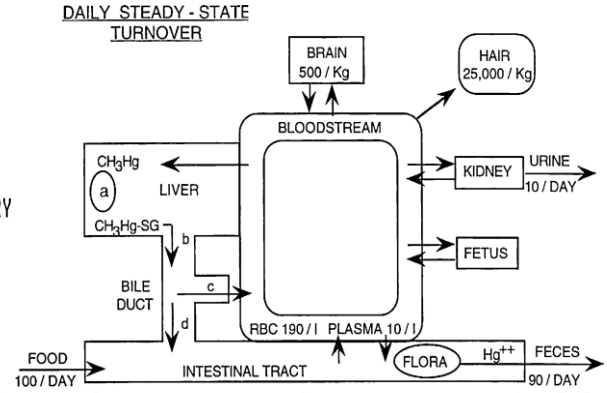

Figure 4. Overview on Hg uptake [3]

1I6 Molecular mechanisms of MeHg-induced neurotoxicity

The molecular mechanism of MeHg-induced neurotoxicity is not completely understood. MeHg-induced interaction between the alteration of receptor and transporter activity (including neurotransmitter), intercellular calcium dyshomeostasis, glutamate metabolism disruption and oxidative stress aggravation are known (reviewed by Liu et al. [29]). Astrocytes, which represent 50% of the CNS volume, are important for regulation of extracellular ion concentrations and extracellular pH and uptake of neurotransmitter, especially glutamate. During brain development, astrocytes are essential for the synthesis and elaboration of cues for neuronal migration and its production of neurotrophic factors, which are important for neuronal division and differentiation. MeHg accumulates in astrocytes and inhibits the transport of cysteine and cystine, which adversely influence the GSH content and their redox status. Further MeHg inhibits the uptake of glutamate in the astrocytes and concurrently stimulates the efflux of glutamate, which leads to an increased extracellular glutamate level in the synaptic cleft (reviewed by Aschner et al. [30]).

Glutamate is an important excitatory neurotransmitter and plays a decisive role in development, learning and memory.[31] High concentrations of this neurotransmitter cause an excitotoxic injury to neural cells as a consequence of the overactivation of the *N*-methyl D-aspartate (NMDA)-type glutamate receptors. Based on this overactivation, MeHg induces an increased influx of Na^+ and Ca^{2+} into neurons. In neuronal cells an increased Ca^{2+} concentration can activate apoptosis pathways or the Ca^{2+} is directly transported to the mitochondria and there may generate increased levels of reactive oxygen species (ROS). The latter mechanism can be directly stimulated by MeHg and causes increased level of ROS, which directly cause decreased glutamate uptake in the astrocytes (reviewed by Farina et. al [32]).

1I7 Toxicity of Hg: Human epidemiological data

The toxicity of Hg is known since the early 1950's caused by a mass-poisoning in Minamata, Japan. The Chisso Cooperation, a chemical factory, used Hg as a catalyst to produce acetataldehyd. A side product of this reaction was MeHg. The Chisso Co. released over several years contaminated MeHg waste water into the Minamata Bay. The released MeHg accumulated in fish and other seafood. The residents of Minamata Bay consumed highly contaminated fish over years, which finally resulted in severe symptoms of poisoning. The signs of Hg intoxication included ataxia, tremor, constriction of the visual field and disturbance in sensory functions caused by neuronal degeneration, especially in the visual, auditory, motor and sensory parts of the brain.[33]

Hg blood levels above 200 µg/L have been observed in the Minamata outbreak.[3] Additionally, this outbreak showed that pregnant woman with no or mild symptoms gave birth to children with pronounced symptoms like mental retardation, cerebral palsy and blindness. Further research showed that MeHg is able to cross the placenta barrier and also the fetal blood-brain barrier. From a meta-analysis [34] it is known that Hg cord blood (CordBl-Hg) levels are higher compared to the maternal Hg blood (MatBl-Hg) levels. Because of its rapid metabolism and the immature detoxification system, the fetus reacts very sensitive to this neurotoxin.[35]

Since the mass poisoning in Minamata, three major epidemiological studies investigated the effects of chronic MeHg exposure in fish consuming populations in New Zealand, at Faeroe Islands and the Seychelles. All three studies were dealing with cognitive skills and fine motor abilities of children in relation to their prenatal MeHg exposure. In the Faeroese cohort, a whale-eating population, children, which

had CordBl-Hg levels above 50 µg/L showed some cognitive and fine motor deficits.[36]

On the contrary, in the Seychelles cohort, a mainly ocean fish-eating population, no adverse effects related to prenatal Hg exposure could be found. Surprisingly, the average maternal hair-Hg (MatHair-Hg) level of the Seychelles population was 6.1 ppm, which is higher than the mean MatHair-Hg level (4.8 ppm) observed in the Faroese population. The New Zealanders do not eat fish as often as the Faeroese or Seychelles populations. However, the result of this study was that the children with a high prenatal Hg exposure (above 6 ppm in MatHair-Hg level) had lower scores in neurological tests as compared to children with low-prenatal Hg exposure.[3] On the basis of these studies the WHO recommend a provisional tolerable weekly intake (PTWI) of 1.6 µg MeHg/kg body weight.[37] The European Union allows only fish with a Hg content of 0.5 mg/kg on the market, except some predatory fish such as tuna, swordfish or shark, which may have a Hg content of 1 mg/kg.[38] The NOAEL (no observed adverse effect level) it is not yet known. Several studies are reporting that Hg blood levels between 5 and 190 µg /L are causing deficits in memory, language, attention and fine motor skills and can also lead to mental retardation and developmental delay. The severity of symptoms is depending on Hg blood levels. [39]

1I8 Aims of the study

A study on perinatal mercury exposure was conducted in Austria and Slovakia examining 100 mother-child-pairs, respectively. The subgoals of the present diploma work within this study were

(1) to determine the Hg exposure of mothers-child-pairs by analyzing Hg concentration in the erythrocyte fraction of maternal blood (MatBl-Ery-Hg) and cord blood (Cordbl-Ery-Hg)

(2) to investigate dietary habits and lifestyle factors (as surveyed in a questionnaire, for details see Appendix) in relation to Hg exposure,

(3) to assess in relation to current guideline values and dietary recommendations whether fish consumption during pregnancy bears a potential risk for the health of children

(4) to evaluate whether Hg exposure influences newborn anthropometry.

2I Materials and Methods

2I1 Study design and study group

114 pregnant women at the Ruzinov clinic in Bratislava and 120 pregnant women at the Semmelweis Clinic in Vienna were recruited during the third trimester of gestation for participation in this longitudinal study. Women with multiple pregnancies, hypertonia, Diabetes mellitus, gestosis, premature birth (birth before the 36th week of gestation), metabolic diseases, thyroid dysfunction and women, who consumed illegal drugs, were excluded. Of the 234 enrolled women, 34 dropped out because of gestational complications. Characteristics of the study group are described in Table 1. Women were informed about the length and the aims of the study and about the expense allowance (i.e., 25 €, respectively). Written informed consent was obtained from participants. The ethics committee of the University clinic in Bratislava and Vienna permitted the study.

2I2 Sampling

During the 36th-38th week of pregnancy each participant donated 3 x 7 ml of blood. In addition, the women completed a questionnaire about health status, diet, amalgam fillings, education, smoking habits, and area of residence (for questionnaires see appendix).

After birth, cord blood samples (1-3 tubes of 7 ml, respectively) were taken. Immediately after sampling, maternal blood and cord blood samples were centrifuged for 10 minutes at 3000 rpm to separate erythrocytes from blood plasma. All samples were stored at -20°C until further treatment.

Two to eight weeks after birth the women completed a second questionnaire about health status of mother and child and birth outcome. The data on gestational length and newborn anthropometry (birth weight, birth length, head circumference) were taken from the medical records.

Table 1. Study group characteristics

		N	Mean ± SD	Range	N (%)
Women					
	Age [a]	200	31±5	18-43	
	Height [cm]	200	167±6	152-181	
	BMI	200	22.4±3.5	16.0-36.7	
	Pregnancy BMI	196	27.0±3.7	20-39	
	Parity	200	1.8±1	1-7	
	Gestational length [ds]	198	280±8	257-295	
	No. of amalgam fillings	200	5±4.5	0-16	
	Fish consumption [g/w]	200	190±220	0-1050	
	Fresh water fish consumption [g/w]	200	30±75	0-600	
	Marine water fish consumption [g/w]	200	160±189	0-1000	
	Ery-Hg [µg/kg]	182	1.8±1.1	0.5-8.1	
	Hg uptake (total)[a] [µg/w]	200	23.1±23.4	0-161	
	Hg uptake (only fish consumption)[b] [µg/w]	200	15.5±22.0	0-149	
	Non smokers				96 (48)
	Current smokers				13 (6.5)
	Ex-Smokers				89 (44.5)
Children					
	Birth weight [g]	200	3422±431	2370-4080	
	Birth length [cm]	200	51±2	46-59	
	Head circumference [cm]	163	34.4±1.4	30-38	
	Ery-Hg [µg/kg]	189	2.6 ±1.5	0.9-11.4	
	Females				97 (48.5)
	Males				103 (51.5)

a) Hg uptake by all surveyed dietary products b) Hg uptake through fish and sea food products

2I3 Preparation and acid digestion of samples

Prior to use all instruments and sample tubes were cleaned with HNO_3 (Merck, Germany; p.a) mixed with millipore water (dH_20) in a ratio of 1:10. 1.0-1.5 g of each thawed erythrocyte sample was digested with a mixture of 4 mL 69 vol% HNO_3 (Roth, Germany; Supra quality) and 0.75-1.0 mL 30% H_2O_2 (Merck, Germany, p.a.) in a microwave oven (Table 2).

We used field blanks (4 ml HNO_3 plus 1 ml H_2O_2) and standard reference material (Seronorm, Trace Elements Whole Blood L-2, 210205) to control measurement quality.

Table 2. Program for digestion of blood samples in microwave mls 1200 mega

Step	Time	Watt
1	10:00	300
2	4:00	450
3	3:00	550
4	7:30	700
5	9:30	500
Ventilation	3:00	

After cooling, vessels were rinsed with 2 x 2 ml dH_20. Sample solutions were filled up with 2 ml HCl (Roth, Germany) and dH_20 to a volume of 10 mL. A sample solution aliquot of 4 ml was then decanted in a mercur© tube and volumetrically filled up with dH_20 to 20 ml, respectively.

2I4 Analysis of mercury

The samples were analyzed by cold vapor atom fluorescence spectroscopy (CV-AFS) (*mercur plus*, Analytik Jena, Germany). (Fig.5) One characteristic of Hg^0 is that it is a gas at room temperature. This is the reason why the cold vapor technique is a common method to detect total Hg content. CV-AFS has the advantage of being able to detect even very low concentrations. The method of AFS is based on the optical emission from gas-phase atoms accelerated to higher energy levels. The atom fluorescence then reradiates the absorbed energy. This fluorescent signal enters a quartz window which abuts on a 250 mm long absorption cuvette and goes through a second quartz window directly to the photomultiplier. (Fig. 6)[40, 41]

Figure 5. The *mercur plus*, Analytik Jena.[4]

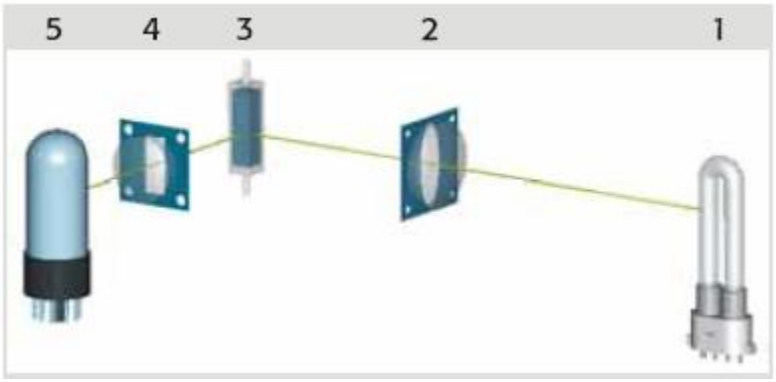

Figure 6. Hg analysis by AFS.[4]

Before the samples can be measured by AFS, the sample solution is transported to the reactor vessels, where the solution is mixed with the reducing solution $SnCl_2$ [20g $SnCl_2$ (Roth, Germany) + 100 ml HCl (Roth, Germany) in 1l dH_20], which leads to the following reaction:

$$Hg^{2+} + Sn^{2+} \rightarrow Hg^0 + Sn^{4+}$$

On the way to the gas-liquid separator, Argon (gas) is added to the solution and carries the Hg^0 gas atoms to the AFS detector. After each measurement the system is cleaned by HCl. [4, 42]

Hg levels of the reference material (LOT 1003129; 16.1±1.3 µg/L; n=20 and LOT 1003192: 15.2±0.9 µg/L; n=8) lay well within the certified ranges (16.0± 3.2 µg/L and 15.2±0.8 µg/L). The limit of detection (LOD), which is defined as the concentration equivalent to the threefold standard deviation of the blank solution, was 0.16 µg/L. The Hg contents were measured in duplicate (RSD<10%) by the working curve method.

2I5 Food consumption

The dietary habits during pregnancy were surveyed with a food-frequency questionnaire (included in Questionnaire 1). Consumption of fish species like Anchovy, Carp, Cod, Herring, Pangasius, Pike, Pike-perch, Plaice, Tuna, Trout, Salmon, Shark and Swordfish was quantitatively recorded in grams per week, whereas the consumption of sea fruits (mussels, crustaceans, cephalopods), innards and milk was surveyed semi-quantitatively in portions per week (categories were 1-2 times per week=200g, 3-7 times per week=300g, >7 times per week=600; for milk

consumption 1-2 glass per week=300 mL, 3-7 glass per week = 650 mL and > 7 times per week = 1000 mL which were also recalculated into gram per week [g/w]).

The Hg levels of the various food items were taken from literature data particularly from the most recent Austrian data (Table 3). Hg uptake (µg/kg) was calculated in the following way:

$$\frac{fish\,[g] \times Hg\ level\ of\ the\ fish[\mu g/kg]}{1000}$$

Table 3. Hg concentrations in fish and seafood, innards and milk used to calculate Hg uptake.

Food	N	Fresh weight [µg/kg]	
Anchovy	8	108	[43, 44]
Carp (farmed AT)	62	43	[44, 45]
Cod	8	19	[44, 45]
Fishsticks	n.a	50	[45]
Herring	n.a	50	[45]
Innards	3	2.6	[44, 45]
Milk	8	12	[44, 45]
Mussels	8	4.5	[44, 45]
Octopus	n.a	94	[46]
Pangasius	8	13	[44, 45]
Pike	1	339	[44, 45]
Pike perch	n.a	150	[47]
Plaice	8	35	[44, 45]
Tuna (canned)	8	116	[44, 45]
Tuna (steak)	8	678	[44, 45]
Trout (farmed AT)	51	21	[44, 45]
Salmon	8	37	[44, 45]
Shrimp	8	69	[44, 45]
Shark	3	387	[44, 45]
Swordfish	8	399	[44, 45]

n.a.: not available

The PTWI for total Hg uptake was calculated for each study participant in the following way:

$$bodyweight\,[kg] \times 5\ \mu g/kg\ \ total\ Hg$$

2I6 Statistical analysis

The Hg values were not normally distributed (Lilliefors test $P<0.05$), thus, non-parametric tests were used in all statistical evaluations. The correlation between lifestyle variables and erythrocytes-Hg (Ery-Hg) concentrations were examined using Spearman rank correlation. Group comparisons were conducted applying the Mann-Whitney U test (two group comparisons) and Kruskal Wallis Test (comparisons of more than two groups). The Chi-Square test was used to analyze contingency tables testing the potential interrelationships between 1) fish consumption and number of amalgam fillings and 2) fish consumption and newborn gender and 3) fish consumption and birth weight 4) MatBl-Ery-Hg and newborn gender.

Prior to statistical analyses, several metric variables were coded into categorical variables. The variable “no. of amalgam fillings” was grouped into four categories, i.e., group 1: no fillings (n=46), group 2: 1-5 fillings (n=53), group 3: 6-10 fillings (n=54) and group 4: 11-16 fillings (n=29). The amount of fish consumption was categorized into groups of 0 g/w (n=41), 10-100 g/w (n=50), 101-200 g/w (n=50) and 201-1050 g/w (n= 59). The education level was coded into the categories low (elementary and secondary modern school, n=32), middle (apprenticeship, grammar and vocational school, n=64) and high (college, university, n=104). Birth length was categorized into three groups [46-49 cm (n=44), 50-51 cm (n= 94), 52-59 cm (n=62)], while four categories were coded for weight [(2370-3000g, n=35), (3010-3540g, n=86), (3550-4000g, n=61), (4020-4810g, n=18)]. The variable “MatBl-Ery-Hg level” was categorized into five groups [(0-1.0 µg/kg, n=23), (1.01-1.5 µg/kg, n=63), (1.51-2.0 µg/kg, n=46), (2.01-2.5 µg/kg, n=22) (2.51-8.1 µg/kg, n=28)].

For modeling the effects on Ery-Hg levels, we applied categorical regression analysis (CATREG). Each factor associated with $P<0.05$ to Ery-Hg levels of mother and newborns, respectively, was included in the respective CATREG models.

For modelling effects on MatBl-Ery-Hg levels, the variables maternal age, education, no. of amalgam fillings, fresh water fish consumption, and marine water fish consumption were included, which were maternal age, education level, no. of amalgam fillings, fresh water fish consumption, and marine water fish consumption, sex of child, and birth weight, for modeling effects on CordBl-Ery-Hg levels. We used the Pratt-coefficient of relative importance as the elimination criterion in CATREG analysis to control for collinearity of explaining variables. The non-significant and

unimportant factors were stepwise eliminated from the regression models. Elimination criteria, in this order, were P>0.5 and Pratt-coefficient <0.05. The SPSS 19.0 program (SPSS Inc, Chicago, IL) was used for statistical calculations. The tests were performed as two-sided at P<0.05. [48]

3I Results

3I1 Ery-Hg concentrations in maternal blood and cord blood.

The mean CordBl-Ery-Hg concentrations amounted to 2.6 ±1.5 µg/kg. In comparison to cord blood, MatBl-Ery-Hg concentrations were lower with a mean value of 1.8 ±1.1 µg/kg. (Table 4)

Table 4. Ery-Hg concentrations in maternal blood and cord blood

	N	MIN	MAX	25 Percentile	50 Percentile	75 Percentile
Maternal blood [µg/kg]	182	0.46	8.1	1.2	1.6	2.1
Cord blood [µg/kg]	189	0.91	11.4	1.6	2.2	3.0

3I2 Determinants of Hg exposure

Study participants consumed freshwater fish in an amount varying between 0-600 g/w, which was 0-1000 g/w for marine fish consumption. The correlations between maternal dietary product consumption and Ery-Hg levels of mothers and children are given in Table 5. The maternal consumption of fish and milk correlated weakly ($r=0.135$, $p<0.1$). Maternal age correlated with MatBl-Ery-Hg level ($p<0.05$) as well as with Cordbl-Ery-Hg ($p<0.05$). Dental amalgam fillings also correlated well with MatBl-Ery-Hg levels ($p<0.001$). Furthermore mother's education level influenced Ery-Hg levels of both mothers ($p<0.05$) and children ($p<0.001$). Table 6 illustrates correlations among the fish species consumed during pregnancy.

Table 5. Correlation of Ery-Hg levels with consumption of various food (in grams/week)

	CordBl-Ery-Hg [µg/kg]	MatBl-Ery-Hg [µg/kg]
MatBl-Ery-Hg	**0.717*** **	
Total fish consumption	**0.433*** **	**0.416** **
Fresh water fish	**0.320** **	**0.265** *
Marine water fish	**0.386** **	**0.363** **
Anchovies	0.101	**0.165** *
Carp	0.141	**0.184** *
Cod	**0.153** *	**0.151** *
Fishsticks	0.006	0.023
Herring	0.094	0.134
Innards	0.027	0.097
Milk	**0.225** **	**0.155** *
Mussels	-0.040	-0.033
Octopus	0.030	0.061
Pangasius	0.105	0.053
Pike	0.030	0.052
Pikeperch	**0.181** *	0.098
Plaice	**0.173** *	0.087
Tuna (canned)	**0.245** **	**0.344*** **
Tuna steak	0.000	0.049
Trout	**0.264*** **	**0.237** **
Shark	-0.064	1.000
Salmon	**0.424*** **	**0.295*** **
Shrimp	**0.180** *	0.117
Swordfish	-0.028	-0.079

Spearman correlation coefficients
***P<0.001, **P<0.01, *P<0.05

Table 6. Correlation of fish species consumption habits (in grams/week)

Anchovies	Cod **0.154***	Tuna (canned) **0.276*****							
Cod	Anchovy **0.154***	Fishsticks **0.183****	Herring **0.155***	Plaice **0.276*****	Salmon **0.158***	Shark **0.141***	Tuna (canned) **0.213****	Trout **0.213****	
Carp	Pike **0.152***	Pike-perch **0.242****	Trout **0.246*****						
Pikeperch	Carp **0.242****	Herring **0.188***	Mussels **0.227****	Pike **0.242****	Plaice **0.258*****	Salmon **0.182****	Shrimp **0.240****	Trout **0.357*****	
Plaice	Cod **0.276*****	Fishsticks **0.209****	Mussels **0.167***	Pangasius **0.139***	Pike-perch **0.258*****	Salmon **0.217****	Shark **0.160***	Shrimp **0.267*****	Trout **0.247*****
Salmon	Cod **0.158**	Herring **0.231*****	Octopus **0.217***	Pangasius **0.208****	Pike-perch **0.182****	Plaice **0.217****	Shark **0.154***	Shrimp **0.330*****	Trout **0.285*****
Shrimp	Mussels **0.349*****	Octopus **0.623*****	Pike **0.162***	Pike-perch **0.240****	Plaice **0.267*****	Salmon **0.330****	Shark **0.309****	Trout **0.185****	
Trout	Carp **0.246*****	Cod **0.213****	Herring **0.217****	Pike **0.253*****	Pike-perch **0.357*****	Plaice **0.247*****	Salmon **0.285****	Shrimp **0.185****	
Tuna (canned)	Anchovy **0.276*****	Cod **0.213****	Herring **0.150***						

***P<0.001, **P<0.01, *P<0.05

As shown in Fig. 7A (as deduced from Table 3), Hg content of various foods shows that consumption of marine predatory fish on the top of the food chain (e.g., shark, tuna, swordfish) leads to higher Hg uptake compared to fish species of lower trophic levels (salmon, cod, etc.). Fig 7B illustrates the amount of surveyed food items (i.e., serving size) consumed per week (details given in Table 7). It is indicated that canned tuna is the favorite fish consumed by 43% of our study participants during pregnancy. 53% of study participants prefer eating only marine fish while 3% only consumed fresh water fish species. Furthermore 20% of our study participants consumed neither marine water fish nor fresh water fish. Only 20.5% of mothers specified to consume trout, which was the overall most consumed fresh water fish. The average uptake of Hg of the investigated dietary products was 23±23 µg per week.

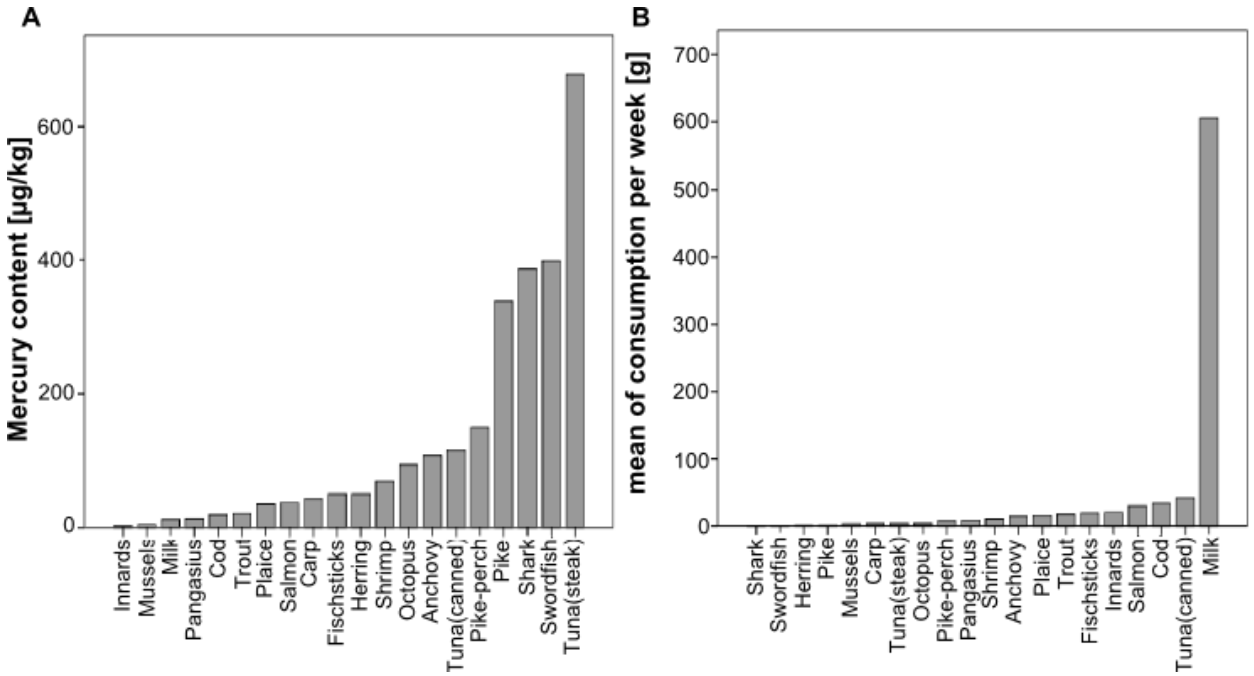

Figure 7. A) Hg content of various food (fresh weight) also surveyed in the Um-MuKi Study. **B)** Mean serving size [g] of dietary products consumed per week.

Table 7. Detailed information on consumption of surveyed food items during pregnancy.

[g/w]	Number of individuals (N)																			
	Anchovy	**Carp**	**Cod**	**Fish sticks**	**Herring**	**Innards**	**Milk**	**Mussels**	**Octopus**	**Pangasius**	**Pike**	**Pike perch**	**Plaice**	**Tuna canned**	**Tuna steak**	**Trout**	**Salmon**	**Shark**	**Shrimp**	**Swordfish**
0	163	188	125	160	196	182	42	197	196	188	197	180	165	114	188	159	144	199	190	199
10	-	3	2	1	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-
20	3	-	5	3	2	-	-	-	-	2	-	4	4	4	2	4	5	1	-	1
25	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	1	-	-	-	-
30	3	3	5	2	1	-	-	-	-	1	1	2	2	10	3	4	5	-	-	-
35	-	-	1	-	-	-	-	-	-	-	-	-	-	1	1	-	-	-	-	-
40	1	-	1	-	-	-	-	-	-	-	-	-	1	3	2	1	1	-	-	-
45	2	-	4	2	-	-	-	-	-	-	-	-	-	3	-	-	1	-	-	-
50	7	2	14	7	-	-	-	-	-	2	1	5	8	12	1	9	6	-	-	-
60	-	-	1	-	-	-	-	-	-	-	-	-	-	1	-	-	1	-	-	-
70	1	1	1	-	-	-	-	-	-	-	-	1	-	2	-	-	-	-	-	-
75	4	-	2	2	-	-	-	-	-	-	-	-	-	5	-	-	2	-	-	-
80	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	1	-	-	-
100	9	-	17	11	-	-	-	-	-	3	-	4	11	18	-	6	14	-	-	-
130	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-
150	7	3	18	9	1	-	-	-	-	-	1	3	6	18	3	12	12	-	-	-
200	-	-	3	2	-	16	-	3	4	2	-	1	1	4	-	1	5	-	10	-
250			1	1				-		-			1	1		-	1	-	-	
300	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-	2	-	-	-
309	-	-	-	-	-	-	36	-	-	-	-	-	-	-	-	-	-	-	-	-
350	-	-	-	-	-	-	-	-	-	1									-	
400	-	-	-	-	-	2	-	-	-	1	-	-	-	-	-	-	-	-	-	-
670	-	-	-	-	-	-	33	-	-	-	-	-	-	-	-	-	-	-	-	-
1030	-	-	-	-	-	-	89	-	-	-	-	-	-	-	-	-	-	-	-	-
total	200	200	200	200	200	200	200	200	200	200	200	200	200	200	200	200	200	200	200	200

The Ery-Hg levels of mothers ($p<0.001$) and children ($p<0.001$) are highly significantly influenced by fish consumption (Fig. 8A, B). There is a highly significant ($p<0.001$) association between maternal education and fish consumption (Fig. 8C). Figure 8D illustrates that all study participants do not exceed the PTWI of Hg. It shows that the PTWI value is only exploited by an average value of 6.6 ±7%. (ranged 0-50%)

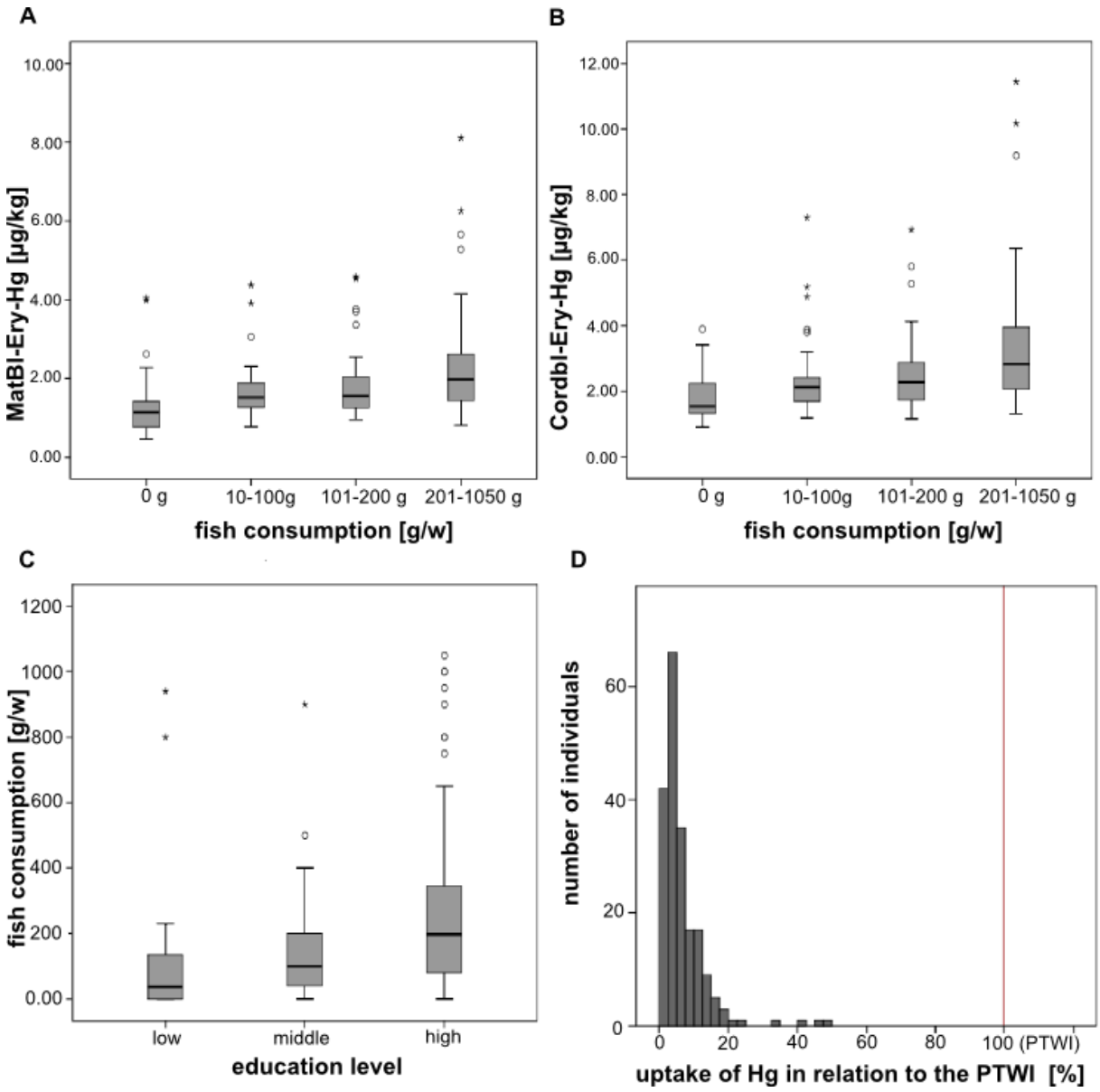

Figure 8. A) MatBl-Ery-Hg content in dependence of fish consumption [0g/w (n=41), 10-100g/w (n=50), 101-200 g/w (n=50), 201-1050g/w (n=59), ($p<0.001$)]. **B)** CordBl-Ery-Hg content in dependence of fish consumption ($p<0.001$). **C)** Total fish consumption in dependence of maternal education level [(low= elementary & secondary modern school, n=32), (middle = apprenticeship, grammar-& vocational school, n=64), (high= college, university, n = 104), ($p<0.001$)] **D)** Individual Hg uptake in relation to the PTWI (0% = non-exhausted PTWI, 100 % exhausted PTWI)

There is a significant association between MatBl-Ery-Hg levels and the presence of amalgam fillings ($p<0.05$) (Fig.9A). As shown in Figure 9B MatBl-Ery-Hg concentrations depend on the number of amalgam fillings. In order to prove that the relationship between the number of dental amalgam fillings and Ery-Hg levels is not masked by fish consumption a cross-tabulation for the variables “fish consumption” and “amalgam fillings” was made (Fig.9D). No association was found between these variables ($p=0.274$).

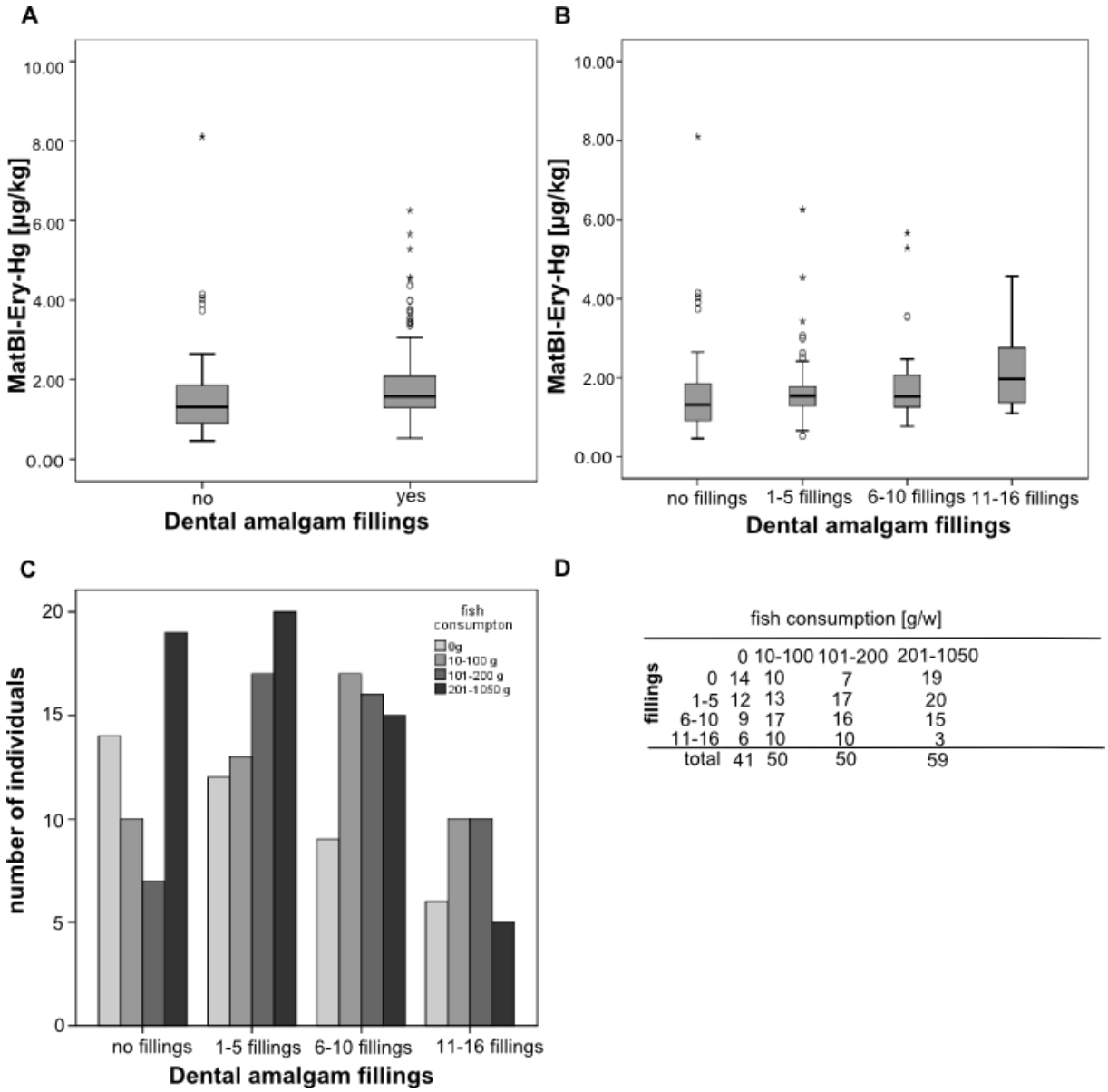

Figure 9. A) MatBl-Ery-Hg in association with presence of amalgam fillings [no (n=46); yes (n=136), $p<0.05$] **B)** MatBl-Ery-Hg in dependence on number of dental amalgam fillings [no fillings (n=46), 1-5 fillings (n=53), 6-10 fillings (n=54) and 11-16 fillings (n=29) ($p<0.01$)]. **C)** Fish consumption habits in groups with different numbers of amalgam fillings. **D)** Cross-tabulation of fish consumption and dental amalgam fillings (Chi^2 test $p=0.274$)

Moreover it seems that the age of the mother plays a role in Hg exposure. Women, who were younger than 31 years of age have significantly lower Ery-Hg levels than women of higher age (Fig.11A, $p<0.01$). This result is also reflected in CordBl-Ery-Hg levels (Fig.11B, $p<0.05$). There is also a statistical association between birth weight and mother's age ($p<0.05$). Additionally, the sex of a child influences Hg exposure. Girls (n=97) had significantly lower CordBl-Ery-Hg levels than boys (n= 103) (Fig. 11D, $p<0.05$). This association is not influenced by differences in maternal fish consumption (Fig. 10A, p= 0.532) or MatBl-Ery-Hg levels (p=0.341). We observed a weak association between MatBl-Ery-Hg and child sex (Fig.11C, $p<0.1$). Furthermore, birth size was not related to CordBl-Ery-Hg levels (p=0.270), yet, we observed a weak positive correlation between birth weight and CordBl-Ery-Hg ($p<0.05$), which is not influenced by maternal fish consumption (p=0.116) but obviously masked by maternal age (p=0.002, Fig.10B). The maternal fish consumption influences neither birth weight (p=0.900), birth size (p=0.265) nor gestational length (p=0.728).

A

sex	fish consumption [g/w]			
	0	10 -100	101-200	201-1050
male	24	23	24	26
female	17	27	26	33
total	41	50	50	59

B

birth weight [g]	age of mother [y]			
	18-25	26-30	31-35	36-43
2370-3000	4	15	13	3
3010-3540	17	31	17	21
3550-4000	3	19	29	10
4020-4810	1	2	10	5
total	25	67	69	39

Figure 10.A) Cross-tabulation of fish consumption and sex of the child, (Chi^2 test p=0.523) **B)** Cross-tabulation of maternal age and birth weight (Chi^2 test p=0.002)

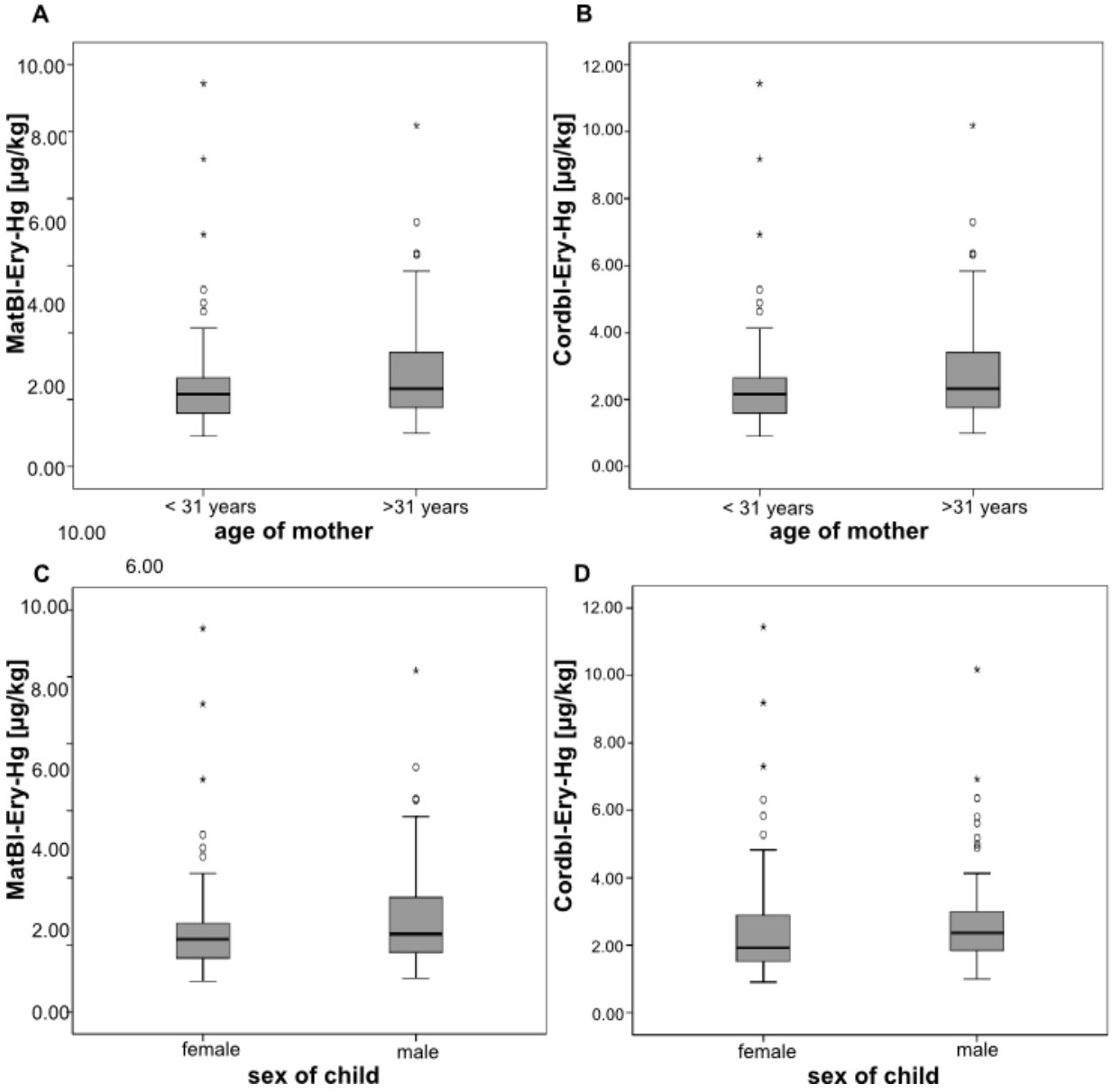

Figure 11. A) Age-specific Ery-Hg contents [below 31 years (n=96), over 31 years (n= 85), p<0.01] **B)** CordBl-Ery-Hg levels in dependence of maternal age [< 31 years (n=100), >31 years (n=88), p<0.05] **C)** MatBl-Ery-Hg contents in relation to child sex [female (n= 97) male (n=103) (p<0.1)] **D)** Sex –specific CordBl-Ery-Hg contents (p<0.05)

CATREG analysis indicates that marine water as well as fresh water fish consumption and dental amalgam fillings are independent predictors of MatBl-Ery-Hg levels. (Table 8)

Table 8. Independent associations of exposure factors with MatBl-Ery-Hg concentration (CATREG analysis)

Exposure marker	Design	Factor [model]	β± S.E	P	Partial r [R^2]	Importance coeff. (rank)
Ery-Hg [µg/kg]	Model 0	Marine water fish consumption	0.381±0.061	<0.001	0.405	0.465 (1)
		Amalgam fillings	0.356±0.062	<0.001	0.389	0.343 (2)
		Fresh water fish consumption	0.381±0.057	<0.001	0.244	0.192 (3)

Consumption of marine water fish during pregnancy is the major determinant of mercury exposure in newborns. The education level of the mothers and consumption of fresh water fish also remain as independent predictors of CordBl-Ery-Hg levels. (Table 9)

Table 9. Independent associations of exposure factors with CordBl-Ery-Hg concentration (CATREG analysis)

Exposure marker	Design	Factor [model]	β± S.E	P	Partial r [R^2]	Importance coeff. (rank)
Ery- Hg [µg/kg]	Model 0	Marine water fish consumption	0.296±0.069	<0.001	0.309	0.473 (1)
		Education level	0.203±0.087	0.001	0.217	0.264 (2)
		Fresh water fish consumption	0.296±0.070	<0.01	0.216	0.264 (3)

4I Discussion

The Human Biomonitoring Commission recommends two Human BioMonitoring values (HBM1 and HBM2) to assess Hg body burden in the general population. The HBM1 value is defined as a control value whereas the HBM2 value is an intervention level. The HBM1 value for Hg blood levels is set at 5 µg/L and represents a concentration, below which no adverse health effects is to be expected. The HBM2 value of 15 µg/L describes a Hg level, where adverse health effects may occur, requiring intervention measures to reduce Hg blood levels immediately. Hg blood levels in a range between 5-15 µg/L should be first verified by further analyses and second Hg exposure should be immediately minimized and the source(s) of exposure eliminated.[49]

In our study group only two Viennese babies exceed the HBM1value of 5 µg/L (i.e., 5.33 µg/L and 6.75 µg/L)[1]. None one of our study participants had blood concentrations above the intervention value of 15 µg/L. This result indicates that the current Hg exposure of mother-child-pairs in Vienna or Bratislava region is not alarming. The Viennese study participants had higher Ery-Hg levels (max. 11.4 µg/kg) than the monitored 100 mother child pairs in Bratislava (max. 6.9 µg/kg).

All differences in lifestyle factors between the Bratislava and Viennese group, especially with regard to the number of dental amalgam fillings and to fish consumption habits, are listed in Table 10.

[1] The Ery-Hg levels [µg/kg] were calculated into whole blood Hg levels [µg/L] by the formula: $Ery - Hg \times weight\ of\ erythrocytes\ (factor\ 1.09) \times hematocrit\ level$

Table 10. Differences between the monitored groups in Bratislava and Vienna

	Bratislava			Vienna			p
Mother	N	Min-Max	Mean±SD	N	Min-Max	Mean±SD	
Ery-Hg [µg/kg]	100	0.5-4.6	1.7±0.8	82	0.5-8.11	1.9±1.4	
Age [a]	100	18-43	31±5	100	18-43	31±5.5	
No. Amalgam fillings	100	0-16	7±4	100	0-16	3±4	<0.001
Total fish consumption [g/w]	100	0-1000	149±176	100	0-1050	231±251	<0.05
Education level							<0.001
low	27			5			
middle	21			43			
high	52			52			
Child							
Ery-Hg [µg/kg]	100	1.03-6.9	2.27±1	89	0.91-11.4	2.9±1.9	<0.05
female	43			54			
male	57			46			
Fish species consumption [g/w]							
Anchovies	100	0-150	22±43	100	0-150	8±26	<0.01
Carp	100	0-150	5±23	100	0-150	3±16.5	
Cod	99	0-150	31±51	100	0-250	37±59	
Fishsticks	100	0-150	8±30	100	0-250	30±55	<0.001
Pangasius	100	-	-	100	0-400	16±61	<0.001
Piker perch	100	0-70	2±9	100	0-200	13±38	<0.01
Plaice	100	-	-	100	0-250	31±53	<0.001
Tuna (canned)	100	0-150	47±58	100	0-300	36.5±70.5	<0.05
Tuna (steak)	100	0-150	7±27	100	0-40	0.7±5	<0.05
Trout	100	0-150	12±36.5	100	0-200	22.5±47	<0.05
Salmon	100	0-200	13±38	100	0-300	47±73	<0.001
Shrimp	100	0-200	4±28	100	0-200	16±54.5	

The Hg blood levels are in good agreement with previous studies conducted in Vienna and Bratislava region.[48, 50] Table 11 gives an overview on Hg exposure levels in different countries. It indicates that Hg exposure of our study participants from Vienna and Bratislava are significantly lower compared to Inuit populations in Canada and Greenland. In these countries it is common to frequently consume marine mammals and fish. This diet is the major source of Hg exposure in these populations.

As it was the case in many other studies [34], we also observed higher Ery-Hg levels in cord blood than in maternal blood, i.e., a mean cord blood: maternal blood Hg ratio of 1.4. This phenomenon is usually explained by the high affinity of MeHg to bind to fetal hemoglobin. The fetus usually has higher hemoglobin concentrations compared to the mother. Further research showed that there is no evidence that the fetal hemoglobin has a greater MeHg binding capacity compared to adult hemoglobin. MeHg is transported through the placental layers by neutral amino acid carriers, but

the analogous carrier on the fetal side are absent or have a reduced activity. This may explain why MeHg accumulates to higher extent in cord blood compared to maternal blood (reviewed by Stern et.al [51]).

Table 11. Selected data on Hg concentrations in maternal blood and umbilical cord blood

City /Country	Hg-Maternal blood [median, range; µg/L]	Hg-cord blood [median, range µg/L]	
Vienna (Austria)	0.6; 0.15-3.9	1.3; 0.5-6.7	Present study
Bratislava (Slovak Republic)	0.6; 0.2-1.6	1.2; 0.6-4.5	Present study
Vienna	0.7; 0.1-5.2	1.1; 0.2-6.8	[48]
Bratislava	0.5; 0.1-9.9	0.53; 0.1-6.1	[50]
Sweden	0.73; 0.2-2	1.4; 0.3-3.8	[52]
Saudi Arabia	1.9;0-206.4	2.9; 0-26.5	[53]
Korea	3.1; 1.7-5.7	5.2; 3-9	[54]
Greenland[a]	12.8±13.6; 1.9-75.6	25.3±32.1;2.4-181	[55]
Canada[a]	10.4±0.4; 2.6-44.2	18.5± 0.4; 2.8-97	[56]

[a] mean values ± standard deviation

In our study group, the major Hg exposure factor is fish consumption. Our results confirm that marine as well as fresh water fish consumption is correlated to MatBl-Ery-Hg levels and CordBl-Ery-Hg levels. Because of the small number (i.e., 3%) of study participants, who consumed only fresh water fish, the observed correlations between fresh water fish consumption and MatBl-Ery-Hg and CordBl-Ery-Hg are very likely confounded by the concomitant consumption of marine fish species, because most individuals (i.e., 24 %) in the freshwater fish consumer group were also eating marine fish. The average Austrian is consuming 14.2 kg fish and seafood products per year, which is a bit higher than in our Vienna study group (231.3 g/w ≙ 12.03 kg/y). However the average Slovakian consumer is eating 8.3 kg fish and seafood per year, which also is in good agreement with the average fish consumption in our Bratislava study group (i.e., 148 g/w ≙ 7.7 kg/y).[57] Among the EU member states the Slovakian population has one of the lowest intake rate of fish and seafood and the Austrian population ranks in the lower middle.[58] Our results confirmed that the Austrians consume more fish and sea food than the Slovakians. None of our study participants exceeded the PTWI, because the average study participant was eating only one portion of fish per week during pregnancy.

Grandjean et al. [59] reported an association between birth weight and intake of the PUFAs Eicosatetraenoic acid (ETA) and Docosapentaenoic acid (DPA) through fish consumption. However we did not find an association between fish consumption and increased birth weight. The average maternal fish intake in our study groups (i.e., one meal per week) was low as compared to this study (on average three fish meals per week). Thus the fish consumption in Vienna and Bratislava might have been too low to detect such relation between birth weight and fish consumption. Moreover we did not find a link between fish consumption and gestational length. This finding might be explained by the fact that we excluded premature birth from our study groups. Similar to Ding et.al [60], who observed the relationship between prenatal low-level Hg exposure and fetal growth in a rural community in northern China, we could not find a relation between Hg exposure and the anthropometry of newborns.

Dental amalgam fillings are a further source of Hg exposure in our study group. Our results confirm that the number of dental amalgam fillings determines Ery-Hg levels. According to the WHO [61] the daily Hg uptake from dental amalgam fillings ranges between 1-27 µg, which is caused by chewing or tooth brushing. However the majority of dental amalgam holders resorb less than 5 µg Hg/d. Therefore amalgam fillings are an important source for a permanent low-level exposure to Hg.

The level of education is significantly correlated with both fish consumption and CordBl-Ery-Hg levels. Socioecologists have assumed that individuals with a high level of education may have a better ability to obtain and/or to understand dietary information regarding a balanced diet.[62] Roos et. al. pointed out, that lower socioeconomic groups prefer to consume traditional food (bread, potatoes, meat) rather than recommended ‘healthy’ food such as fruits, vegetables, or fish.[63] In this way education might have influenced fish consumption habits and Hg levels in our study group.

Similar to other studies [64], we observed gender-related Hg exposure: The male babies exhibit higher Ery-Hg levels than girls, although maternal fish consumption and maternal Hg levels did not vary in these groups. Adjusted to the MatBl-Ery-Hg, the male babies have slightly increased CordBl-Ery-Hg levels compared to females, which might indicate that the males accumulate more Hg from their mothers than females do. It is still not known whether male fetuses react more sensitive to the toxic effects of MeHg compare to female fetuses. Vahter et al. [65] reviewed that gender

differences in exposure of toxic metals is explicable because of differences in kinetics, mode of action or susceptibility. Similar to our findings, Thomas et. al. [66] treated females rats with MeHg, which showed a faster and more efficient elimination rate than male rats. After 98 days of dosing, the female rats excreted 54% of the dosed MeHg by feces and males only 51%. The female urinary excretion was about 7.5% of the dosed MeHg and the male one was about 3.2%. The authors concluded that there is a gender-related difference in distribution, retention and metabolism of MeHg. Furthermore there is a possibility that gender-related differences in Hg accumulation are associated to fat composition, because it has been observed that there is a limited distribution of MeHg to fatty tissue.[64] As a matter of fact most of the previous findings refer to adult humans or animals. Therefore, our finding has to be interpreted with caution and further research is needed to confirm this finding.

The age of the mother is as well significantly correlated with Ery-Hg levels probably indicating continuous Hg exposure. Another plausible explanation is that with increasing age the metabolism rate is decreasing, which leads to a slower Hg excretion rate.[67, 68]

Consumption of cow's milk also has an influence on Ery-Hg levels. Although milk has a low level of Hg (12 µg/kg) it is consumed on a regular basis. 41% of the study participants consumed more than one litre per week (other dairy products were not surveyed in the questionnaires). Chapman et. al [69] has reviewed that consumption of milk is associated with an increasing MeHg absorption rate in the intestinal tract and moreover decreases excretion rate by feces. Thus, the influence of milk products on Ery-Hg levels might be greater than initially thought.

The participants in our study prefer marine fish over fresh water fish. The most frequently consumed fish is canned tuna. Hg content of canned tuna is lower compared to tuna steak, which might be explained by the fact that smaller (younger) tuna fish is processed to canned tuna. From an ecological perspective fresh water fish, especially regional products should be consumed rather than marine fish. The latter needs to be transported over long distances and it usually contains more Hg than fresh water fish. In our study group, the most frequently consumed fresh water fish species was trout. Compared with trout, tuna contains much higher levels of MeHg, lower levels of PUFAs and cannot be cultivated.

Fish as an ecological and economic factor

The FAO (United Food and Agriculture Organization) [70] monitored 600 marine fish stocks in 2003 and concluded that 20% of the marine fish stocks are moderately exploited, 52% are fully exploited, 17% are overexploited, 7% are depleted and 1% is recovering from depletion. This statistics show that nearly all fish stocks are on the limit of fishing capacity. This is a strong ecological argument to preferably consume cultured fish. Since 1950 the fish production increased from 15 to 120 million tons per year. Almost 46% of fishery products are cultured. Aquacultures are the fastest growing animal food producing sector with an average growth rate of 6.6% per year. China is representing one third of the world fish farmers. Farmed fish has lower Hg levels than wild fish species.[44] Noakes et al. [71] comment that farmed salmon has replaced wild salmon in many traditional markets which has changed the economic viability of the wild salmon fishery. However there are various problems with farmed fish. Experts are criticizing treatment with antibiotics, which are often used to avoid or control diseases in fish farms. Parasites from the fish farms could also infect the wild stocks. Another potential risk of farmed fish is that there might be a crossbreeding between farmed fish and wild fish, which reduces the genetic diversity of wild fish stocks and will lead to its replacement by farmed fish species. Furthermore the quality of a farmed fish is badly affected by the fact that it is being fattened to reduce the breeding time.[72] Fish famers enrich the waters with organic compounds to enhance their breeding outcome, which may lead to a disturbance in the natural ecosystem function. [73]

In 2008 almost 81% of world fish production was used for human consumption, while the rest was destined for fish meal and fish oil production or for direct feeding in fish farms. 29.7% of the total fish production is marketed as fresh fish while 41.2% are used to be prepared frozen or cured for the human fish consumption. During the last decade the importance of fish as part of the human diet has increased. However its importance as a source of fish meal and fish oil production has been diminished [74]

Risk and benefits of fish consumption during pregnancy

The discussion about benefits and risks of fish consumption during pregnancy is ongoing. On the one hand fish provides essential PUFAs, which are needed for the development of the brain and for cardiovascular health. On the other hand MeHg produces adverse effects in the same areas of the human body. n3-PUFAs are involved in placental flow and process of parturition and the intake during pregnancy is related to fetal growth and prolonged gestation. Fish is also a relevant source of Se, the assumed major antagonist of Hg. Due to the beneficial effects associated with the intake of n3-PUFAs and Se, several experts and food safety authorities recommend to eat two to three fish meals per week containing high level of PUFAs and Se and concomitantly low levels of Hg (i.e., anchovies, carp or salmon, which are rich in PUFAs and shrimp or mussels, which are high in Se). [43, 45, 59, 75] Table 12 is listing Hg contents, the Se contents and the EPA and DHA levels of commonly consumed fish species. Hg contents in fish do not vary in dependence of the preparation (cooked, cured or raw fish) or whether it is marketed fresh or frozen, because the Hg is stably bound to amino acids in the muscle tissue. [25]

Table 12. Hg, Se and n3-PUFAs content of food

Food	Hg [µg/kg]	Se [µg/kg]	EPA+ DHA pro 100g [mg]	
Anchovy	108	458.5	2055	[43-45]
Carp (farmed AT)	43	139	451	[43, 44, 76]
Cod	19	656	158	[43-45]
Fishsticks	50	170	214	[45]
Herring	50	470	2014	[45]
Innards	2.6	222	n.A	[43, 44]
Milk	12	16.5	0	[43-45]
Mussels	4.5	1238	782	[43-45]
Octopus	94	1400	314	[46, 76, 77]
Pangasius	13	103	100	[43, 44, 78]
Pike	339	418	140	[25, 43, 44]
Pike perch	150	200	215	[47, 79, 80]
Plaice	35	519	190	[25, 43, 44]
Tuna (canned)	116	641	228	[43-45]
Tuna (steak)	678	1728.5	733	[43-45]
Trout (farmed AT)	21	127	935	[43-45]
Salmon	37	539	2648	[43-45]
Shrimp	69	778	315	[43-45]
Shark	387	885	120	[25, 43, 44]
Swordfish	399	934	819	[43-45]

It has been assumed that the adverse effects of MeHg are confounded by the opposing benefits from PUFAs intake and that the data of the PUFAs benefits will be confounded by the risks of MeHg. Even if it is possible to calculate the benefits from PUFAs consumption, e.g., from dietary supplementations, it is impossible to assess the unconfounded MeHg risks. In their review Stern et. al. [81] conclude that fish species such as anchovies or salmon, which contain high levels of PUFAs and low levels of MeHg will pose a net benefit. Accordingly, fish species like tuna, shark or swordfish with low levels of PUFAs and high levels of MeHg will pose a net risk. Moreover when fish is rich in n3-PUFAs and low in Hg, the fish species may be higher contaminated with other pollutants like polychlorinated biphenyls (PCB) or other dioxins. Compared to MeHg, fish is not the common exposure route for dioxin, PCB or other pollutants. [73]

There is a growing discussion on the issue whether fish oil supplementation should replace fish consumption in order to avoid Hg exposure during pregnancy, but several studies (reviewed by Coletta et. al.[27]) showed no difference in gestational age or risk of premature birth between fish oil supplemented groups and controls. At date there are not enough data to give a recommendation of an additional intake of fish oil supplementation.

n3-PUFAs, Se and other beneficial compounds in fish can be protective against the harmful effects of the neurotoxin Hg. Several studies indicated that children, who had mothers, which consumed during pregnancy a high number of low-contaminated fish, perform better in fine motoric skills and cognitive test. It has been shown that fish consumption during pregnancy leads to general good health condition as well as to a good development right up into adolescence.[82, 83]

In summary it can be stated that the risks and benefits of fish consumption during pregnancy are mutually confounding and neither can be adequately understood in isolation. The most useful fish consumption advisories for pregnant women need to address the benefits from PUFAs as well as the risks from MeHg intake because both are concomitantly taken up via fish consumption. [81]

5l Conclusion

The mean Ery-Hg levels of our study participants were not alarming. Compared to other European populations our study group showed a low level of fish consumption resulting in the observed low to moderate Hg exposure levels in pregnant women and their newborns. Nonetheless, we found strong associations between the Ery-Hg levels and fish consumption and between MatBl-Ery-Hg level and the number of dental amalgam fillings. Fish is healthy food because it contains a large amount of essential nutrients. Especially during pregnancy these nutrients are required for the regular development of fetal brain, for fetal growth and adequate gestational length. However, fish contains also MeHg, which is a well-known neurotoxin. It is thus recommended to consume fish two to three times per week, particularly those species, which are rich in PUFAs and poor in MeHg. In sum, pregnant women should preferably eat fish of low-trophic levels such as anchovies and salmon as well as domestic fish species like carp and trout. The consumption of predator fish species such as tuna, shark or sword fish however should be avoided.

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7I Appendix

Questionnaires:

Projekt UM-MUKI: Umweltschadstoffe in Mutter-Kind-Paaren – Belastungssituation im Raum Bratislava-Wien

Fragebogen 1 (~SSW21)

Datum:…… **ID**…..

(1) Allgemeines

Name:..... Geburtsdatum: _ _ _ _ _ _ _ _

Geburtsort/-land: ..... Wohnort: .....

Größe (cm): ..... Gewicht (kg):.....

Gewicht vor SS (kg):…… SSW:……

Wievielte SS…… Anzahl Fehlgeburt/en:……..

(2) Höchste abgeschlossene Ausbildung, Beruf

☐ Volksschule ☐ Hauptschule ☐ Lehrabschluss ☐ AHS/BHS ☐ Hochschule

☐ andere:…….

Beruf (auch frühere berufliche Tätigkeiten): .....

.....

Beruf Partner: .....

(3) Arbeitsplatz

Sind Sie ☐ Studentin bzw. in Ausbildung ☐ berufstätig ☐ nicht berufstätig ☐ Hausfrau

Dauer Berufstätigkeit:☐ >10 Jahre ☐ >5 Jahre ☐ <5 Jahre

Letzte Berufstätigkeit vor ……..Jahren

Frühere Berufstätigkeit(en) ……

Mutterschutz seit ☐ gesetzl. Frist ☐ davor……. …….☐ später…….

Branche

☐ Lebensmittel ☐ Textil ☐ Chemie ☐ Kunststoff ☐ Holz ☐ Medizin

☐ Pharma ☐ Kosmetik ☐ Drogerie ☐ Baustoff ☐ Möbel ☐ Tiermedizin

☐ Tierzucht ☐ Sonstiges: ……

Fragebogen 2 (~SSW 36)

Datum:……

ID…….....

(1) Allgemeine Daten

Name:..... Wohnort: .....

Gewicht (kg):..... SSW:……

Verlauf SS: ……..

(2) Haben Sie Ihren Wohnort geändert?

□ nein → **(3)**

□ ja: Wie viele Personen leben in ihrem Haushalt? Anz. Erwachsene: _ _ Anz. Kinder: _ _

Wie lange wohnen Sie schon an Ihrem derzeitigen Wohnort? _ _ Jahre

□ Einfamilienhaus: □ erbaut vor 1945 □ erbaut nach 1945

□ Wohnung: □ erbaut vor 1945 □ erbaut nach 1945

□ dicht verbautes Gebiet □ Stadtrand □ Land

Abstand zu stark befahrener Straße: □ < 50 m □ 50 – 300 m □ > 300 m

Nähe zu Industrie/Gewerbebetrieben: □ <50 m □ 50-300m □ keine Betriebe in der Nähe

□ grundlegende Sanierungsarbeiten innerhalb der letzten 5 Jahre ……. □ keine

Bodenbelag: □ neu innerhalb der letzen 5 Jahre □ kein neuer Bodenbelag

überwiegender Bodenbelag: □ Teppich □ Laminat □ Kunststoff Holz □ Keramik □ Linoleum

Polstermöbel: □ >10 Jahre alt, □ >5 Jahre alt □ neue Polstermöbel

□ Ledermöbel

□ Verwenden Sie regelmäßig einen Wäschetrockner in der Wohnung? □ nein □ ja

(3) Anamnese und Blutbild

Aktuelle Erkrankungen: □ nein □ ja, welche:……

Medikamente: □ nein □ ja, welche:……

(4) Haarbehandlungen

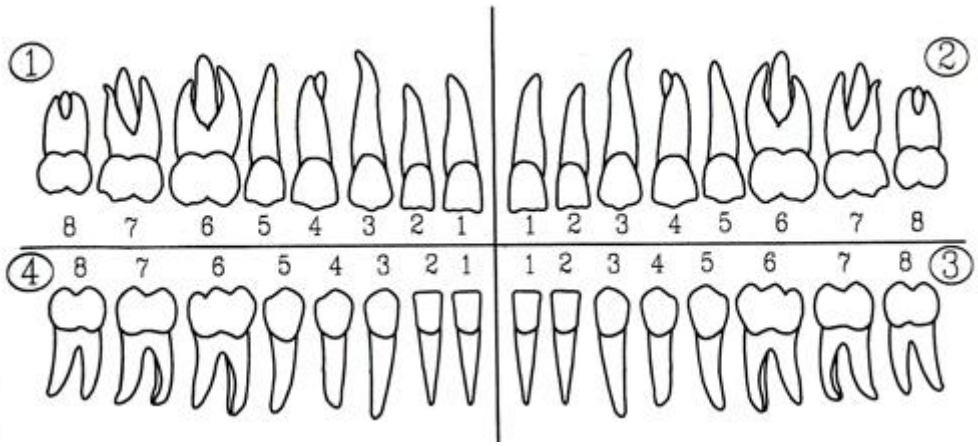
Verwenden Sie Haarfärbemittel? nein ja, welche: .....

Dauerwelle o.ä.: □ nein ja

Wann haben Sie Ihre Haare das letzte Mal gefärbt? vor ..... Woche(n)

(5) Zahnstatus

Besitzen Sie Amalgamplomben? nein □ ja, Anzahl Füllungen: .....



Wann war letzte Plombierung? <3 Monate □ <6 Monate <9 Monate <1 Jahr

Wurden in der letzten Zeit Amalgam-Plomben entfernt?

nein ja, vor <3 Monaten □ <6 Monaten, <9 Monaten, <1 Jahr <2 Jahren

Besitzen Sie Kunststoffplomben /Inlays? nein ja, Anzahl Füllungen: .....

Wann war letzte Plombierung? <3 Monate □ <6 Monate <9 Monate <1 Jahr

Wurden in der letzten Zeit Kunststoff-Plomben entfernt?

nein ja, vor <3 Monaten □ <6 Monaten, <9 Monaten, <1 Jahr <2 Jahren

(6) Ernährung

Wie oft stehen diese Lebensmittel wöchentlich auf ihrem Speiseplan? Bitte Durchschnittswert für die vergangenen 9 Monate angeben

Fleisch (1 Portion: ca. 150g)

Kalbfleisch/Rindfleisch □ nie □ 1-2mal □ 3-7mal >7mal

Schweinefleisch □ nie □ 1-2mal □ 3-7mal >7mal

Geflügel □ nie □ 1-2mal □ 3-7mal >7mal

Wild nie □ 1-2mal □ 3-7mal >7mal

Innereien □ nie □ 1-2mal □ 3-7mal
>7mal

Pilze

Champignons □ nie □ 1-2mal □ 3-7mal □
>7mal

Andere Pilze □ nie □ 1-2mal □ 3-7mal
>7mal

Getränke

Leitungswasser (hier: tägliche Aufnahme) □ <1/2 L □ <1L □ <2L >2L

Mineralwasser PET □ nie □ ……L/Woche

Mineralwasser Glas □ nie □ ……L/Woche

Softdrinks PET-Flaschen □ nie □ ……L/Woche

Softdrinks Tetrapak □ nie □ ……L/Woche

Fruchtsaft Tetrapak □ nie □ ……L/Woche

Glas Milch □ nie □ 1-2mal □ 3-7mal
>7mal

Tasse Kaffee nie □ 1-2mal □ 3-7mal
>7mal

Tasse Schwarztee nie □ 1-2mal □ 3-7mal
>7mal

Tasse Grüntee nie □ 1-2mal □ 3-7mal
>7mal

1/8 L Rotwein nie □ 1-2mal □ 3-7mal
>7mal

1/8 L Weißwein/Champagner nie □ 1-2mal □ 3-7mal
>7mal

0,3 L Bier nie □ 1-2mal □ 3-7mal
>7mal

Stamperl Schnaps (2 cL)	nie	□ 1-2mal	□ 3-7mal
>7mal			

Schalentiere (1 Portion: ca. 100g)

Muscheln (Austern,…)	□ nie	□ 1-2mal	□ 3-7mal
>7mal			
Krebse (Garnelen, Shrimps, Krabben)	□ nie	□ 1-2mal	□ 3-7mal
>7mal			
Tintenfisch (Oktopus, Sepia, Calamari)	□ nie	□ 1-2mal	□ 3-7mal
>7mal			

Fisch (1 Portion: 100-150g)

Sardinen, Sardellen	□ nie	□ ……g/Woche
Dosen-Thunfisch	□ nie	□ ……g/Woche
Thunfischsteak	□ nie	□ ……g/Woche
Lachs (geräuchert, Steak)	□ nie	□ ……g/Woche
Sushi	□ nie	□ ……g/Woche
Hering (Matjes, „Russen“,…)	□ nie	□ ……g/Woche
Haifisch (Steak, Schillerlocke)	□ nie	□ ……g/Woche
Schwertfischsteak	□ nie	□ ……g/Woche
Dorsch/Kabeljau	□ nie	□ ……g/Woche
Fischstäbchen	□ nie	□ ……g/Woche
Scholle, Seezunge	□ nie	□ ……g/Woche
Forelle	□ nie	□ ……g/Woche
Karpfen	□ nie	□ ……g/Woche
Hecht	□ nie	□ ……g/Woche
Zander	□ nie	□ ……g/Woche
……..	□ nie	□ ……g/Woche

…….. □ nie □ ……g/Woche

…….. □ nie □ ……g/Woche

Take away food/ Fast Food/Dosennahrung

Hamburger/Ähnliches □ nie □ 1-2mal □ 3-7mal >7mal

Pommes Frites □ nie □ 1-2mal □ 3-7mal >7mal

„Take away“ in Papierkartons □ nie □ 1-2mal □ 3-7mal >7mal

„Take away“- Getränke im Papierbecher □ nie □ 1-2mal □ 3-7mal >7mal

Mikrowellen-Popcorn □ nie □ 1-2mal □ 3-7mal >7mal

Lebensmittel aus Konserven? □ nie □ 1-2mal □ 3-7mal >7mal

(7) Rauchen

Sind Sie

□ Nichtraucherin

frühere Raucherin: Wie viele Jahre haben Sie insgesamt geraucht? ……. Jahre

Raucherin: Seit wie vielen Jahren rauchen Sie? ……Jahre

Ø Anzahl an Zigaretten: …….pro Tag

Wie viele Personen in Ihrem Haushalt sind Raucher? ……Personen

(8) Arbeitsplatz

Hat sich Ihr Arbeitsplatz verändert? □ nein → **(9)**

□ ja: Sind Sie □ Studentin bzw. in Ausbildung berufstätig nicht berufstätig Hausfrau

Dauer Beruftstätigkeit: >10 Jahre >5 Jahre <5 Jahre

Letzte Beruftstätigkeit vor ……..Jahren

Frühere Berufstätigkeit ……..

Mutterschutz seit gesetzl. Frist davor……. ……. später……

Branche

Lebensmittel Textil Chemie Kunststoff Holz Medizin

Pharma Kosmetik Drogerie Baustoff Möbel Tiermedizin

Tierzucht Sonstiges: …….

Art des Arbeitsplatzes

Büro Produktion Verkauf: ……

Labor Reinigung chemische Reinigung Arztpraxis Zahnarztpraxis

Fotografie Friseurin Kosmetikerin Sonstiges: …….

Bestand Exposition mit (auch bei Studentinnen und Hausfrauen abfragen!)

Reinigungsmitteln

Chemikalien, wenn bekannt (z.B.: Imprägniermittel, Epoxyklebern,…):……

Bioziden, wenn bekannt: ……

Wurden in den letzen 5 Jahren grundlegende Sanierungsarbeiten am Arbeitsplatz durchgeführt? □ nein ja …….

überwiegender Bodenbelag am Arbeitsplatz:

□ Teppich □ Laminat □ Kunststoff Holz □ Keramik □ Linoleum

Sehr häufige / reichliche Anwendung von Raumpflegeprodukten (Imprägnier-, Polier-, Desinfektionsmitteln etc.) nein ja:……..

(9) Freizeit

Verhalten vor Schwangerschaft

Tragen von „Funktions“-Sportbekleidung (antibakteriell/geruchshemmend/wasser o. schmutzabweisend)/Woche □ nie □ 1-2mal □ >3mal

Verwendung von Bastelmaterialien, Bastelklebern

□ nie □ 1-2mal/Monat □ 1-2mal/Woche

Verhalten während Schwangerschaft

Tragen von Funktions-Sportbekleidung (antibakteriell/geruchshemmend/wasser- o. schmutzabweisend)/Woche □ nie □ 1-2mal □ >3mal

Verwendung von Bastelmaterialien, Bastelklebern

□ nie □ 1-2mal/Monat □ 1-2mal/Woche

HERZLICHEN DANK FÜR IHRE MITARBEIT!

Fragebogen 3 (post partum)

Datum:……

ID…….....

Name:.....

Daten Kind

Geburtsdatum: ..... Name: .....

Geschlecht: ..... Größe:.....

Gewicht: ..... Kopfumfang: .....

APGAR: ..... Schwangerschaftsdauer: in Wochen: ……

Gesundheitszustand

Mutter:…….

Gesundheitszustand

Kind:……

Geburtsverlauf

□ Spontangeburt □ Einleitung □ Sectio

Anästhesie: □ nein □ ja: □ Vollnarkose □ Epiduralanästhesie

Auffälligkeiten Nabelschnur? .....

Auffälligkeiten Mekonium? .....

Auffälligkeiten Fruchtwasser? .....

Auffälligkeiten Plazenta? .....

Blutbild Kind

Apgar:…….. BE:…… Hb:……

Nabelschnur-Blut pH art.:…… Nabelschnur-Blut pH ven.:……..

HERZLICHEN DANK FÜR IHRE MITARBEIT!

8I Curriculum Vitae

Persönliche Daten:

Name: Veronika Plichta
E-Mail: veronika.plichta@gmail.com

Ausbildung:

11/2011-06/2013	Diplomarbeiten für die Studienzweige Anthropologie und Ökologie an der Medizinischen Genetik, Medizinischen Universität Wien
2008-	Diplomstudium Ökologie und Anthropologie (Schwerpunkt Humanökologie) an der Universität Wien
2006-2008	Diplomstudium der Biologie an der Universität Wien
2005-2006	Diplomstudium der Molekularen Biologie an der Universität Wien
1997-2005	BG Rahlgasse Wien, Matura am 07.06.2005
1993-1997	VS Notre Dame de Sion, Wien

Berufliche Qualifikation

11/2012 – bis dato	wissenschaftliche Mitarbeit am Projekt: ***„Mercury toxicokinetics in human term placenta: functional proof and localization of involved candidate proteins“***
11/2011 – 11/2012	wissenschaftliche Mitarbeit am Projekt: „***UM-MUKI: Umweltschadstoffe in Mutter-Kind-Paaren-Belastungssituation“*** Diplomarbeit betreut von Prof. Gundacker, Medizinische Genetik, Medizinische Universität Wien
4/2013,4/2012	Tutorin im Humangenetisches Praktikum für Mediziner (Block 5) Medizinischen Universität Wien
4/2012	Vortrag: ***„Pränatale Quecksilberbelastung: Leben wir in Österreich auf einer Insel der Seligen?“*** Langen Nacht der Forschung, Wien