From the Institute of Environmental Medicine
Karolinska Institutet, Stockholm, Sweden

Mercury exposure during early human development

Karolin Ask Björnberg

Stockholm 2005

Published and printed by Karolinska University Press
Box 200, SE-171 77 Stockholm, Sweden

ISBN 91-7140-224-1

To Johan and Hedda

ABSTRACT

People are exposed to methylmercury (MeHg) mainly via consumption of fish, especially large predatory fish species. Inhaled mercury vapour (Hg^0) released from dental amalgam fillings is the main source of exposure to inorganic mercury (I-Hg). In the body, a small fraction of MeHg is demethylated to inorganic Hg^{2+} while most Hg^0 is oxidized to Hg^{2+}. Both MeHg and Hg^{2+} are neurotoxic, especially for the developing brain.

The aim of the present thesis was to increase the knowledge about the exposure to MeHg and I-Hg in Swedish women of childbearing age, the transport to the fetus and infant and to evaluate possible associations between Se, an antioxidant reported to protect against Hg toxicity, and the different forms of Hg.

Hg exposure during pregnancy was studied by determination of total Hg in maternal hair and MeHg and I-Hg in maternal blood. Fetal exposure was assessed by MeHg and I-Hg in cord blood. Infant exposure was assessed by MeHg and I-Hg in infant blood and total Hg in breast milk. The concentrations of MeHg and I-Hg were determined by cold vapour atomic fluorescens spectrophotometry (CVAFS). Se concentrations in serum or whole blood were analysed by graphite furnace atomic absorption spectrophotometry (GFAAS).

The exposure to I-Hg was mainly as Hg^0 from dental amalgam fillings with low exposure from other sources. The concentration of I-Hg in cord blood was about the same as in maternal blood and increased with increasing number of maternal dental amalgam fillings. This shows that a substantial fraction of Hg^0 passes the placenta to the fetus before being oxidized to Hg^{2+}. Also, I-Hg accumulated in the placenta in relation to the number of amalgam filings. The use of amalgam in dentistry is decreasing.

MeHg exposure was highly dependent on fish consumption in general, but consumption of freshwater fish and certain marine species e.g. swordfish and tuna contributed more to the exposure. MeHg in cord blood increased with increasing maternal fish consumption. It was almost twice the concentration in maternal blood, supporting an active transport of MeHg across the placenta.

Although, the average exposure to MeHg was relatively low, there was quite a range. A small fraction of women recruited because of high fish consumption had Hg concentrations exceeding the RfD (U.S. EPA) and PTWI (JECFA), corresponding to a daily intake of 0.1-0.2 µg MeHg/kg bw. Thus, there seems to be a fairly narrow margin of safety for increased risk of neurodevelopmental effects in fetus of women with high fish consumption unless they decrease their intake of certain fish species before being pregnant. It can be concluded that the recently updated dietary advisories on fish consumption for pregnant and lactating women and women planning pregnancy are justified and necessary as long as MeHg levels in fish are elevated. There was a good compliance to the dietary advisories regarding fish consumption during pregnancy but it is important that the advisories reach all women planning pregnancy, so they can decrease their consumption of certain fish species to avoid early fetal exposure. However, it is also important to emphasize the benefits of fish consumption. It is not known to what extent the positive effects of fish consumption may outweigh the negative effects of MeHg.

Infant exposure to I-Hg and MeHg was lower than the prenatal exposure. Total Hg concentrations in breast milk decreased during the first three months postpartum. I-Hg seems to be more easily transported to breast milk than MeHg. Still, MeHg in breast milk may contribute more to infant exposure, because of higher gastrointestinal absorption. Infant blood MeHg decreased until 13 weeks of age indicating that infants are able to excrete MeHg taken up during fetal life, contrary to previous believes.

No associations between Se and the different forms of Hg were observed in the placenta and Se did not affect the accumulation or transport of I-Hg or MeHg in placenta. The concentration of Se in serum decreased during the course of pregnancy. Probably, this can partly be explained by a prioritized fetal transport and need of Se for placental functions.

LIST OF PUBLICATIONS

This thesis is based on the following papers, which will be referred to by their Roman numerals.

I. Osman K, Åkesson A, Berglund M, Bremme K, Schütz A, Ask K, Vahter M (2000). Toxic and essential elements in placentas of Swedish women. Clinical Biochemistry 33:131-138

II. Ask K, Åkesson A, Berglund M, Vahter M (2002). Inorganic mercury and methylmercury in placentas of Swedish women. Environmental Health Perspectives 110:523-526

III. Ask Björnberg K, Vahter M, Petersson-Grawé K, Glynn A, Cnattingius S, Darnerud PO, Atuma S, Aune M, Becker W, Berglund M (2003). Methyl mercury and inorganic mercury in Swedish pregnant women and in cord blood: influence of fish consumption. Environmental Health Perspectives 111:637-641

IV. Ask Björnberg K, Vahter M, Petersson Grawé K, Berglund M (2004). Methyl mercury exposure in Swedish women with high fish consumption. The Science of the Total Environment, in press 16th Nov 2004
available at: http://www.sciencedirect.com

V. Ask Björnberg K, Vahter M, Berglund B, Nicklasson B, Blennow M, Sandborgh-Englund G (2005). Transfer of methylmercury and inorganic mercury to the fetus and breast-fed infant, submitted to Environmental Health Perspectives

CONTENTS

LIST OF ABBREVIATIONS

AAS	Atomic absorption spectrophotometry
ATSDR	Agency for Toxic Substances and Disease Registry
BMDL	Benchmark dose level
CV	Coefficient of variation
CVAFS	Cold vapor atomic fluorescence spectrophotometry
FAO	Food and Agriculture Organization of the United Nations
FFQ	Food frequency questionnaire
GW	Gestational week
ICP-MS	Inductively coupled plasma mass spectrophotometry
I-Hg	Inorganic mercury
JECFA	Joint FAO/WHO Expert Committee on Food Additives
MeHg	Methylmercury
MIA	Multiple injection analysis
NOEL	No observed effect level
NRC	National Research Council
PCB	Polychlorinated biphenyls
PTWI	Provisional tolerable weekly intake
QC	Quality control
RBC	Red blood cell
RfD	Reference dose
Se	Selenium
T-Hg	Total mercury
U.S. EPA	United States Environmental Protection Agency
UNEP	United Nations Environment Programme
WHO	World Health Organization

INTRODUCTION

ENVIRONMENT

Mercury (Hg) is a naturally occurring element found throughout the environment (ATSDR 1999). Hg has been mined from cinnabar (ore of mercury sulfide) and used in different products and processes since ancient times (Clarkson 1997). Cinnabar has a bright red color and one early use was as pigments in cosmetics and dyes. Hg has also been used in manufacturing of mirrors and felt hats, in pharmaceutical preparations, as fungicides and in dental amalgam (WHO 1990, WHO 1991, Clarkson 1997). Since the start of the industrial era, Hg has mainly been used in the chloralkali-industry, as a cathode in the electrolysis of sodium chloride. It is also used in thermometers, barometers, batteries, lamps and electrical switchers (ATSDR 1999). Large quantities of Hg have been and are still used for e.g. gold extraction (Hylander and Meili 2003). Another usage is preservatives for certain vaccines (Magos 2001, Clarkson 2002).

Hg occurs in several forms (NRC 2000): inorganic elemental or metallic mercury (Hg^0), inorganic compounds (Hg^+; mercurous and Hg^{2+}; mercuric) and organic compounds (e.g. methylmercury, ethylmercury). The main forms and sources of human Hg exposure are presented in Table 1.

Table 1. *Exposure forms of mercury*

Form		**Exposure source**
Hg^0	Elemental mercury	Dental amalgam
Hg^+, Hg^{2+}	Inorganic mercury compounds (I-Hg)	Food, skin-lightening creams
CH_3Hg^+	Methylmercury (MeHg)	Fish

Hg is emitted to the atmosphere from both natural and anthropogenic sources, e.g. through mining, combustion, industrial discharges (ATSDR 1999) and crematories (Vahter and Friberg 1992), in the form of elemental mercury vapor (Hg^0). In atmosphere, Hg^0 is converted to soluble inorganic Hg^{2+}, which returns to the surface of the earth in rainwater. Hg deposited on land and water may partly be reduced to Hg^0 again and reemitted to the atmosphere (WHO 1990, ATSDR 1999). Thus, there is continuous global cycling of Hg. Inorganic mercury (I-Hg) in soil and sediments may be converted to methylmercury (MeHg) by microorganisms. In this form, Hg can accumulate in the aquatic food chain.

Hg pollution is a global problem (UNEP 2002). In many countries, actions have been taken to reduce the use and emission of Hg (UNEP 2002, Commission of the European Communities 2005). In Sweden, the use of Hg decreased substantially during the last decade (KEMI 2004). However, due to the extensive historic release of Hg from chloralkali industries and use of Hg as a preservative in paper industry, many lakes and coastal areas in Sweden are still contaminated and this problem will persist for many decades (Naturvårdsverket 2002).

EXPOSURE

Inorganic mercury

Inhaled Hg^0 released from dental amalgam fillings is the main source of exposure to I-Hg in the general population (WHO 1991). Dental amalgam contains about 50% Hg (ATSDR 1999) and has been used for dental restorations since early 1800s (Hoover and Goldwater 1966). Bruxism and use of chewing gum may increase the release of Hg^0 and thereby the exposure (Barregård et al. 1995, Sällsten et al. 1996).

The use of skin-lightening soaps and creams, contaning I-Hg compounds, is a source of I-Hg exposure (al Saleh and al Doush 1997). Also, exposure to I-Hg may occur when liquid elemental Hg is used for ritual religious purposes (Riley et al. 2001). To a small extent, humans might be exposed to I-Hg via food. In Sweden, total Hg concentrations of 9 µg/kg and 5 µg/kg have been reported in pork and beef, respectively (Jorhem et al. 1991), however, this is probably partly MeHg due to the use of fish meal in animal feed.

Methylmercury

Fish consumption is the main source of exposure to MeHg in the general population. MeHg biomagnifies throughout the aquatic food chain, reaching highest concentrations in larger and long-lived predatory fish and marine mammals many of which are consumed by humans (ATSDR 1999, UNEP 2002). In Sweden, the environmental quality objective (Regeringsproposition 2000) is that the concentrations of MeHg in fish should not exceed the background level of 0.2 mg/kg (Naturvårdsverket 2002). About half of all Swedish lakes have MeHg levels in one-kilo pike (Naturvårdsverket 2002) above the European limit of 0.5 mg/kg for most fish species (Commission Regulation No 466 2001). There are also many lakes in Sweden where MeHg levels exceed 1 mg/kg in fish (Naturvårdsverket 2002), the maximum limit for certain species. Halibut, tuna, swordfish and shark are examples of ocean-living species which may have elevated levels of MeHg (UNEP 2002).

During the 1950s a mass poisoning of MeHg occurred in Minamata, Japan. This was due to the consumption of highly contaminated fish following industrial emission of MeHg in Minamata Bay (Tsubaki and Irukayama 1977). This caused attention to the MeHg problem in Sweden (Berglund et al. 1970). Another episode of mass MeHg poisoning occurred in Iraq in the early 1970s when seed grain treated with a MeHg-containing fungicide was ground into flour and used for baking bread (Bakir et al. 1973, Myers et al. 2000). In both events there were many deaths. Since then, it has become clear that also low level chronic MeHg exposure through consumption of MeHg contaminated fish is of concern.

Ethylmercury

EtHg is an organic Hg compound. It is mainly used in the preservative thimerosal present in many vaccines (25-50 µg Hg/ml; AAP 1999) also routinely given to infants and children (Goldman and Shannon 2001, Pichichero et al. 2002). In Sweden, the use of thimerosal containing vaccines for children stopped in 1992 (pers communication. Patrik Olin, Swedish Institue for Infectious Disease Control). As a precautionary measure, the American Academy of Pediatrics has recently recommended that thimerosal-containing vaccines should be reduced or eliminated (AAP 1999).

HEALTH EFFECTS

Inorganic mercury

The primary target organ of Hg^{2+} toxicity is the kidney (WHO 1991, Clarkson 1997). Hg^{2+} accumulates in kidneys where it may interfere with both tubular and glomerular renal function causing kidney damage. The target organ for Hg^0 toxicity is the brain, although the neurotoxicity is due to Hg^{2+} (WHO 1991), which is rapidly formed via oxidation of Hg^0 by the catalase-hydrogen peroxide pathway (Clarkson 1997).

Toxic effects due to exposure to both Hg^0 and Hg^{2+} have mainly been associated with occupational exposure but in the general population the main I-Hg exposure is as Hg^0 via dental amalgam fillings (WHO 1991). Contact allergy to Hg in dental amalgam is a well-established adverse effect (Public Health Service 1993). Other adverse health effects caused by Hg in dental amalgam have been a subject of considerable debate for many years, but there is no certain evidence for such an association (Sandborgh-Englund et al. 1996, Berlin 2002, Bates et al. 2004). However, it can not be excluded that dental amalgam can cause adverse health effects in a sensitive part of the population (Berlin 2002).

Hg^{2+} binds to SH-groups, which may interfere with critical enzyme or structural protein functions (WHO 1991, NRC 2000). It has been suggested that inhaled Hg^0 may damage the microtubular system (Pendergrass et al. 1997). Hg^{2+} may also cause oxidative stress (Olivieri et al. 2000).

Methylmercury

The critical organ for MeHg toxicity is the brain. Both adult and fetal brains are affected, although the developing nervous system appears to be more sensitive (WHO 1990). During the poisoning event in Minamata, Japan, it became clear that prenatal MeHg exposure may damage the fetal brain (WHO 1990, Harada 1995). Infants with severe cerebral palsy were born after their mothers, who were only slightly poisoned, had consumed heavily contaminated fish. Neurotoxic effects were also found among Iraqi children prenatally exposed to MeHg (Amin-Zaki et al. 1974).

Recently, two large-scale epidemiological studies have been carried out on fish-eating populations chronically exposed to MeHg via fish (Grandjean et al. 1997, Davidson et al. 1998). Impaired cognitive function was reported to be associated with prenatal exposure to MeHg in the Faeroe Island (Grandjean et al. 1997), but not in the Seychelles (Davidson et al. 1998). Recent follow-up studies in these populations did not change the conclusions (Myers et al. 2003, Murata et al. 2004). Intrauterine exposure to MeHg was associated with delays in brainstem auditory evoked potentials, among 14-year old children from the Faeroe Island (Murata et al. 2004).

The reason for different results in the two studies is a matter of debate. In the Seychelles, the MeHg exposure resulted from daily high fish consumption and in the Faeroe Island the population was also episodically exposed to high levels of MeHg through consumption of pilot whale. In general, the pilot whale has much higher concentrations of MeHg than ocean fish and also contains polychlorinated biphenyls (PCBs). The Faeroes investigators showed that MeHg neurotoxicity might be potentiated by PCBs, although they reported that the data continue to show adverse effects of MeHg even after correction for PCBs (Grandjean et al. 2001). No PCB in serum was detected in the Seychelles study, however only a few samples were tested (Davidson et al. 1998).

Fish consumption may reduce the risk of coronary heart disease according to several epidemiological studies (Kromhout et al. 1985, Kromhout et al. 1995, Daviglus et al. 1997). Probably this is due to the fact that fish is an important source for polyunsaturated fatty acids (Mahaffey 2004a). However, a possible role for MeHg exposure in the development of cardiovascular effects has been indicated. An increased risk of coronary heart disease due to high intake of MeHg from fish was found in some epidemiological studies (Salonen et al. 1995, Guallar et al. 2002) but not in others (Ahlqwist et al. 1999, Hallgren et al. 2001, Yoshizawa et al. 2002). Prenatal MeHg exposure was associated with increased blood pressure at age 7 (Sörensen et al. 1999) and with effects on the autonomic regulation of heart function at age 14 (Grandjean et al. 2004) among children from the Faeroe Island. These findings raise the possibility that MeHg exposure via fish may diminish the cardioprotective effect of fish intake.

Biochemical mechanisms of MeHg neurotoxicity involve binding to SH-groups in proteins and include disrupted protein synthesis and inhibition of microtubule (WHO 1990, Clarkson 1997, Castoldi et al. 2001). This inhibits division, growth and migration of neuronal cells, which makes the developing nervous system especially sensitive. MeHg may also induce the formation of reactive oxygen species leading to oxidative stress (Castoldi et al. 2001). Oxidative stress may cause damage on DNA, lipids and proteins leading to disturbed cellular function (Hensley et al. 2000). Antioxidants have been shown to provide some protection against effects of MeHg (Daré et al. 2000).

KINETICS

Inorganic mercury

Human studies have shown that very little, in the order of 0.04%, of elemental Hg^0 is absorbed in the gastrointestinal tract (af Geijersstam et al. 2001). Also, minimal absorption (about 2% of the uptake by the lung) occurs at dermal exposure (Hursh et al. 1989). Approximately 70-80% of inhaled Hg^0 is absorbed in the lungs to the blood where it is distributed throughout the body (Hursh et al. 1976, Sandborgh-Englund et al. 1998b). Hg^0 is oxidized to inorganic Hg^{2+} by the catalase-hydrogen peroxide pathway and accumulates in this form in most tissues but particularly in the kidneys (ATSDR 1999). Experimental studies have shown that before being oxidized, inhaled Hg^0 may pass the blood-brain and placental barriers leading to accumulation of Hg within the brain (Berlin et al. 1969) and fetus (Khayat and Dencker 1982). Also, Hg has been found in fetal brain after exposure to Hg^0 (Vimy et al. 1990, Warfvinge 2000), although the largest amounts seem to accumulate in fetal liver (Khayat and Dencker 1982, Vimy et al. 1990, Takahashi et al. 2001). Hg^{2+} does not readily pass the blood-brain barrier (Berlin et al. 1969) or placenta (Khayat and Dencker 1982, Urbach et al. 1992). The half-time in blood after exposure to Hg^0 is about 3 days (fast phase) and 18 days (slow phase) (Barregård et al. 1992) and the whole-body half-time of inhaled Hg^0 is about 60 days (Hursh et al. 1976).

Less than 10% of ingested I-Hg compounds is absorbed in the gastrointestinal tract (Rahola et al. 1973), but the absorption in infants is believed to be higher (Clarkson 1992). Absorption through skin may occur although there is no quantitative data available (ATSDR 1999). The half-time in blood is about 28 days and whole-body half-time is about 40 days after exposure to Hg^{2+} (Rahola et al. 1973). Excretion of I-Hg occurs via feces and urine and the urinary route dominates when exposure is high (WHO 1991). Hg^0 may be excreted (7-14%) via exhaled air (Hursh et al. 1976, Sandborgh-Englund et al. 1998b, Jonsson et al. 1999).

Methylmercury

Oral exposure is the main route of exposure to MeHg. In humans, about 95% of MeHg is absorbed in the gastrointestinal tract (Åberg et al. 1969, Miettinen 1973) to the bloodstream where it is distributed to all organs throughout the body. It may also be absorbed by inhalation and through skin, however to what extent has not been studied (ATSDR 1999). Experimental studies have shown that MeHg is readily transported across the blood-brain barrier (Vahter et al. 1995) and the placenta (Nordenhäll et al. 1995), probably via the neutral amino acid carriers (Kerper et al. 1992, Kajiwara et al. 1996). One fraction of the MeHg in brain is slowly demethylated to I-Hg which accumulates in certain brain structures (Vahter et al. 1995). The half-time of MeHg in blood of adults is about 50 days (Stern 1997). The primary route of excretion (90%) of MeHg is mainly via feces. Demethylation of MeHg in the intestine is a key step in the excretion process. Both MeHg and the formed I-Hg are excreted into bile conjugated with glutathione. Unless demethylated MeHg is reabsorbed in the intestine, undergoing enterohepatic circulation (WHO 1990). Within the intestine, MeHg is demethylated by microflora (Rowland et al. 1984). The rate of excretion for MeHg in infants is not known, but is believed to be much lower than in adults. The low excretion of MeHg is due to low capacity for demethylation of MeHg by intestinal microflora in infants (Rowland et al. 1983) resulting in less excretion of MeHg (Nordenhäll et al. 1998).

MERCURY IN PLACENTA

As illustrated in Figure 1 the placenta consists of the maternal (decidual) and fetal (chorionic) parts. Placental chorionic villi bathe in maternal blood in the intervillous space. Nutrient and gas exchange between the fetus and the mother takes place across trophoblastic cell layers of the chorionic villi. The trophoblasts receive diffused and transported substances from maternal blood. Fetal capillaries in the chorionic tissue take up and carry these substances to the fetus (Brody 1993). In this way, the placenta serves as a point of contact between maternal and fetal circulations although the bloods are not mixed. The placental barrier between the two blood systems consists of two trophoblastic cell layers, connective tissue of the chorionic villi and the endothelium of the fetal capillary.

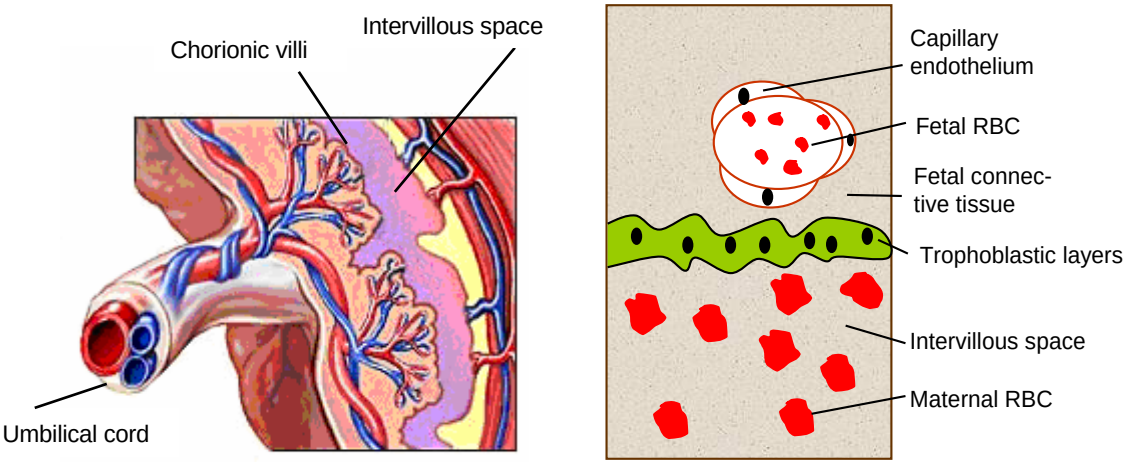

Figure 1. *The placenta.*

Nutrients, oxygen and waste products are transported across the placental barrier through carrier-mediated transport or simple diffusion (Miller et al. 1983). However, the same processes that transport nutrients can also act as pathways for toxic substances (Iyengar and Rapp 2001). Binding of metals to the placenta may interfere with placental function, such as transport of essential trace elements (WHO 1996) and nutrients (Danielsson et al. 1984) required for fetal growth and development.

It is generally believed that MeHg easily crosses the placental barrier, while I-Hg (Hg^{2+}) is trapped. Inhaled Hg^0 is oxidized to Hg^{2+} already in the blood, but some Hg^0 remains in the circulation long enough to pass the placental barrier (Khayat and Dencker 1982, Vahter et al. 2000, Warfvinge 2000). However, the few studies that have measured different forms of Hg (total Hg, MeHg, I-Hg) in placenta do not show consistently that I-Hg is the main form of Hg in placenta. (Kuhnert et al. 1981, Cappon and Smith 1981, Tsuchiya et al. 1984, Capelli et al. 1986, Soria et al. 1992, Yang et al. 1997).

MERCURY IN BREAST MILK

Breast milk is produced by the mammary gland secretory epithelial cells, which are organized into alveoli. The milk is secreted continuously into the alveolar lumen where it is stored. Myoepithelial cells that enfold each alveolus contract in response to the hormone oxytocin and thereby expel milk from the alveoli. Milk components are transported across the epithelial cells through different secretory processes (Neville 1999). Tight junctions between the cells normally form a barrier, however, it has been proposed that e.g. serum albumin may pass through the paracellular pathway due to leaky junctions (Lönnerdal 1985).

Breast milk is the best source of nutrition for infants and breast-feeding has many benefits (Gartner et al. 1997, Oddy 2002, Pronczuk et al. 2004). However, breast milk may also be a source of environmental contaminants (Anderson and Wolff 2000, Dorea 2004). Exposure to and the kinetics of MeHg and I-Hg in breast-fed infants are largely unknown. Experimental animal studies suggest that both MeHg and I-Hg are transported from plasma to breast milk bound to serum albumin through the paracellular pathway (Sundberg 1999a). I-Hg may also be transported bound to casein.

Reported correlations between total Hg in milk and plasma (Skerfving 1988), believed to contain mainly I-Hg and between I-Hg in milk and I-Hg in whole blood (Oskarsson et al. 1996) have shown that I-Hg is excreted in breast milk. This is supported by decreasing concentrations of I-Hg in maternal blood during lactation (Vahter et al. 2000). One study reports that total Hg in milk correlates with number of amalgam fillings but not with fish consumption (Oskarsson et al. 1996) and one study found correlations to both (Drexler and Schaller 1998). Both human and animal studies indicate that the infant may be exposed to MeHg via breast milk (Sundberg et al. 1991, Grandjean et al. 1994, Nordenhäll et al. 1998). To what extent I-Hg and MeHg in breast milk is taken up by the child is not known.

RISK ASSESSMENT AND RISK MANAGEMENT

Inorganic mercury

A recent risk evaluation on I-Hg compounds and elemental Hg concluded that 2 µg/kg bw per day is a tolerable intake for I-Hg and that 0.2 µg/m^3 is a tolerable concentration for long-term inhalation exposure to Hg^0, based on data for occupational exposure (WHO 2003). The Swedish occupational limit for Hg^0 is 30 µg/m^3 (Arbetarskyddsstyrelsen 2000). Dietary exposure to I-Hg is estimated to 4 µg/day (WHO 1990). It has been estimated that continuous inhalation exposure to the tolerable concentration would lead to an inhaled amount of approximately 4 µg/day (WHO 2003). A daily exposure to Hg^0 from dental amalgam is usually below 5 µg/day in dental amalgam holders (ATSDR 1999).

According to recommendations by the Swedish National Board of Health and Welfare, use of amalgam as dental material in pregnant women should be avoided (Socialstyrelsen 1991). Furthermore, in line with the Swedish environmental quality objectives that the use of Hg should cease in Sweden (Regeringsproposition 2000) the use of amalgam for dental fillings has decreased substantially (KEMI 2004).

Methylmercury

Based on data from Iraq, WHO (1990) concluded that there is an increased risk of neurodevelopmental effects when maternal hair Hg levels exceed about 10 ppm. A reference dose (RfD), a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime, was set to 0.1 µg/kg bw per day by the U.S. Environmental Protection Agency (U.S. EPA 1997, as cited in NRC 2000) also based on the Iraqi data. This RfD was regarded as scientifically justifiable by the National Research Council (NRC 2000), although, they concluded that the Faeroe Island study should be used as the critical study. They estimated a benchmark dose level (BMDL) of 58 µg/L in cord blood (corresponding to 12 mg/kg in maternal hair). This refers to the lower 95% confidence limit of Hg in cord blood that is estimated to produce a 5% increase in the incidence of abnormal scores on the Boston Naming Test. Further, they recommended use of an uncertainty factor of 10 due to biological variability, when estimating the dose, and data-base insufficiencies. This yields 5.8 µg/L in cord blood (or 1.2 mg/kg in maternal hair) corresponding to an intake of 0.1 µg/kg bw per day.

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) recently lowered the provisional tolerable weekly intake (PTWI) to 1.6 µg/kg bw per week based on data from both the Seychelles and Faeroe Island studies (JECFA 2003). They used a composite NOEL (no observed effect level)/BMDL of 14 mg/kg in maternal hair and a total uncertainty factor of 6.4. A factor of 2 should account for inter-individual variability for the used hair:blood ratio of 250 (range between 140 to 370) when the concentration in maternal hair was converted into maternal blood concentration. Further, a factor 3.2 should account for inter-individual pharmacokinetic variability when maternal blood concentration is converted into maternal dietary intake (JECFA 2003).

The European Union has established maximum limits of MeHg in fish for sale. At present a maximum level of 0.5 mg/kg applies to most fish and fishery products, with the exception of certain fish species for which 1 mg/kg applies (Commission Regulation No 466 2001).

To protect the most sensitive groups of MeHg toxicity, fetuses and infants, the Swedish Food and Drug Administration gives dietary advisories concerning fish consumption to pregnant and lactating women and women planning pregnancy (SLV 2004). These groups are recommended to avoid intake of certain fish species which may contain elevated levels of MeHg. These species are pike, perch, pike-perch, burbot and eel from fresh- and coastal waters and large marine species such as halibut, tuna (not canned tuna), swordfish, ray and shark. The rest of the population, including women of child-bearing age, are recommended not to eat these species more than once a week. The compliance to the recommendations has not been studied.

MERCURY AND SELENIUM

Selenium (Se) is an essential element for humans. It occurs naturally in food primarily as selenomethionein and selenocysteine (Combs and Combs 1984). The amount of Se in food is dependent on the concentration of Se in soil. In Sweden, the Se concentration is naturally low in rocks, soils and plants (Alexander and Meltzer 1995) and the average intake of Se among Swedish women (32 µg/day; Becker and Pearson 2002) is below the recommended dietary intake of 40 µg Se/day for women and 55 µg Se/day for pregnant and lactating women (NNR 1996). Se is essential for fetal growth and development (Black 2001).

Selenocysteine is the bioactive form of Se present in many enzymes, such as glutathione peroxidase, thioredoxin reductase, iodothyronine deiodinase, and also in selenoprotein P (Combs and Combs 1984, Behne and Kyriakopoulos 2001). Many of these enzymes have antioxidative properties and assist the cell in the defense of oxidative stress. Se has been shown to be important for reproduction and immune functions, for protection against cancer and cardiovascular diseases (Rayman 2000), and for protection against the progression of HIV (Kupka et al. 2004). Low Se status has been reported to be associated with pregnancy preeclampsia (Rayman et al. 2003), probably due to increased oxidative stress (Orhan et al. 2003).

Already in 1967, it was reported that Se could reduce the toxicity of Hg (Parizek and Ostadalova 1967). Since then, several experimental studies have shown that Se interacts with both MeHg and I-Hg, and protects against Hg toxicity (Watanabe 2002). For example, Se supplementation in maternal diet was shown to reduce MeHg-induced neurotoxic effects in the offspring (Fredriksson et al. 1993). However, the effect in humans is not well documented (NRC 2000). The exact mechanism of the protection is not well understood and several possible mechanisms have been suggested (Cuvin-Aralar and Furness 1991). For example, the antioxidative properties of Se may protect against the oxidative stress induced by both MeHg and Hg^{2+}. Se is also thought to reduce the toxicity by forming complexes with I-Hg or MeHg. However, the binding of Se to Hg also reduces the portion of Se available for formation of selenoenzymes.

AIMS OF THESIS

The general aim of the present thesis was to determine the exposure to MeHg and I-Hg during early human development and to evaluate the possible interactions with Se. The more specific aims were to:

Provide data on the exposure to MeHg and Hg^0 in Swedish women of childbearing age both in the general population and in individuals with higher exposure via significant fish consumption.

Increase the present knowledge about the transport of MeHg and I-Hg to fetuses and infants and placental accumulation.

Clarify possible associations between Se and Hg (both MeHg and I-Hg) during pregnancy.

Increase the basis for risk assessment regarding Hg exposure in women, fetuses and infants.

METHODS

This section is a summary of the methods used. For details, the reader is referred to the individual papers.

STUDY GROUPS

For the purpose of the present thesis, pregnant and lactating women, women of child-bearing age, newborns and infants have been studied in the different projects. The study groups are summarized in Table 2. In all studies, informed consent was obtained from women before participation. All studies have been approved by local ethics committees.

Table 2. *Summary of study groups*

Study	Number	Subjects	Age	Study area
I, II	254	Pregnant women, newborns	31 (20-45)	Solna, Stockholm
III	131	Pregnant women, newborns	27 (20-40)	Uppsala county
IV	127	Fish-consumers (women)	38 (19-45)	Sweden
V	20	Pregnant and lactating women, newborns and infants	31 (24-37)	Stockholm

Study I, **II**, **III** and **IV** were part of the Swedish health related environmental monitoring programme, the aim of which is to follow the exposure of the general population to environmental pollutants, such as toxic metals.

Study I and **II**: Between October 1994 and January 1996, totally 254 pregnant women were recruited for a longitudinal study at their first visit to any of the antenatal care clinics in Solna close to Stockholm, at approximately gestational week (GW) 11 (first trimester). They were followed-up at GW 36 (last trimester) and at delivery, when umbilical cord blood and placenta (n=125) were collected.

Study III: Between January 1996 and May 1999, 953 pregnant women in Uppsala County were recruited at the antenatal care clinics as controls in a case-control study of risk factors for early miscarriages (Cnattingius et al. 2000). All first-time pregnant women recruited from early fall 1996 and onwards (n=376) were asked, when they were in late pregnancy, GW 32-34, to participate in an exposure study of environmental pollutants (Glynn et al. 2001). Of the 376 women approached, 131 women agreed to participate and to donate hair and cord blood. A complete set of hair, cord blood, and questionnaire data was successfully collected from 123 women.

Study IV: From June to August 2001, 127 Swedish women of child-bearing age, and with high fish consumption (=eat fish several times per month) were recruited by advertisement in magazines and local press in parts of Sweden where the levels of MeHg in fish are known to be high (Johansson et al. 2001), i.e. the counties of Västernorrland, Västerbotten, Gävleborg, Västmanland, Örebro, Värmland, Västra Götaland and Halland.

Study V: During 2001 pregnant women were recruited at delivery by midwives at the Huddinge University Hospital. The women and their infants were followed-up at approximately 4 days, 6 weeks and 13 weeks after delivery.

BIOMARKERS OF EXPOSURE

The concentrations of Hg in cord blood, or blood or hair of pregnant women are often used to assess the exposure to the developing fetus. As MeHg has a half-time in blood of about 50 days (Stern 1997), blood Hg concentrations at one point in time reflects relatively short-term exposures relative to the total pregnancy period. Hair grows approximately 1 cm per month and more than 80% of Hg in hair is in the form of MeHg (Cernichiari et al. 1995). Therefore, by measuring the concentration of Hg along the length of the hair, the exposure to MeHg during the whole pregnancy period or during different parts of pregnancy may be estimated (Clarkson 1997). Breast milk might be a useful medium for biological monitoring of metals if the newborn child is mainly breast-fed (Elinder et al. 1994). Se status can be measured by the concentrations of Se in whole blood and in serum which reflect the long-term and recent intake of Se, respectively. Serum Se levels are usually 10-40 µg/l lower than in whole blood (Alexander and Meltzer 1995).

In the present projects, the concentration of Hg was speciated in blood to enable assessment of maternal, fetal and infant exposure to MeHg and I-Hg. Hair samples were analyzed for total Hg to give an estimate of MeHg exposure. Hg in breast milk was used for assessment of Hg exposure of the infant. Se in serum and whole blood was measured for assessment of maternal and fetal Se status.

Table 3 summarizes the different samples collected in the different studies. Utensils used for sample collection were acid washed in 10% HNO_3 or checked to be free from Hg contamination. All samples, except hair, were stored frozen at -20°C until analysis.

Table 3. *Samples collected in papers I-V.*

Study	Blood	Serum	Hair	Cord blood	Cord serum	Infant blood	Placen-ta	Breast milk	Quest-ionnaire
I, II	√	√		√	√		√		√
III			√	√					√
IV	√	√	√						√
V	√			√		√		√	√

Blood: Blood and serum samples were collected using Venoject tubes (Terumo®, Leuven, Belgium) or Vacutainer tubes (Becton Dickinson, Stockholm, Sweden).

Hair: Full-length hair samples were taken at the back of the head. Samples, about the size of a toothpick, were tied and cut close to the scalp end. They were put into plastic bags and stored at room temperature. In pregnant women (**Study III**), the hair sample was taken at GW 32-34. Whenever possible, 9 cm of hair was taken. This gives an approximate integrated measure of the MeHg exposure during the first seven months of

pregnancy as well as two months before conception. For women with high fish consumption (**Study IV**), 12 cm of hair from the scalp end was taken to give an estimate of the average exposure during one year previous to sampling.

Placenta: Placentas were collected in plastic containers. A pilot study found substantial variation in concentrations of elements between subsamples of one placenta. Therefore, whole placentas were homogenized in a metal-free food processor (Hugin, Robot-Coupe).

Breast milk: Breast milk samples were collected into acid-washed plastic containers by the mothers themselves either manually or by using a pump. The women were given instructions to carefully wash their hands and pump device before taking the sample and to freeze the sample.

QUESTIONNAIRES

Study I and **II**: The women were asked to fill out a self-administered questionnaire concerning consumption of freshwater fish during pregnancy (never, once or twice a month, once or twice a week, several times a week) and number of dental amalgam fillings (0, <10, >10).

Study III: The women completed an extensive food frequency questionnaire (FFQ) produced by the Swedish National Food Administration. They were asked how often they consumed different types of fish and other foodstuffs during the year they became pregnant and the answer alternatives were: never, 3 times/year, 6 times/year, 1-3 times/month, once/week, twice/week, 3-4 times/week, 5-6 times/week, once/day or more. Their consumption of specific freshwater fish species, potentially high in MeHg, and canned tuna during the actual pregnancy was also reported. The women were also asked to report on their knowledge of the recommendations to avoid certain fish species during pregnancy. Furthermore, they were asked to check, by using a mirror, the number and size of their dental amalgam fillings, and report both the total number of fillings and the size of them as marks on an illustration of the upper and lower jaws.

Study IV: The women completed a self-administered and extensive FFQ, modified from the one used in **Study III** including even more detailed information about their consumption of different fish species, reflecting their average intake during a year. They also answered a cross-check question about their average total fish consumption using the same answer alternatives. The women also reported on their awareness of that certain food may have high levels of environmental pollutants and therefore should be consumed with restriction or even avoided. Furthermore, the women were asked to report on their number of dental amalgam fillings (0, 1-3, 4-6, 7-9, 10-12, 13-15, >15).

Study V: The women were interviewed, by the midwives, at delivery about their fish consumption (1. frozen fish in package, cultivated fish, 2. freshwater fish, 3. other fish, shellfish), vaccinations and dental care during the last 6 months. At 13 weeks post partum, a similar interview was completed that also included information about breast-feeding and the use of infant formula. The number of dental amalgam-filled surfaces was recorded by a dentist.

ELEMENT ANALYSES

All utensils used in the different analysis were acid washed to reduce the risk for contamination. All samples were analyzed in duplicate.

Cold vapor atomic fluorescence spectrophotometry (CVAFS)

In most samples we measured the concentrations of total Hg and I-Hg. The MeHg concentrations were calculated as the difference between total Hg and I-Hg with the assumption that the major part of the organic Hg fraction is in the form of MeHg. Analysis of total Hg in hair and total Hg and I-Hg in blood and placenta were carried out by cold vapor atomic fluorescence spectrophotometry (CVAFS) (Merlin, PSA 10.023; P.S. Analytical Ltd., Orpington, Kent, UK) using an improved Magos method (Magos and Clarkson 1972). The Hg concentrations were determined after alkaline solubilization of the samples and reduction via an automatic multiple-injection analysis (MIA) system (Einarsson and Hansén 1995) with a Tefzel 13-channel selector valve (Figure 2; Analys Modul Sweden AB). Preset volumes of the reagent solutions are delivered by the selector to a reaction tower. Before use, the reagent mixture is purified, from all Hg present, in an on line purification step. The solubilized sample is then added automatically to the tower by the selector, and the formed Hg^0 is transported, by argon gas passing through the tower, to the detector. I-Hg is reduced to Hg^0 by addition of stannous chloride and total Hg is reduced by addition of a combination of stannous and cadmium chloride.

CVAFS (Merlin, PSA 10.003) (Sandborgh-Englund et al. 1998a) was also used for analysis of total Hg in breast milk after acid microwave digestion.

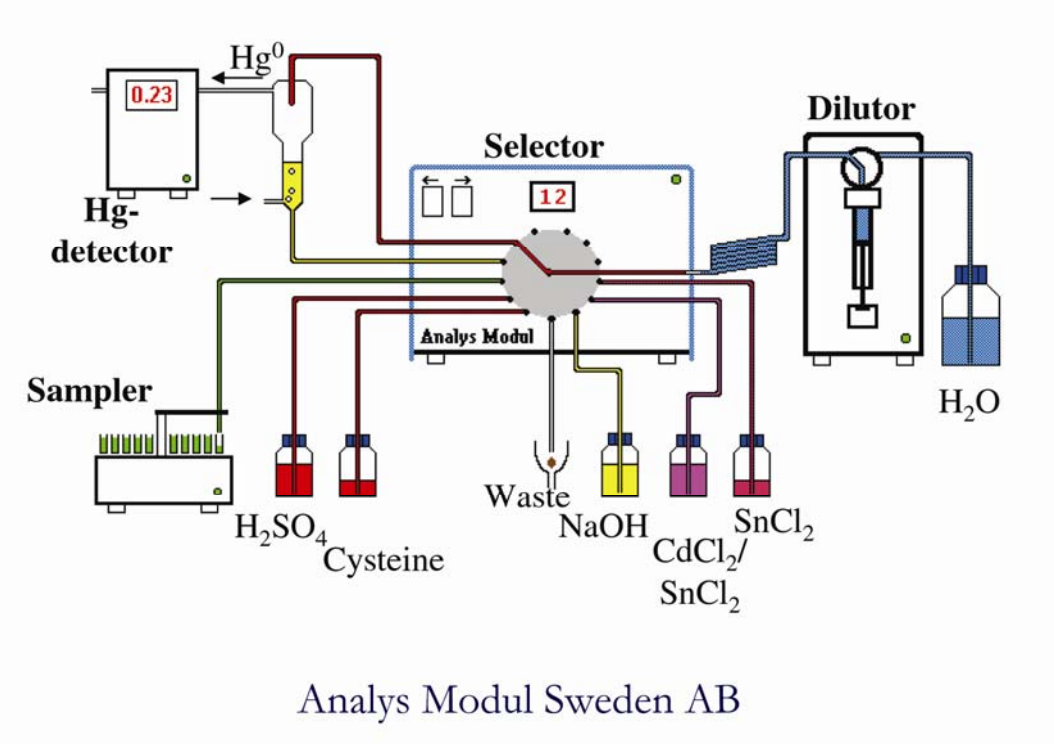

Figure 2. *An illustration of the method used for analysis of total Hg and inorganic Hg.*

Sample preparation

Blood: Samples of 1.0 ml blood were treated with 1.0 ml L-cysteine (1%), 1.5 ml NaOH (45%) and 0.5 ml deionized water via the MIA system. They were stored at room temperature in dark over night to complete the solubilization.

Hair: Hair samples of approximately 20 mg were treated with 2.0 ml L-cysteine (1%), 4.0 ml NaOH (45%) and 14 ml NaCl (1%). The mixture was heated at 90-95 °C for 20 minutes to complete the solubilization

Placenta: Samples of 2.0 g were treated with 5.0 ml L-cysteine (1%), 5.0 ml NaOH (45%) and 6.0 ml NaCl (1%). The mixture was heated at 80-85 ° C for 30 minutes to complete the solubilization.

Breast milk: Samples of 1.0 ml breast milk were mixed with 1.5 ml concentrated HNO_3 (suprapure) and digested in a microwave oven (MDS-2000, CEM-Innovators Microwave Technology, Matthews, USA).

Electrothermal atomic absorption spectrophotometry (AAS)

The concentrations of Se in serum and blood were determined by electrothermal atomic absorption spectrophotometry (AAS) according to Alfthan (1982). A Perkin-Elmer 5000 equipped with Zeeman background corrector, graphite furnace (HGA-500) and an AS-40 autosampler was used. A selenium lamp of EDL I type and a standard graphite tube with L,vov`s platform were also used.

Sample preparation

Samples of 50 µl serum or blood were mixed with 450 µl matrix modifier containing nickel nitrate, Triton-X-100 and nitric acid. The function of the matrix modifier is to keep Se in a bound form to avoid losses during the charring step. Triton-X-100 decreases the surface tension of the injected solution.

Inductively coupled plasma mass spectrophotometry (ICP-MS)

A low-resolution ICP-MS instrument (VG PQ2+, Fisons Elemental, Winsford, Cheshire, UK) and a Gilson 222 autosampler (Gilson, Villiers, France) was used for determination of Se in placenta. The analysis was carried out at the Department of Occupational and Environmental Medicine at the University of Lund. For further details see **Study I**.

QUALITY CONTROL

In order to ascertain analytical accuracy, appropriate reference materials were included in each analysis. Table 4a-c show the result of different types of reference material included in **Study I-V**. In addition, samples of blood (**Study III**), placenta (**Study II**) and breast milk (**Study V**) were spiked with known concentrations of Hg and recoveries were calculated. In general, our obtained values showed satisfactory results on both accuracy and precision (both intra-day and between days). No systematic changes over time were detected.

Tabell 4a. *Obtained values (mean±SD) of reference materials used for analytical quality control of total Hg (T-Hg) and inorganic (I-Hg) in whole blood and placenta.*

Study	Seronorm 404107 **Blood** 2.2-3.3 µg T-Hg/L[a]	Seronorm 404107 **Blood** I-Hg[b]	Seronorm 404108 **Blood** 6.7-8.4 µg T-Hg/L[a]	Seronorm 404108 **Blood** I-Hg[b]
II			7.9±0.3	5.8±0.2
III	2.3±0.2	0.5±0.1	7.6±0.7	6.4±0.9
IV	2.3±0.2	0.5±0.03	7.7±0.8	6.5±0.2
V	2.4±0.2	0.6±0.03	8.0±0.3	6.5±0.2

[a] recommended value for T-Hg
[b] I-Hg (no recommended value is assigned by supplier, see text for discussion)

Tabell 4b. *Obtained values (mean±SD) of reference materials used for analytical quality control of total Hg (T-Hg) in hair and breast milk.*

Study	IMM hair QC **Hair** 4.9±0.24 µg T-Hg/L[a]	SRM-1549 **Breast milk** 0.3±0.2 µg T-Hg/L[b]
III	4.8±0.1	
IV	4.7±0.3	
V		0.3± 0.1

[a] interlaboratory comparison. The recommended value is based on analysis by four different analytical methods at six laboratories
[b] certified reference value

Tabell 4c. *Obtained values (mean±SD) of reference materials used for analytical quality control of total Se in serum, whole blood and placenta.*

Study	Seronorm 112 **Serum** 90 µg Se/L[a]	Seronorm 704121 **Serum** 79-92 µg Se/L[a]	Seronorm 404108 **Whole blood** 82-89 µg Se/L[a]	Muscle RBC-184 **Placenta** 0.183±0.012 µg Se/g[b]	Fish liver Dolt-2 **Placenta** 6.06±0.49 µg Se/g[b]
I	91±5			0.19±0.04	5.8±0.32
III			86±10		
IV		78±2.3			

[a] recommended value
[b] certified reference value

There is no commercially available reference material for Hg species in blood. We have used different batches of Seronorm (Nycomed Co., Oslo, Norway), with recommended concentrations on total Hg, as reference material for analysis of both total Hg and I-Hg in blood (Table 4a). The analytical values for I-Hg in Seronorm have been in agreement with our previous obtained results showing good precision. In total, the results for Seronorm 404108 are 6.4±0.6 µg I-Hg/L (n=44; CV=10%) and the results for Seronorm 404107 are 0.53±0.05 µg I-Hg/L (n=36; CV=10%). In addition, repeated analysis of cow blood spiked with low concentrations of I-Hg and MeHg were performed in parallel with the Seronorm samples in a number of analyses. This gave average recoveries of 101 % and 96 % for I-Hg and total Hg respectively. Spiking of one placenta homogenate with I-Hg and MeHg gave average recoveries of 96 % for both I-Hg and T-Hg. Analysis of a MeHg standard solution gave average recoveries between 95-100% in the different studies.

After using a certified reference material for Hg in hair (GBW 09101-CRM; Shanghai Institute of Nuclear Research; reference value 2.16± 0.21 mg/kg) which consistently gave lower values, although within 2 SD, than the reference value we decided to evaluate this reference material. Duplicate analysis of ten different solubilisates of the GBW 09101-CRM sample gave an average concentration of 1.86 mg Hg/kg (range 1.27-2.59 mg/kg; CV=18%). There was also large variation between duplicate samples. This indicates a non-homogenous sample. A similar evaluation of a hair quality control sample prepared at our laboratory (IMM hair QC) did not reveal non-homogeneity. This sample was prepared, using hair from the Faeroe Island (in collaboration with Drs P Weihe and P Grandjean), to be used as reference material for total Hg in hair. The recommended value is based on inter-laboratory comparison. The concentration of total Hg in hair (mean±SD; see Table 4b) was determined from the results reported by six laboratories using four different analytical methods. Therefore, we concluded that IMM hair QC would be more appropriate to use as reference material for total Hg in hair.

A standard reference material (SRM-1549, non-fat milk powder; National Bureau of Standards) was used as reference material for Hg in breast milk (Table 4b). Seven different breast milk samples from one woman were analyzed at 3 different occasions and the coefficient of variation (CV) varied between 2-16%. Furthermore, repeated analysis (n=6) of a breast milk sample spiked with two different concentrations of Hg gave an average recovery of 100±4% and a CV of 4%.

Different batches of Seronorm (Nycomed Co., Oslo, Norway) were used as reference material for Se in serum and blood. Lyophilized bovine muscle (Community Bureau of Reference, Commission of the European Communities) and dogfish liver (Institute for Environmental Chemistry, National Research Council, Canada) were used to check analytical performance of Se in placenta (Table 4c).

The limit of detection (LOD) was calculated as 3 x SD of the reagent blanks. The total variation of LOD in all studies were 0.03-0.16 µg I-Hg/L in blood and placenta and 0.004-0.13 µg total Hg/L in blood, placenta, hair and breast milk. LOD for Se varied between 6-60 µg Se/L in serum and blood. Due to very low exposure, a few blood samples had I-Hg below LOD, especially in **Study V**. Although values below LOD are afflicted with a larger uncertainty than those above, the exact values have been used in the calculations.

STATISTICS

The concentrations of total Hg, MeHg and I-Hg in blood, placenta, hair and breast milk were not normally distributed. Spearman correlation (r_s) was used to test for correlations between variables but whenever the requirements for normally distributed residuals were met we used Pearson correlation (r). Non-parametric tests (Kruskal-Wallis, Wilcoxon and Mann-Whitney) were used to test for differences between variables and groups. Logarithmic transformation of dependent variables was used to conform to the requirement of normal distribution to be able to use multiple linear regression (**Study III**) and ANOVA for repeated measures (**Study V**). Statistical significant level was set to $p < 0.05$.

All statistical analyses have been conducted with SPSS for Windows (SPSS Inc, Chicago, IL). In boxplots, which are used to illustrate data, SPSS define the box length as the interquartile range and cases with values between 1.5 and 3 box lengths from the upper or lower edge of the box are defined as outliers (o) and cases with values more than 3 box lengths from the upper or lower edge of the box are defined as extremes (*). However, all data have been included in the statistical analyses.

RESULTS AND DISCUSSION

This section is a summary and discussion of the main results which will be referred to. Some data not previously published are included. For details, the reader is referred to the individual papers.

PLACENTAL TRANSPORT AND ACCUMULATION

Inorganic mercury

Although previous experimental studies have shown that Hg^{2+} does not pass the placenta (Khayat and Dencker 1982, Urbach et al. 1992, Dock et al. 1994), we found that the concentrations of I-Hg in cord blood and maternal blood were significantly correlated (Figure 3; **Study V** and Vahter et al. 2000), and the average concentration in cord blood was similar to that in maternal blood (Figure 5a). Thus, I-Hg was readily transported across the placenta. As there was a clear increase in cord blood I-Hg with increasing number of maternal dental amalgam fillings from which Hg^0 is released (Figure 3 in **Study III**), it seems likely that Hg^0 passed the placental barrier and reached the fetus before being oxidized by catalase to Hg^{2+}. Experimental animal studies have shown that Hg^0 released from amalgam fillings crosses the placenta resulting in increased Hg concentrations in blood and tissues of the fetus (Vimy et al. 1990, Takahashi et al. 2001, Takahashi et al. 2003). Also, Hg concentrations in fetal tissue have been found to increase with increasing number of maternal amalgam fillings (Drasch et al. 1994, Lutz et al. 1996).

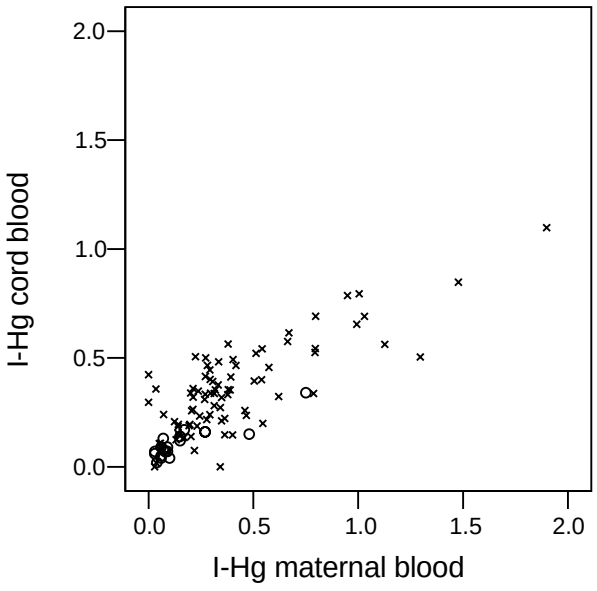

Figure 3. *The association between I-Hg (µg/L) in cord blood and maternal blood.* **(o)** $r_s=0.77$; $p<0.001$; $n=20$; *Study V,* **(x)** $r_s=0.68$; $p<0.001$; $n=82$; *Vahter et al. (2000).*

The average cord blood I-Hg was 0.9 times the maternal blood I-Hg in both **Study V** and Vahter et al. (2000), but there was a large variation in the ratio as shown by Figure 3. The 5th and 95th percentiles were approximately 0.35 and 2.0 in both studies. Hg^0 is

oxidized by the catalase-hydrogen peroxide pathway. Possibly, the large variation could be related to inter-individual differences in catalase activity, such as polymorphism (Christiansen et al. 2004), used for oxidation of Hg^0. This would lead to variation in binding of Hg^{2+} to maternal tissues or the placenta.

The association between I-Hg in cord and maternal blood levelled off at higher maternal I-Hg concentrations and the ratio I-Hg cord blood:maternal blood decreased markedly with increasing maternal blood I-Hg concentrations (Figure 4a).

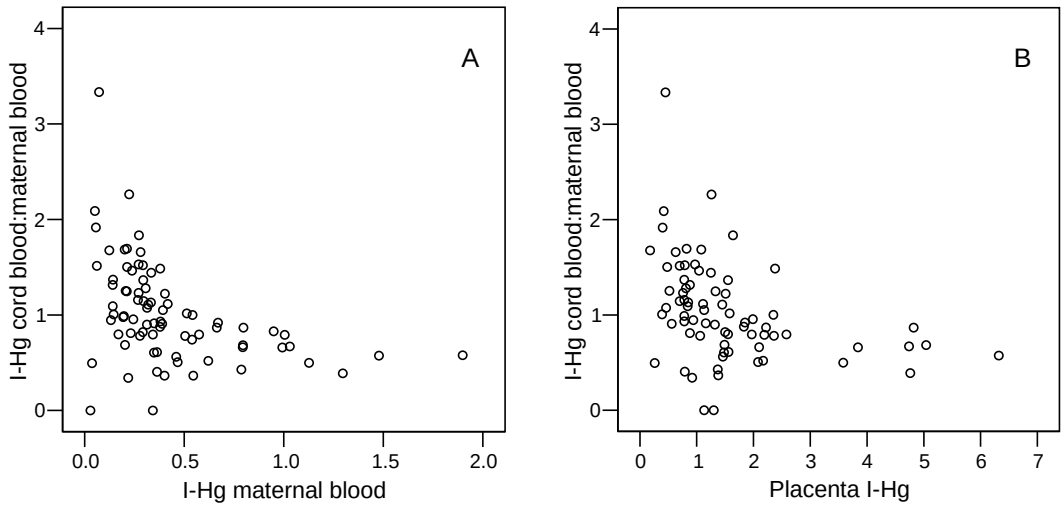

Figure 4. *The ratio I-Hg cord blood:maternal blood in relation to the concentration of I-Hg in (**A**) maternal blood (µg/L) and (**B**) placenta (µg/kg). One outlier with a ratio of 10 has been excluded from the figure.*

Besides the passage of Hg^0 to the fetus, I-Hg also accumulated in placenta (**Study II**; median: 1.3 µg/kg, range: 0.18-6.7 µg/kg). I-Hg in placenta increased significantly with increasing I-Hg concentrations in maternal blood (Figure 1a in **Study II**) and the average concentration in placenta was four times higher than in maternal blood (Figure 5a). The accumulated placental I-Hg increased considerably with increasing number of maternal dental amalgam fillings (Figure 2 in **Study II**). Thus, it seems likely that the I-Hg bound in placenta originated from Hg^0 released from amalgam fillings and oxidized to Hg^{2+}. We found that the I-Hg cord blood:maternal blood ratio was lower at higher placental I-Hg concentrations (Figure 4b). Probably, catalase in placenta (Watson et al. 1998) is induced at higher maternal blood concentrations of Hg^0 which leads to increased oxidation of Hg^0 to Hg^{2+} and accumulation in the placenta.

The placenta contains metallothionein, a protein rich in cysteine (Nordberg and Nordberg 2000). As Hg^{2+} is known to induce metallothionein (Kägi 1993) it might be speculated that placental metallothionein binds Hg^{2+} and prevents transfer to the fetus. This is supported by experimental findings in which fetal Hg accumulation after exposure to Hg^0 was found to be greater in metallothionein-null mice than in wild-type mice (Yoshida et al. 2002). Probably, binding of Hg^{2+} to metallothionein in placenta protects against oxidative stress. Oxidative stress is known to occur in the placenta (Myatt and Cui 2004).

Experimental studies suggest that Hg^{2+} in placenta can affect the transport of amino acids, oxygen consumption (Urbach et al. 1992), enzyme activity (Boadi et al. 1992b) and hormonal secretion (Boadi et al. 1992a). Also, transport of nutrients may be affected (Danielsson et al. 1984). At what concentrations Hg^{2+} causes adverse effects in human placenta and how this will affect the fetus is not known.

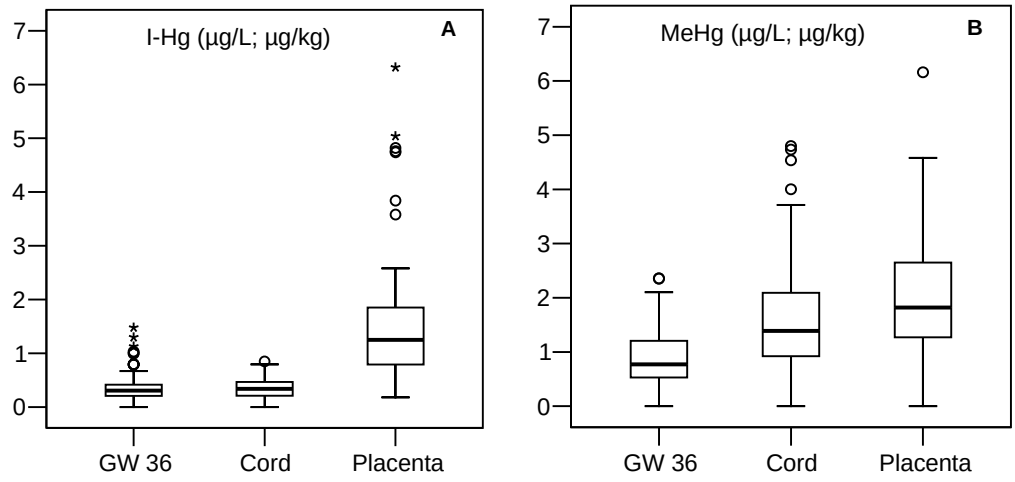

Figure 5. *The concentrations of **(A)** I-Hg and **(B)** MeHg in maternal blood (gestational week, GW 36), cord blood (µg/L; data from Vahter et al. (2000)) and placenta (µg/kg; Study II), n=77.*

It is hypothesized that Se protect against Hg toxicity. In individuals from the general population a 1:1 molar relationship of total Hg and Se was found in the renal cortex (Nylander and Weiner 1991) suggesting complex formation. Experimental studies have shown that Hg^{2+} and Se are bound in this 1:1 relationship to selenoprotein P (Yoneda and Suzuki 1997). A significant association between Se and Hg in fetal kidneys has also been reported (Vahter et al. 1997), maternal dental amalgam fillings were found to be the major source of fetal kidney Hg (Lutz et al. 1996). As the placenta is a target organ for I-Hg we wanted to evaluate if Se in placenta was associated with I-Hg.

We found no correlation between Se and I-Hg in placenta (**Study II**). The Se uptake in the placenta seems to give concentrations within a narrow range, 2-3 µmol/L (**Study I**; further discussion under the Selenium section). These concentrations are far higher than the concentrations of I-Hg (median 0.006 µmol/L) in placenta. The I-Hg cord blood:maternal blood ratio was not influenced by Se in placenta and we found no evidence that Se affected the accumulation or transport of I-Hg in placenta.

Methylmercury

The concentration of MeHg in cord blood was highly associated with MeHg in maternal blood (Figure 6). As shown in Figure 5b, the concentration in cord blood was on average twice as high as in maternal blood (**Study V** and Vahter et al. 2000; data used in **Study II**) supporting the hypothesis that MeHg passes the placenta via active transport using neutral amino acid carriers (Kajiwara et al. 1996). This results in a continuous accumulation in the fetus. It has been proposed that this transport occurs

because of MeHg bound to SH-groups in cysteine in blood is mimicking methionine. In red blood cells (RBC), MeHg binds to hemoglobin (Kershaw et al. 1980). The higher MeHg in cord blood can partly be explained by the high hemoglobin in the fetus (Geigy Scientific Tables 1984).

Although the average cord blood MeHg was approximately twice as high as maternal blood MeHg in both **Study V** and Vahter et al. (2000) there was large inter-individual variation in the ratio (Figure 6). The 5th and 95th percentiles were approximately 1 and 3, respectively. Recently, an assessment based on ten studies, including that by Vahter et al. (2000), concluded that 1.7 is an average estimate of the cord blood:maternal blood MeHg ratio (Stern and Smith 2003) and the 25th and 95th percentiles were 1 and 3, respectively. There is no indication that the ratio varies with maternal exposure. The variation may be explained by inter-individual variation in kinetics.

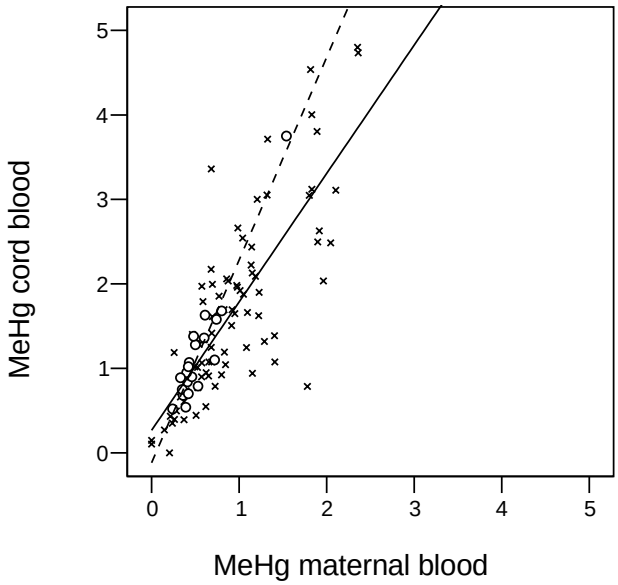

Figure 6. *The associations between MeHg in cord blood and maternal blood.* **(o)** *r=0.95; p<0.001; n=20; Study V,* **(x)** *r=0.79; p<0.001; n=82; Vahter et al. (2000).*

As shown in Figure 5b, the concentration of MeHg in placenta (median 1.8 µg/kg, range 0-6.2 µg/kg; **Study II**) was only twice as high as that in maternal blood. MeHg in placenta was significantly correlated to MeHg in maternal blood (Figure 1b in **Study II**). MeHg may induce oxidative stress (Yee and Choi 1996, Castoldi et al. 2001). Experimental studies have also shown that MeHg may inhibit the activity of the selenoenzyme glutathione peroxidase in placenta (Watanabe et al. 1999). This is an antioxidative enzyme which is needed to protect from oxidative stress in the placenta (Myatt and Cui 2004).

A complex binding between MeHg and Se as soluble bis(methylmercuric) selenide has been found in both blood (Naganuma and Imura 1980a) and tissues (Naganuma et al. 1980b, Masukawa et al. 1982) in experimental studies. We found no significant correlation between Se and MeHg in placenta (**Study II**). The concentration of MeHg in placenta (median 0.009 µmol/L) was much lower than the concentration of Se in

placenta (2-3 µmol/L). Further, the transport of MeHg to fetus did not seem to be affected by placental Se as the MeHg cord blood:maternal blood ratio was not influenced by Se in placenta.

In conclusion, fetuses may be exposed to both Hg^0, from maternal dental amalgam fillings, and to MeHg. Although Hg^{2+} is accumulated in placenta, Hg^0 released from maternal amalgam fillings seems to pass the placenta and reach similar concentrations of I-Hg in the fetus as in the mother. The concentration of MeHg in fetal blood, almost twice as high as in the maternal blood, supports the hypothesis of an active transport mechanism across the placenta. We also conclude that Se in placenta does not seem to affect the accumulation or transport of the different forms of Hg in placenta. It might be speculated that high placental Se protects against adverse effects caused by I-Hg and MeHg.

INFANT EXPOSURE VIA BREAST MILK

We found low concentrations of Hg in breast milk. Due to the low concentrations and problems especially with the solubilization step we were not able to speciate Hg in breast milk.

Table 5. *The concentration (µg/L) of total Hg in breast milk (median and range).*

	4 days (colostrum)	**6 weeks** (mature milk)	**13 weeks**
Total Hg (µg/L)	0.29 0.06-2.1	0.14 0.07-0.37	0.17 0.06-0.43

Table 6. *The concentration (µg/L) of total Hg in breast milk (median and range) at beginning, middle and end of the same feeding session at 6 weeks.*

	Beginning	**Middle**	**End**
Total Hg (µg/L)	0.12 0.04-0.31	0.15 0.07-0.32	0.18 0.07-0.49

It is known that both the volume and composition of breast milk changes over time (Mitoulas et al. 2002). In **Study V** we found that the concentration of Hg in colostrum (4 days) was higher than in mature milk at 6 weeks ($p<0.001$; Table 5; Figure 6 in **Study V**) after which it remained rather unchanged. Probably, this can be explained by the higher concentration of albumin in colostrum compared to that in mature milk (Neville et al. 1983). Animal studies suggest that both MeHg and I-Hg are transported from plasma to breast milk bound to serum albumin using the paracellular pathway (Sundberg et al. 1999b). Also, increasing volume of breast milk during the length of lactation (Kunz et al. 1999) may contribute to a dilution of Hg concentrations in mature milk.

Interestingly, we also found an increase in Hg concentrations in breast milk during one and the same feeding session ($p<0.001$; Table 6; **Study V**). This could possibly be explained by an increase in casein concentrations during the feed (Geigy Scientific Tables 1981) as it is suggested that I-Hg also is secreted from plasma to milk bound to casein (Sundberg et al. 1999b). This would indicate that more I-Hg than MeHg is transported to breast milk. Indeed, milk to plasma ratio of about 0.6-1.0 for I-Hg (Oskarsson et al. 1996, Sundberg et al. 1998) and 0.2 for MeHg (Sundberg et al. 1998, Sakamoto et al. 2002) have been reported showing that I-Hg is more easily transported to breast milk than MeHg. Besides the binding to albumin and casein in milk, the transport of I-Hg and MeHg from plasma to breast milk is influenced by the distribution in maternal blood. About 65% of I-Hg but only about 10% of MeHg in whole blood is present in plasma (Kershaw et al. 1980, Berglund et al. 2005) and, thus available for transport to the milk.

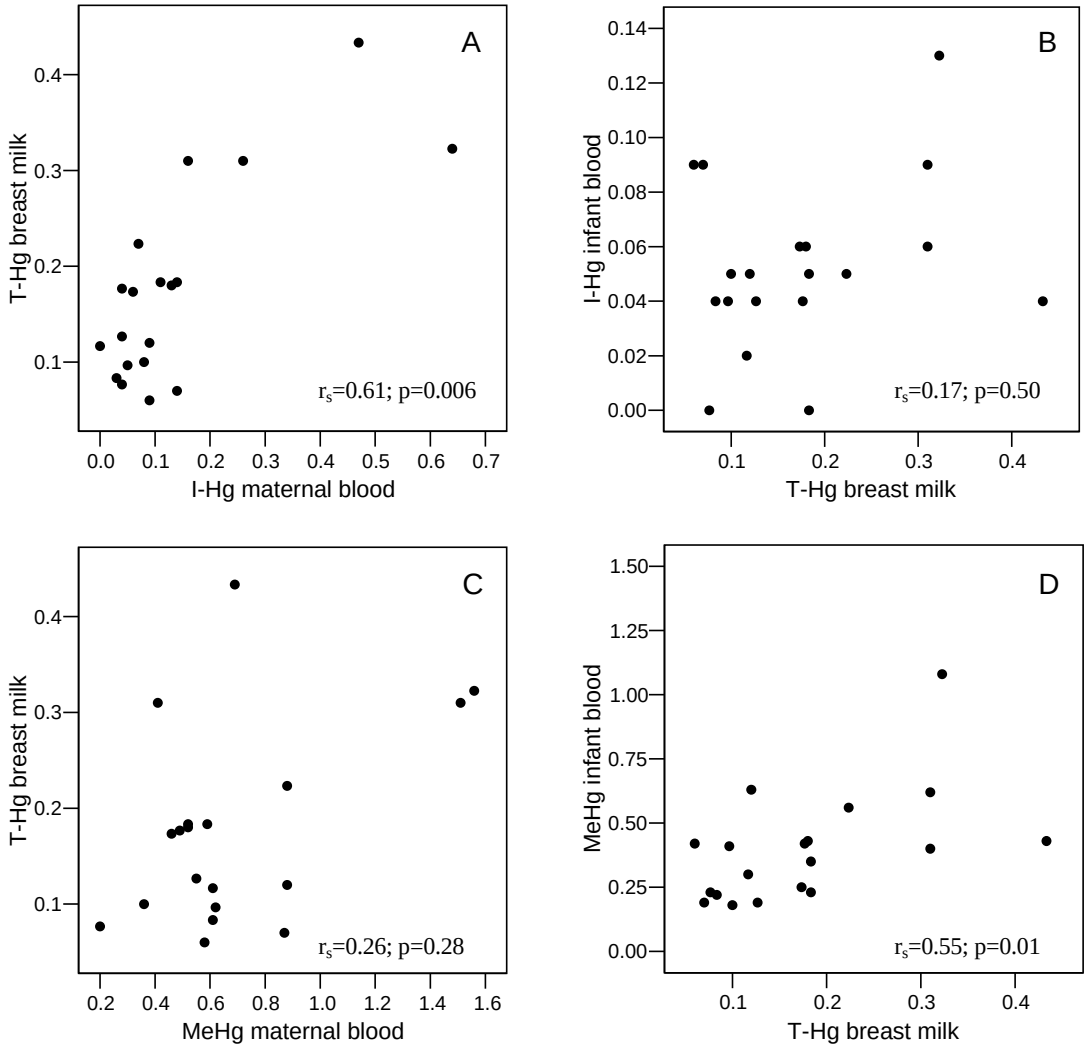

Figure 7. *The associations, at 13 weeks, between concentrations (µg/L) of (**A**) T-Hg (total Hg) in milk and I-Hg in maternal blood, (**B**) I-Hg in infant blood and T-Hg in milk, (**C**) T-Hg in milk and MeHg in maternal blood, (**D**) MeHg in infant blood and T-Hg in milk.*

The actual concentrations of I-Hg and MeHg in breast milk are obviously dependent on the maternal exposure to the different forms. We found an association between I-Hg in maternal blood and Hg in breast milk (Figure 7a), at 13 weeks, indicating transport of I-Hg to breast milk in line with previous findings (Skerfving 1988, Oskarsson et al. 1996). The question is to what extent I-Hg in breast milk is taken up by the infant. The uptake of Hg^{2+} in the gastrointestinal tract is known to be low (Rahola et al. 1973), but it has been suggested to be higher in infants than in adults (Clarkson 1992). The lack of association, at 13 weeks, between I-Hg in infant blood and Hg in breast milk (Figure 7b) indicates that the infant's gastrointestinal uptake of I-Hg from milk is limited. This was supported by a marked decline in infant blood I-Hg concentrations from birth to 13 weeks of age (Figure 8).

On the other hand, we found no significant association between MeHg in maternal blood and Hg in breast milk (Figure 7c), which is in contrast to a Japanese study, reporting a correlation (r) of 0.80 between total Hg in RBCs and breast milk (Sakamoto et al. 2002). This was probably due to the lower MeHg exposure and higher I-Hg exposure (as Hg^0 via amalgam fillings) in our study. However, we found that MeHg in infant blood at 13 weeks correlated with Hg in breast milk (Figure 7d). This might be explained by the fact that the small amount of MeHg that passes from maternal plasma to breast milk is efficiently taken up by the infant, about 95% of ingested MeHg is absorbed in the gastrointestinal tract (Åberg et al. 1969, Miettinen 1973).

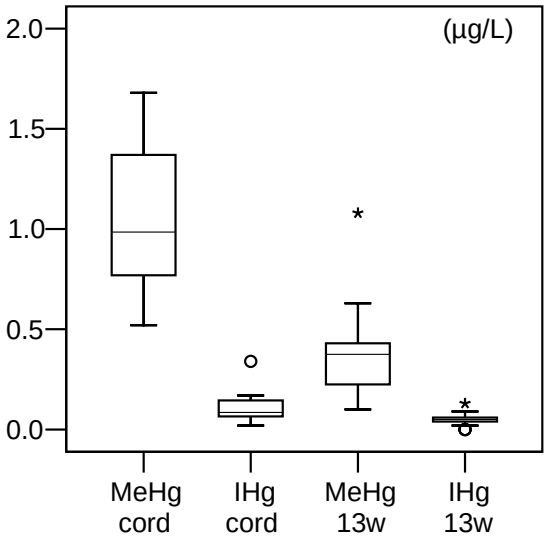

Figure 8. *The decline in concentrations (µg/L) of MeHg ($p<0.001$) and I-Hg ($p=0.001$) in infant blood ($n=20$) from delivery (cord blood) until 13 weeks of age.*

Still, infant blood MeHg decreased markedly until 13 weeks of age (Figure 8), indicating that much of the fairly large amount of MeHg taken up during fetal life was excreted after birth, contrary to previous believes. Animal studies have shown that excretion of MeHg (which takes place mainly via feces) by the suckling newborn is limited (Nordenhäll et al. 1998) as demethylating bacteria in the gastrointestinal tract become established first after weaning (Rowland et al. 1983). Unless it is

demethylated, MeHg is reabsorbed via the enterohepatic circulation. A low rate of excretion of MeHg in the infant is not compatible with the marked decline in blood MeHg concentrations during the first 3 months of life as we found. Also, the decline cannot entirely be explained by increasing body weight/blood volume, and/or decreasing hematocrit alone. Infant body weight increases about 70% during the first 3 months of age, from about 3.5 to 6 kg on average (Mei et al. 1998). During the same period, hematocrit values decrease about 14%, from 0.59 to 0.45 (Geigy Scientific Tables 1984). Thus, if no MeHg had been excreted the average infant blood MeHg at 13 weeks would have been approximately 0.5 µg/L (1 µg/L /1.7*0.86) which is higher than the analytical value of 0.38 µg/L.

It can be concluded that the highest exposure to both MeHg and I-Hg occurs during fetal life. Although I-Hg seems to be more effectively transported to breast milk than MeHg, MeHg contributed more to infant exposure because of higher gastrointestinal absorption. However, the exposure depends on the maternal exposure to the different Hg compounds. At higher maternal exposure to MeHg via fish, breast milk can be a significant source of MeHg exposure for the infant. In the fish-eating population in the Faeroe Island the average concentration of total Hg in breast milk was 2.5 µg/L (Grandjean et al. 1995) and the concentration in hair Hg in infants increased with duration of breast-feeding in those breast-fed throughout the first year (Grandjean et al. 1994)

MATERNAL EXPOSURE

Inorganic mercury

Exposure to I-Hg was evaluated based on I-Hg concentrations in blood. In general, as shown in Table 7, the concentrations of I-Hg in blood of the women were low and lower than the concentrations of MeHg.

The concentration of I-Hg in blood of the women was highly influenced by their dental amalgam fillings (Figure 9). Interestingly, the association between the concentration of I-Hg in blood and dental amalgam fillings was about the same both when the number of fillings were self-estimated (r_s=0.56; p<0.001; n=124; **Study IV**) or filled surfaces were checked by a dentist (r_s=0.55; p<0.01; n=20; **Study V**) indicating that reliable data is obtained also when the number of fillings is self-reported.

Interestingly, we found a decrease in blood I-Hg among pregnant women over time. This decrease was due to a lower number of dental amalgam fillings. The women with high fish consumption recruited during 2001 (**Study IV**) were somewhat older and had more fillings than the pregnant women of **Study V** recruited during the same year. In addition, the association between I-Hg and MeHg in blood (r_s=0.25; p=0.005) in **Study IV** indicated that a small part of I-Hg in blood comes from MeHg due to demethylation during sample solubilisation/analysis (<5%) and demethylation in the RBCs (Berglund et al. 2005). This means that the actual blood I-Hg was lower.

Table 7. *Comparison of the concentrations of MeHg and I-Hg in blood (µg/L) and T-Hg (total Hg) in hair (mg/kg) from the different studies presented as median, 25-75thand 5-95th percentiles and total range. GW=gestational week.*

Year		Blood MeHg	Blood I-Hg	Hair T-Hg
	Pregnant women, Solna (II)[a]			
	age 31 (20-45)			
	GW 36; N= 112	**0.73**	**0.32**	
	25-75th	0.51-1.2	0.20-0.51	
	5-95th	0.19-2.1	0.03-1.2	
94-96	Total range	0.0-2.8	0.0-1.9	
	Umbilical cord; N= 98	**1.4**	**0.34**	
	25-75th	0.79-2.1	0.22-0.50	
	5-95th	0.26-3.8	0.09-0.79	
	Total range	0.0-4.8	0.0-1.1	
	Pregnant women, Uppsala (III)			
	age 27 (20-40)			
	GW 32-34; N= 127			**0.35**
	25-75th			0.28-0.52
	5-95th			0.14-1.1
96-99	Total range			0.07-1.5
	Umbilical cord; N= 130	**1.3**	**0.15**	
	25-75th	0.81-2.0	0.11-0.23	
	5-95th	0.35-3.4	0.07-0.36	
	Total range	0.10-5.7	0.03-0.53	
	Pregnant women, Stockholm (V)			
	age 31 (24-37)			
	Delivery, GW 39; N= 20	**0.45**	**0.09**	
	25-75th	0.39-0.61	0.06-0.17	
	5-95th	0.24-1.5	0.03-0.74	
2001	Total range	0.24-1.5	0.03-0.75	
	Umbilical cord; N= 20	**0.99**	**0.09**	
	25-75th	0.76-1.4	0.06-0.15	
	5-95th	0.52-3.6	0.02-0.33	
	Total range	0.52-3.8	0.02-0.34	
	Women, high fish-consumers (IV)			
	age 38 (19-45); N= 127	**1.7**	**0.24**	**0.70**
2001	25-75th	1.2-3.2	0.11-0.45	0.46-1.0
	5-95th	0.53-6.3	0.05-0.77	0.23-1.9
	Total range	0.30-14	0.01-1.6	0.08-6.6

[a] Data from Vahter et al 2000 on the same study population as used in Study I and II.

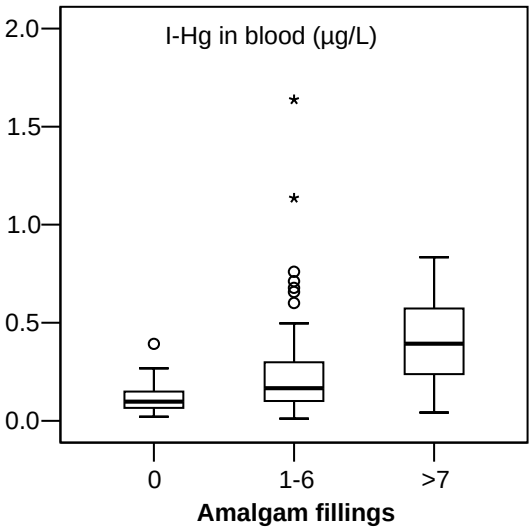

Figure 9. *The concentration of I-Hg in blood in relation to the number of dental amalgam fillings (p<0.001). Data are from women with high fish consumption in Study IV. 0 (n=23), 1-6 (n=49) and >7 (n=52).*

As illustrated in Figure 9, blood I-Hg concentrations were very low in women with no amalgam fillings and the variation was small. Therefore we may conclude that the exposure to I-Hg is mainly as Hg^0 from dental amalgam fillings with low exposure from other sources, such as diet and air. The general population is exposed to less than 0.2 µg/day due to background levels of Hg^0 in air (Vahter and Friberg 1992). Using blood I-Hg data (median 0.10 µg/L; range 0.02-0.40 µg/L) from 23 women with no amalgam fillings (**Study IV**), we have estimated a daily exposure to I-Hg from the diet according to the following equation (Rowland and Tozer 1989):

$$R_0 = C_{ss} * CL$$

where:

R_0 = daily dose (*µg/day per kg bw*)

C_{ss} = concentration at steady state (*µg/L*)

CL= clearance (*0.123 L/day per kg bw;* Jonsson et al. 1999).

Assuming a body weight of 65 kg, the daily exposure to I-Hg from diet would be approximately 1 µg/day. However, due to the small demethylation, as discussed above, the actual intake was probably even lower. Our estimate is well below the estimated total dietary intake of 4 µg I-Hg/day based on limited data from the 1970-80s (WHO 1990).

We did not find any associations between Se and I-Hg in blood in any of the included studies.

Methylmercury

Methylmercury in blood and hair

Exposure to MeHg was evaluated based on the concentrations of MeHg in blood, which is a measure of current exposure, and total Hg in hair, which reflects the integrated exposure over several months (Table 7). The concentration of Hg in hair was associated with MeHg in blood (r_s=0.78; p<0.001; n=126; **Study IV**). Also, the concentration of total Hg in maternal hair was associated with MeHg in cord blood (r=0.73; p<0.001; n=126; Figure 1 in **Study III**).

In humans, the blood to hair ratio has been estimated to 1:250 (WHO 1990). We found a median ratio of 1:253 (**Study IV**), however there was a considerable inter-individual variation. The 10^{th} and 90^{th} percentiles were 166 and 450, respectively. A large variation around the estimate has also been reported previously (WHO 1990, JECFA 2003). The variation could partly be explained by variations in the growth rate of the hair, both within and among individuals (Valkovic 1977). In humans, an individual hair follicle experiences a cycle with phases of growth, transition and resting. At one moment in time, 88% of the hairs are in the growth phase and 11% in the resting phase (Valkovic 1977). The variation in the ratio could also be explained by variation in the distribution of MeHg in plasma and RBCs (Berglund et al. 2005) as it is MeHg present in plasma that is transported to hair.

We revealed that Hg concentrations in hair segments corresponding to late pregnancy (n=15) were significantly lower and more closely correlated with MeHg in cord blood compared to hair Hg concentrations in segments from early pregnancy (**Study III)**. The concentration of Hg in hair was lower in the two-centimeter segment closest to the scalp corresponding to the $6\text{-}7^{th}$ months of pregnancy (median 0.62 mg/kg) than in the segments corresponding to the $4\text{-}5^{th}$ months (median 0.79 mg/kg; p=0.002) or the $1\text{-}3^{rd}$ months (median 0.96 mg/kg; p=0.003). The decrease of hair Hg concentration during the course of pregnancy could be related to less MeHg exposure due to diminished fish consumption or to transport of MeHg to the growing fetus. Also, hemodilution of maternal blood during pregnancy (Hytten 1985) may cause lower maternal blood concentrations of MeHg in late pregnancy (Vahter et al. 2000) and thereby less MeHg available for transport to hair.

Methylmercury exposure in relation to fish intake

In Sweden, pregnant and lactating women have been recommended, by the National Food Administration, to avoid consumption of the freshwater fish species pike, perch, pike-perch, burbot and eel and also marine halibut, since these often contain elevated levels of MeHg. These advisories are given to protect the most sensitive groups of MeHg toxicity, fetuses and infants.

Our first study on MeHg exposure in pregnant urban women showed low average blood concentration, but large inter-individual variations (Vahter et al. 2000). The limited data on fish consumption revealed that freshwater fish was rarely consumed during pregnancy. In order to find out the MeHg exposure from different types of fish as well as the consumption rates of various types of fish we evaluated MeHg exposure using more detailed fish consumption data in combination with biomonitoring. MeHg exposure was assessed in pregnant women and their fetuses (**Study III**) and in women of child-bearing age, recruited because of high fish consumption (reported to eat fish several times per month; **Study IV**). We found that the exposure to MeHg was highly influenced by fish consumption in general. MeHg in blood, hair and cord blood increased significantly with increasing total fish consumption (Figure 2 a+b in **Study**

III and Figure 1 in **Study IV**). In **Study V**, we found no obvious association between MeHg exposure during pregnancy and fish consumption. However, the number of participants were small (n=20) and they had low fish consumption according to the not so detailed fish consumption data.

The pregnant women in **Study III** were recruited from both urban and rural areas. The median concentrations of MeHg in cord blood (1.3 µg/L) and Hg in maternal hair (0.35 mg/kg) were fairly low. However, we found quite a range in the exposure. The maximum concentrations of 5.7 µg/L in cord blood and 1.5 mg/kg in hair correspond to intakes close to the U.S. EPA reference dose (RfD) of 0.1 µg MeHg/kg bw per day (NRC 2000) (further discussion under the Risk assessment section). Among women of the fish consumption study (**Study IV**) there was a larger range in fish intake which is reflected in the larger range in MeHg exposure with maximum concentrations of 14 µg/L in blood and 6.6 mg/kg in hair. However, the median concentration of MeHg in blood of 1.7 µg/L and 0.70 mg/kg in hair were twice as high as the pregnant women in **Study III**. Twenty percent of the women in **Study IV** had hair Hg concentrations exceeding the U.S. EPA RfD. Still, hair Hg concentrations were lower than those reported in fish-eating populations in the Seychelles (6.8 mg/kg; range 0.5-27) (Davidson et al. 1998) and the in Faeroe Island (4.3 mg/kg; interquartile range 2.6-7.7) (Grandjean et al. 1997).

In **Study III**, freshwater fish was rarely consumed during pregnancy and there was no significant association with the concentrations of MeHg in hair or cord blood. In **Study IV**, freshwater fish was consumed by most of the women (80%), who were recruited because of high fish consumption. MeHg exposure increased with increasing consumption of freshwater fish (Figure 2 a+b in **Study IV**). Many of the women also reported intake of other fish species potentially high in MeHg, such as swordfish and fresh tuna. We found that the MeHg exposure was highest among women consuming both freshwater fish species and other fish potentially high in MeHg (Figure 3 a+b in **Study IV**).

About 10% of the women in **Study IV** reported that they consumed freshwater fish more than once a week, which is the maximum recommended intake for the general population, including women of childbearing age (SLV 2004). Although they did not have much higher MeHg exposure than women eating such species less frequently, other studies have reported considerably higher exposure to MeHg from consumption of freshwater fish (Oskarsson et al. 1990, Johnsson et al. 2004). Thus, there seems to be a wide range in the exposure to MeHg reflecting the large variations in MeHg levels both between and within fish species (Ohlin 1993, Lindeström 2001). The fish consumption pattern in Sweden is currently being evaluated through a widely distributed questionnaire concerning fish-consumption habits, provided by the Swedish National Board of Fisheries.

An important finding in **Study IV** was that consumption of certain marine species, such as swordfish and fresh tuna, contributed to the MeHg exposure. Several national food agencies, e.g. U.S. (U.S.FDA 2003), Canada (Health Canada 2003), Great Britain (FSA 2003), Norway (SNT 2003) and Denmark (Fodevaredirektoratet 2003) have included shark, swordfish and large tuna in their dietary advisories to pregnant and lactating women. Since October 2003, after the completion of **Study IV**, swordfish, shark, ray and fresh/frozen tuna were included also in the Swedish dietary advisories to pregnant and lactating women (SLV 2004). The updated dietary advisories also include women planning pregnancy.

It can be concluded that MeHg exposure is dependent on fish consumption in general. Consumption of certain fish species such as freshwater fish and marine swordfish and

tuna contribute more to the exposure. However, dependent on the location where the fish is caught and the size of the fish MeHg levels in fish vary substantially (Ohlin 1993, Lindeström 2001).

Compliance to the dietary advisories

The dietary advisories (SLV 2004) should be provided to all pregnant women in connection with their first visit at the antenatal care, usually around the 10-12th week of pregnancy. Eighty-one percent of the pregnant women in **Study III** did not consume freshwater fish of the type included in the dietary advisories. Also, most pregnant women (88%) reported that they had received the information compared to only about 30% in the early 1990s (Oskarsson et al. 1994). Taken together, this indicates that the compliance to the dietary advisories has increased among pregnant women and that information and communication have improved significantly over the last decade.

The recently updated dietary advisories concerning fish consumption also includes women planning pregnancy (SLV 2004). In the study of women of child-bearing age who reported eating much fish (n=127), 50 women had no children. Only 54% of those, reported that they were aware of that fish might contain elevated levels of environmental pollutants and that it is recommended to eat less of, or avoid, such food (**Study IV**). The information had been received mainly via media.

Food frequency questionnaire

Food frequency questionnaires (FFQ) were used in **Study III** and **IV** to evaluate the MeHg exposure from fish consumption. FFQs have been suggested to give good quality fish consumption history data (Li et al. 2005).

The FFQ used in **Study IV** included very detailed information about consumption of different fish species, reflecting average intake during one year, in combination with a cross-check question about the average total fish consumption. The result of these questions was inconsistent. Although significantly correlated (r_s=0.55; $p<0.001$), the total fish consumption (approximately 4 times/week), as summarized from the reported frequencies, was twice as high as reported in the cross-check question. It has been shown that intake estimates often are overestimated when the number of questions is increased (Krebs-Smith et al. 1995) and underestimated when several items are summarized in one question (Serdula et al. 1992). However, due to the possibility to aggregate single questions but not to separate grouped questions, it is concluded that it is better to use several single questions than a grouped one (Cade et al. 2002). We used the fish consumption data as reported in the FFQ for evaluation of intake of different fish species, the ones potentially high in MeHg and others. However, when evaluating the MeHg exposure it should be borne in mind that the fish intake given rise to the concentrations of MeHg in **Study III** and **IV** most probably was lower than that reported in the FFQ and higher than that reported in the cross-check question.

In both **Study III** and **IV** we found increasing MeHg concentrations with increasing fish intake. The correlation coefficients (r_s) were in the order of 0.3-0.5 comparable to the association reported at similar MeHg exposure level as our studies (Mahaffey et al. 2004b). The correlation coefficient is of course influenced by both the validity of the FFQ (Mahaffey et al. 2004b) and the concentration of MeHg within the fish.

Methylmercury and selenium

We found a slight increase in the concentrations of Se in serum among women recruited because of high fish consumption (r_s=0.35; p=0.002; **Study IV**). However, the average concentration of serum Se (70 µg/L) was similar to that reported for Swedish individuals with no consumption of fish (Lindberg et al. 2004), which shows that other Se sources than fish are equally important (Becker and Pearson 2002). We also found weak associations between Se and MeHg in serum and blood (**Studies II-IV)**. Several studies, including fish-eating populations, have reported associations, between Se and MeHg in serum and blood (Grandjean et al. 1992, Svensson et al. 1992, Hagmar et al. 1998, Steuerwald et al. 2000, Muckle et al. 2001, Barany et al. 2002). The association has mostly been explained by the fact that fish is a source of both Se and MeHg. Associations between Hg and Se have been reported in some fish species but not in others (Dorea et al. 1998, Lima et al. 2005).

Experimental studies show that Se may protect against neurological effects induced by MeHg (Fredriksson et al. 1993) however, the practical significance of Se in MeHg exposed humans has not been established (NRC 2000). In the Faeroe Island, a positive association between Se and total Hg was found, but no protective effects against Hg associated neurological effects could be detected (Steuerwald et al. 2000).

RISK ASSESSMENT

Inorganic mercury

A recent risk assessment concerning Hg in dental amalgam concluded that there is a risk of adverse effects on fetal development after maternal exposure to Hg^0 from dental amalgam fillings (Berlin 2002), however the matter is not fully elucidated. It was further concluded that the use of amalgam should cease to diminish exposure to Hg^0, in line with the precautionary principle, and release of Hg to the environment.

According to recommendations by the Swedish National Board of Health and Welfare, use of amalgam as dental material in pregnant women should be avoided (Socialstyrelsen 1991). In women and fetuses included in the present studies the exposure to I-Hg came mainly from dental amalgam fillings. However, the exposure was very low as reflected in the low I-Hg concentrations in blood.

Our results show that the use of amalgam as dental material is decreasing. Actually, this is supported by the fact that the amount of Hg used for production of dental amalgam has decreased from about 1700 kg in the early 1990s to about 100 kg in 2003 (KEMI 2004). Therefore, it can be concluded that the exposure to Hg^0 from dental amalgam seems to be a decreasing problem in Sweden. However, as a risk for adverse fetal effects can not be excluded the recommendation to avoid amalgam work in pregnant women is justified.

Methylmercury

Although the average MeHg concentrations found in women and fetuses included in the present studies were fairly low, there was quite a range in the exposure as illustrated in Figure 10a and b.

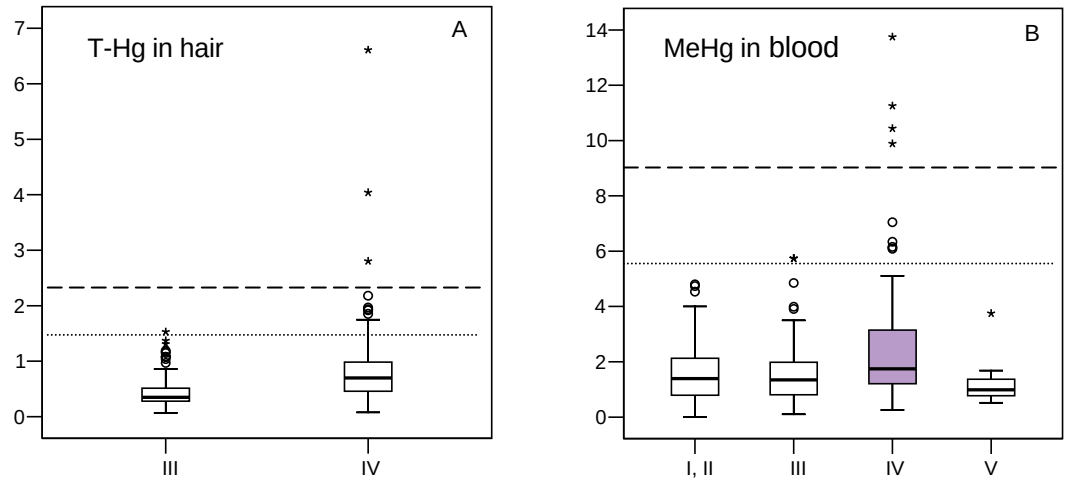

Figure 10. ***(A)*** *The concentration of total Hg (T-Hg) in hair (mg/kg) in pregnant women (Study III) and in women of child-bearing age recruited because of high fish consumption (Study IV). RfD (EPA) corresponds to 1.2 mg/kg and PTWI (JECFA) corresponds to 2.2 mg/kg.* ***(B)*** *The concentration of MeHg in* ***cord blood*** *(µg/L; Study I+II, III and V) and* ***blood of women*** *(µg/L; Study IV). RfD (EPA) corresponds to 5.8 µg/L in cord blood and PTWI (JECFA) corresponds to 8.8 µg/L in maternal blood.*

The U.S. EPA RfD of a daily intake of 0.1 µg MeHg/kg bw per day corresponds to concentrations of 1.2 mg Hg/kg in hair and 5.8 µg Hg/L in cord blood (NRC 2000). The WHO/FAO JECFA made a different assessment, using the same data, and recommends a somewhat higher PTWI of 1.6 µg/kg bw per week (about 0.2 µg/kg bw per day; JECFA 2003) which corresponds to 2.2 mg/kg in hair and 8.8 µg/L in maternal blood. As illustrated in Figure 10, the maximum concentrations of MeHg among pregnant women (**Study III**) were close to the U.S. RfD but below JECFA PTWI. A small fraction of women of child-bearing age recruited because of high fish consumption (**Study IV**) had Hg concentrations in hair exceeding the RfD/PTWI.

As the half-time in blood of MeHg is almost 2 months (Stern 1997) fetuses would be exposed to fairly high levels of MeHg in their early development if a woman consumes much MeHg contaminated fish before pregnancy. The dietary advisories regarding fish consumption (SLV 2004) are usually not provided to pregnant women until their first visit at the antenatal care at approximately 10-12th week of pregnancy.

We conclude that there is a fairly narrow margin of safety for an increased risk of neurodevelopmental effects in fetus for women with high fish consumption unless they decrease their intake of certain fish species well in advance of pregnancy. However, the concentration of Hg in hair in Swedish women recruited because of high fish consumption is below that reported in fish-eating populations, 7 mg/kg in the Seychelles (Davidson et al. 1998) and 4 mg/kg in the Faeroe Island (Grandjean et al. 1997).

Further, we conclude that the recently updated dietary advisories on fish consumption for pregnant and lactating women and women planning pregnancy are justified and necessary as long as MeHg levels in fish are elevated. It is also important that efforts are made to reach the advisories to all women planning pregnancy so they can diminish their consumption of certain fish species to avoid early fetal exposure. However, fish is

also an important source for polyunsaturated fatty acids (Mahaffey 2004a, Sakamoto et al. 2004) and intake of fish during pregnancy may also benefit the child's early cognitive development (Daniels et al. 2004). Therefore, it is important to emphasize that fish also is a wholesome part of the diet when giving the advisories. It is not known to what extent the positive effects of fish consumption may outweigh the negative effects of MeHg, and possibly also PCBs, in fish. This needs to be taken into account at risk assessment.

BIOLOGICAL MONITORING OF MERCURY

An important part of the present work has been the speciation of the different forms of Hg. We determined MeHg and I-Hg in blood and placenta and total Hg in hair and breast milk. The analytical quality was thoroughly checked in all studies. The method used also enabled a low limit of detection, usually below 0.10 µg/L and never exceeding 0.16 µg/L. In other studies the detection limit is generally in the range of 0.1-0.3 µg/L (NRC 2000).

The results show that the method used for speciation of Hg in blood provides unique opportunities to distinguish between exposure to MeHg and exposure to I-Hg during early development and to relate the concentrations to the main sources of exposure.

Total Hg in RBC is often used as a measure of MeHg exposure and total Hg in plasma has been used as a measure of I-Hg exposure. Our recent findings (Berglund et al. 2005) show that approximately 90% (range 76-100%) of MeHg and 35% (range 15-54%) of I-Hg in whole blood are present in the RBCs. Using these findings and data on MeHg and I-Hg in whole blood from pregnant women in the population used for **Study I** and **II** (n=112) the concentrations of MeHg and I-Hg in RBC and plasma were estimated (Table 8). A hematocrit value of 0.42 (Geigy Scientific Tables 1984) was used.

Example of calculation: MeHg in RBC= (0.73 µg MeHg/L in whole blood x 0.90)/0.42

Table 8. *Estimated concentrations (µg/L) of I-Hg, MeHg and total Hg (T-Hg) in red blood cells (RBC) and in plasma. For calculation, data from pregnant women in Study II was used.*

	MeHg RBC	**I-Hg RBC**	**T-Hg RBC**	**MeHg plasma**	**I-Hg plasma**	**T-Hg plasma**
1[a]	1.6	0.27	**1.9**	0.13	0.36	**0.49**
2[b]	6.0	0.23	**6.2**	0.48	0.30	**0.78**
3[c]	1.8	1.2	**3.0**	0.14	2.1	**2.2**

[a] all women (n=112; median concentration)
[b] the woman with highest concentration of MeHg in whole blood
[c] the woman with highest concentration of I-Hg in whole blood

The results from Table 8 confirm the importance of speciation. If only total Hg in RBC and plasma had been determined, the assumption that total Hg in plasma reflects I-Hg exposure would have overestimated the actual concentration, especially in situations exemplified in 1 and 2. Furthermore, the assumption that total Hg in RBC reflects

MeHg exposure would have been misleading in situations with elevated I-Hg exposure (example 3).

Hg in hair was not speciated in the present work. As more than 80% of Hg in hair is in the form of MeHg (Cernichiari et al. 1995) the total concentration of Hg in hair is often used as an integrated measure of MeHg exposure over time. Indeed, the concentration of total Hg in hair gives the best measure of the MeHg that actually entered the hair, as there is a demethylation of MeHg to I-Hg within the hair (Berglund et al. 2005).

SELENIUM

Interactions between toxic and essential elements in the mother-fetus relationship were evaluated in **Study I**. For the purpose of this thesis, mainly the part of **Study I** concerning maternal, fetal and placental Se status was included.

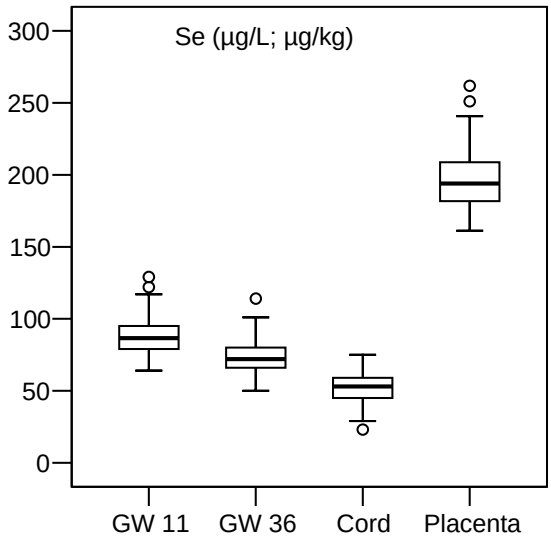

Figure 11. *The concentration of Se in maternal serum, at gestational week (GW) 11 and 36, in cord serum (µg/L) and in placenta (µg/kg). Data from GW 11 has not been published previously, n=74.*

Maternal serum Se (n=74) decreased ($p<0.001$) during the course of pregnancy in line with recent findings (Kantola et al. 2004). This is illustrated in Figure 11 which includes data from **Study I** and unpublished data on maternal serum Se in GW 11. The decrease is partly related to the fact that blood volume increases during pregnancy (Levander and Burk 1994). Also, Se is needed for fetal growth and for placental functions. Maternal serum Se decrease with parity (Figure 12), indicating that the amount of Se transported to the fetus is not restored before the next pregnancy. Decreasing iron status (Åkesson et al. 1998) and zinc status (**Study I**) with parity were also found in the same cohort of Swedish women. Thus, there seems to be a depletion of maternal stores of essential elements with increasing parity, implying a risk for

inadequate maternal nutrition. However, the concentration of Se in cord serum did not decrease with parity indicating that Se is preferentially taken up by the child.

The placental concentration of Se was almost three times the maternal serum Se concentrations in late pregnancy and four times the concentrations in cord serum (Figure 11) in line with previous findings (Korpela et al. 1984, Lee et al. 1995, Dobrzynski et al. 1998). Synthesis of selenoprotein P in placenta has been demonstrated in experimental studies (Kasik and Rice 1995) and is suggested to be needed for transport of Se to the fetus. Furthermore, a high concentration of Se in placenta is most likely needed for glutathione peroxidase, a selenoenzyme with antioxidative properties, in order to prevent oxidative stress in placenta (Myatt and Cui 2004).

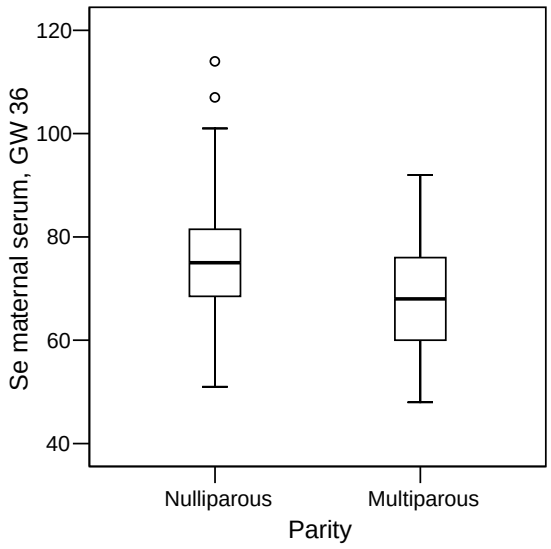

Figure 12. *The concentration (µg/L) of Se in maternal serum at gestational week (GW) 36 in relation to parity (p=0.002). Nulliparous (n=51) and multiparous (n=47).*

CONCLUSIONS

In Sweden, the exposure to I-Hg is mainly as Hg^0 from dental amalgam fillings with low exposure from other sources. Fetuses are exposed to Hg^0 released from maternal dental amalgam fillings. Although Hg^0 is readily oxidized by catalase to Hg^{2+} a significant amount of Hg^0 pass the placental barrier. The concentration of I-Hg in the fetus is similar to that in the mother. A large variation is probably related to inter-individual differences in catalase activity used for oxidation of Hg^0. The formed Hg^{2+}accumulates in the placenta.

MeHg exposure is highly dependent on fish consumption in general. Consumption of certain predatory fish species, such as freshwater fish and large marine species e.g. swordfish and tuna contribute more to the exposure. Fetuses are exposed to MeHg via maternal fish consumption. The concentration of MeHg in cord blood is almost twice as high as the concentration in the mother, supporting the hypothesis of an active transport mechanism across the placenta via the neutral amino acid carriers.

The highest exposure to both I-Hg and MeHg occurs during fetal life. Although I-Hg seems to be effectively transported to breast milk the infant uptake is low. Little MeHg is transported to breast milk. Still, breast milk may contribute significantly to infant MeHg exposure because of high gastrointestinal absorption. It can be concluded that infants are able to excrete MeHg taken up during fetal life, contrary to previous believes, as infant blood MeHg decreased markedly during the first three months of age.

Se status decrease towards the end of pregnancy. This can partly be explained by a prioritized fetal transport and increased need of Se for placental functions. Se in placenta does not seem to affect the accumulation or transport of I-Hg or MeHg in placenta. High placental Se is most likely needed for antioxidative selenoproteins, such as glutathione peroxidase, which protects against oxidative stress in placenta, possibly also that caused by I-Hg and MeHg. Associations between Se and MeHg in blood probably reflect the fact that fish is a source of both Se and MeHg.

The results show that the use of amalgam as dental material is decreasing and the exposure to Hg^0 from dental amalgam seems to be a decreasing problem in Sweden. However, as a risk for adverse fetal effects can not be excluded the recommendation to avoid amalgam work in pregnant women is justified.

There seems to be fairly narrow margin of safety for increased risk of neurodevelopmental effects in fetus of women consuming much MeHg contaminated fish. Thus, the recently updated dietary advisories on fish consumption for pregnant and lactating women and women planning pregnancy are justified as long as MeHg levels in fish are elevated. There was a good compliance to the dietary advisories regarding fish intake during pregnancy but it is important that efforts are made to reach the advisories to all women planning pregnancy, so they can decrease their consumption of certain fish species to avoid early fetal exposure. However, it is also important to emphasize the benefits of fish consumption. It is not known to what extent the positive effects of fish consumption may outweigh the negative effects of MeHg.

ACKNOWLEDGEMENTS

This work has been performed at the Division of Metals and Health at the Institute of Environmental Medicine, Karolinska Institutet with financial support from the Swedish Environmental Protection Agency, The Faculty of Odontology at KI, IMM and KI.

All women participating in the different projects are acknowledged.

My thesis has finally been completed after invaluable help and contribution in different ways from several people. Especially, I would like to thank:

Professor *Marie Vahter*, my supervisor, for your excellent scientific guidance and never failing enthusiasm and support. Thanks also for always taking your time for inspiring discussions and for giving constructive criticism.

Marika Berglund, my co-supervisor, for stimulating discussions and for always managing to see the bright side of a problem. Your encouragement has been important. Thanks also for a nice time in Minamata and for giving me a job.

Gunilla Sandborgh-Englund, also my co-supervisor, for all your scientific advice and support and for teaching me toxicokinetics. It was nice to experience breast milk problems in the lab as well as the Niagara Falls with you.

Birger Lind, for enthusiastically introducing me to the world of analytical chemistry and trace element analysis and for teaching me how to put L,vov`s platform in the right place.

Brita Palm, for all hard work and nice hours in the lab. Your skilful laboratory assistance has contributed a lot this work.

Ulla "Lotta" Björs, for introducing me in the Hg lab. I really appreciated your careful work.

Agneta Åkesson, for being the best rap-partner and exjobbs-supervisor one can have and for teaching me statistics. Your tremendous support and friendship have been invaluable throughout the years.

Kierstin Petersson-Grawé, my co-author from Livsmedelsverket, for important contributions and for support and encouragement.

Katarina Osman, co-author, for mixing placentas and for telling me in the early beginning that I would suit as a researcher, which really inspired me.

All other co-authors for their important contributions in the different projects.

Östen Einarsson, Analysmodul, for providing the Hg instrument and for making it easier or even unnecessary to replot.

Professor *Thomas Clarkson* and Mrs *Elsa Cernichiari* at the University of Rochester, U.S.A. for a nice, fruitful and instructive visit at their lab.

Other dear colleagues at the Division of Metals and Health: *Barbro Nermell*, for helping me with computer problems and for always being in a good mood. *Gudrun Malmborg*, for your help with administrative things and for your care and support. *Margaretha Grandér*, for your support and for being a very nice colleague. *Toyo Katsuta Olofsson*, for teaching me useful Japanese phrases before my trip to Minamata.

Anna-Lena Lindberg, *Maria Kippler* and *Li Li*, my room-mates. I left you for a few weeks when writing the thesis, however I had to move back. I could not have been without all your valuable inputs especially in the basis of molecular biology and analytical chemistry when writing the thesis. Thanks and good luck to you all!!

Gabriela Concha, my former room-mate, I've missed you since you left although you left your +++ energy and the little sleeping pig to me.

Ilona Silins, *Lotta Ryk* and *Mirjam Nordling*, my TEM-colleagues for inspiring work with conferences and exhibition. Ilona especially also for many nice lunches.

Tove Rylander Rudqvist, for nice lunch breaks, friendship and your encouragement during the last months.

Anna Persson, for helping me in the bitter end with pictures and layout.

Jag vill också tacka *alla mina vänner*, *mitt volleybollag*, som tvingats spela utan mig den sista tiden, och Johans familj: *Elisabet*, *Lou*, *Andreas*, *Truls*, *Otto*, *Jossan*, *Viktor*, *Karin* och *Sune* som gör livet roligt och värdefullt.

Mormor, *Matilda*, *Arvid*, *Stina*, *Jenny*, *Magdalena*, *Jörgen*, *Anna*, *Nicholas*, *George*, *Daniel* och *Hampus*, för er uppmutran och för att det alltid är så roligt att komma hem till Småland.

Mamma och *Pappa*, för er omtanke och ert otroliga stöd och för att ni alltid finns där för mig. Ni betyder otroligt mycket och är de bästa föräldrar som finns!

Susann och *Johan*, mina fantastiska syskon som alltid ställer upp för sin lillasyster. Sannas tulpaner lyste upp när det var kämpigt!

Hedda, min lilla solstråle för att du är meningen med livet!

Min älskade *Johan*, för all din kärlek och för att du alltid bryr dig så mycket om mig! Du har uppmuntrat mig under alla år när jobbet varit jobbigt. Den sista tiden har du varit ett stort stöd och dragit ett tungt lass och det uppskattar jag otroligt mycket. Utan dig så hade jag aldrig fixat det här. Du är bäst!

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