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Longitudinal Study of Methylmercury and Inorganic Mercury in Blood and Urine of Pregnant and Lactating Women, as Well as in Umbilical Cord Blood[1]

Marie Vahter,* Agneta Åkesson,* Birger Lind,* Ulla Björs,* Andrejs Schütz,† and Marika Berglund*

*Institute of Environmental Medicine, Karolinska Institutet, S-171 77 Stockholm, Sweden; and
†Department of Occupational and Environmental Medicine, Lund University, S-221 85 Lund, Sweden

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We have investigated exposure to methylmercury (MeHg) and mercury vapor (Hg^0) in pregnant women and their newborns in Stockholm. The women were followed for 15 months post delivery. MeHg, inorganic Hg (I-Hg), and total Hg (T-Hg) in maternal and cord blood were determined by automated alkaline solubilization/reduction and cold vapor atomic fluorescence spectrometry. T-Hg in urine was determined by inductively coupled plasma mass spectrometry. About 72% of the Hg in blood ($n = 148$) in early pregnancy was MeHg (median 0.94 μg/L, maximum 6.8 μg/L). Blood MeHg decreased during pregnancy, partly due to decreased intake of fish in accordance with recommendations to not eat certain predatory fish during pregnancy. Cord blood MeHg (median 1.4 μg/L, maximum 4.8 μg/L) was almost twice that in maternal blood in late pregnancy and was probably influenced by maternal MeHg exposure earlier and before pregnancy. Blood I-Hg (median 0.37 μg/L, maximum 4.2 μg/L) and urine T-Hg (median 1.6 μg/L, maximum 12 μg/L) in early pregnancy were highly correlated, and both were associated with the number of amalgam fillings. The concentrations decreased during lactation, probably due to excretion in milk. Cord blood I-Hg was correlated with that in maternal blood. The results show the importance of speciation of Hg in blood for evaluation of exposure and health risks.

Key Words: **mercury compounds; biomarkers of exposure; blood; urine; speciation.**

INTRODUCTION

People are exposed to different forms of mercury, which differ with respect to kinetics and toxicology (WHO, 1990, 1991; Clarkson, 1997). In general, food contains low concentrations of inorganic Hg^{2+}, and of that ingested, little is absorbed in the intestine (WHO, 1991). Dental amalgam is generally the major source of mercury vapor (Hg^0) exposure (WHO, 1991). In the body, inhaled Hg^0 is oxidized by catalase to Hg^{2+}, which reacts with tissue functional groups and may cause neurotoxic and nephrotoxic effects. Whereas Hg^{2+} does not readily pass cellular membranes, Hg^0 remains in the circulation long enough to cross the placental and blood–brain barriers (Dencker *et al*., 1983; Nylander *et al*., 1987; Dock *et al*., 1994). In fact, tissue concentrations of Hg in the fetus were found to be associated with the number of maternal dental amalgam fillings (Drasch *et al*., 1994; Lutz *et al*., 1996). Effects of Hg^0 on reproductive outcome have been demonstrated in experimental animals and in women occupationally exposed to Hg^0 (Söderström *et al*., 1996; Fredriksson *et al*., 1996; Newland *et al*., 1996). Still, there are very few data on fetal exposure to Hg^0 in the general population.

Exposure to methylmercury (MeHg) is believed to occur almost exclusively via consumption of seafood, especially predatory fish and marine mammals. Like Hg^0, MeHg easily crosses the placenta (WHO, 1990). Our previous studies on toxic metals in aborted fetuses and deceased infants showed brain Hg concentrations (mainly MeHg) similar to those reported for adults (Lutz *et al*., 1996). Epidemiological studies have shown associations between prenatal exposure to MeHg and impaired psychomotor and cognitive function in the children in some fish-eating

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populations (reviewed in WHO, 1990; Grandjean *et al*., 1997, 1998), but not in others (Davidson *et al*., 1998; Crump *et al*., 2000). Reasons for the discrepancies have been debated (Grandjean and White, 1999; Stern and Gochfeld, 1999; Davidson *et al*., 1999). In Sweden, some 10,000 lakes have predatory fish MeHg concentrations exceeding 1.0 mg Hg/kg, and 40,000 lakes have fish MeHg concentrations above 0.5 μg Hg/kg. Therefore, pregnant and lactating women are recommended to not eat predatory fish from the lakes and coastal waters. However, little is known about the compliance. Over the past 15 years, only a couple of studies on mercury levels in hair (mainly MeHg) of pregnant women have been reported (Ohlander *et al*., 1985; Oskarsson *et al*., 1994). In addition, in 30 lactating women from the north of Sweden, the average blood MeHg concentration [total Hg (T-Hg) minus inorganic Hg (I-Hg)] was found to be 1.7 ± 0.7 μg/L (Oskarsson *et al*., 1996). Notably, there was a substantial variation in the blood-to-hair Hg ratio, making assessment of individual MeHg exposure based on hair Hg data uncertain. Data on MeHg in human cord blood, which is the exposure biomarker best associated with developmental effects (Grandjean *et al*., 1997), are available for a few individuals only, in Sweden (Skerfving, 1988) and elsewhere (Suzuki *et al*., 1984; Soria *et al*., 1992; Yang *et al*., 1997).

In addition to the problems involved in sampling of cord blood, the main reason for the lack of data on fetal exposure to Hg^0 and MeHg is the difficulties associated with speciation of Hg in blood, especially at the concentrations occurring in the general population. We have improved the traditional Magos (1971) method by using automated cold vapor atomic fluorescence spectrometry to determine low concentrations of inorganic Hg and MeHg in blood. That has enabled us to assess the exposure to different forms of mercury in urban Swedish women during pregnancy and lactation, as well as the transfer of the Hg species to the fetuses. As the physiological changes in pregnancy and lactation are known to affect the kinetics and toxicity of other metals (Roberts and Silbergeld, 1995), we collected samples in both early and late pregnancy and up to 1 year after delivery.

METHODS

Subjects and Sampling

We recruited pregnant women at their first visit to any of the three antenatal care units (ACU) in the town of Solna, close to Stockholm, Sweden. In total, 254 (41%) of the 618 pregnant women registered at the ACU during 15 months in 1994–1996 agreed to participate and were eligible (enough knowledge of Swedish to understand the written information). No information on the number of women who chose not to participate is available, but about 30–50% of the women visiting the ACUs were immigrants, many of whom had limited knowledge of Swedish. According to the personnel at the ACUs, most of the women that they invited to participate agreed to do so. Obviously, during busy days there was less time for the ACU personnel to inform about the project, and few women were recruited. Of the 254 pregnant women recruited, 237 gave at least one sample of blood or urine to be analyzed for Hg and were included in the present study. Known reasons for quitting the study included miscarriage ($n = 10$), induced abortion ($n = 1$), and change of ACU ($n = 22$). Thus, there were more samples collected in early pregnancy than in later pregnancy. Maternal age ranged from 20 to 45 years (median 31 years) and, on average (median), they were nullipara (range 0–3), with two (range 1–8) pregnancies, including the present. Gestational age was determined by ultrasound or, in 9% of the women, from the date of the last menstrual period. On average, the women were breastfeeding their babies for 6.4 months (range 0.5–12 months).

The women were asked to fill out a simple, self-administered questionnaire concerning consumption of freshwater fish before and during pregnancy (never, once or twice a month, once or twice a week, several times a week) and number of amalgam fillings (none, less than 10, more than 10, unknown). We did not ask for fish species, as the women are informed by the ACU which species should be avoided during pregnancy. Information on occupation, smoking, and alcohol consumption was available from the ACU records.

Concentrations of I-Hg ($Hg^0 + Hg^{2+}$) in blood and total Hg in urine were used as indicators of exposure to inorganic Hg, mainly Hg^0, and blood MeHg was used as index of MeHg exposure. Blood and urine samples were collected twice during pregnancy and three times postpartum (Table 1). Blood was collected at the ACU and the women received metal-free containers for collection of first morning urine. The urine samples were kept frozen in their homes until the following visit to the ACU. Cord blood was collected at delivery at the Karolinska Hospital. All samples were frozen at -20°C until analysis. T-Hg was determined in all urine samples collected (Table 1), and blood I-Hg and MeHg were determined in a subsample of 148 women who had a set of at least three blood samples. There was no statistically significant difference between the urine T-Hg

TABLE 1

Concentrations (μg/L) of Inorganic Mercury (I-Hg) and Methylmercury (MeHg) in Blood and Total Mercury (T-Hg) in Urine (Mainly Inorganic; Adjusted to Density 1.018 g/ml) at Various Points in Time during Pregnancy (Gestational Week, GW) and Lactation and in the Neonate

	Pregnancy		Neonate	Postpartum		
			Blood samples			
Sampling time	GW 11 8–15	GW 36 34–39	GW 40 37–42	3 days 1–7	2.7 months 2.0–4.2	15 months 12–19
I-Hg (μg/L)	0.37 0.06–1.4 (0.0–4.2)	0.32 0.04–1.2 (0.0–1.9)	0.34 0.10–0.75 (0.0–1.1)	0.31 0.04–1.0 (0.0–1.4)	0.30 0.05–0.95 (0.0–1.6)	0.23 0.0–0.84 (0.0–1.1)
MeHg (μg/L)	0.94 0.19–2.5 (0.0–6.8)	0.73 0.20–2.0 (0.0–2.8)	1.4 0.30–3.8 (0.0–4.8)	0.73 0.16–1.7 (0.0–1.8)	0.80 0.15–2.2 (0.0–4.7)	1.2 0.34–3.7 (0.19–6.0)
N	148	112	98	59	90	66
			Urine samples			
Sampling time	GW 12 9–18	GW 37 35–39		3 days 1–6	3.0 months 2.0–4.6	15 months 12–19
T-Hg (μg/L, adj to density)	1.6 0.75–4.6 (0.40–12)	1.6 0.65–4.1 (0.40–7.5)		1.3 0.67–3.0 (0.44–4.5)	1.3 0.60–3.7 (0.38–11)	1.2 0.69–3.2 (0.68–6.9)
N	226	136		63	98	25

Note. Data represent median concentrations, 5th to 95th percentiles, and (total range).

(gestational week, GW 12) of the 141 women who had their blood analyzed for mercury and that of the 85 women whose blood was not tested ($P = 0.48$; Mann–Whitney test). Thus, the subsample of women with data on blood Hg seemed to be representative of the whole group.

We tested whether the women quitting the study at various points in time differed from those continuing with respect to concentrations of all forms of mercury (blood and urine), age, parity, and amalgam fillings. There were no differences found, indicating that it is possible to compare the paired results of mercury in blood or urine longitudinally.

Sample Treatment and Analysis

Blood MeHg, I-Hg, and T-Hg were determined by alkaline solubilization and reduction via an automatic multiple-injection analysis (MIA) system (Einarsson and Hansén, 1995), with a teflon 13-channel selector valve (Analys Modul Sweden AB), and cold vapor atomic fluorescence spectrometry (Merlin, PSA 10.023; P.S. Analytical Ltd., UK). Duplicate subsamples of 1.0 mL blood were treated with 1.0 mL L-cysteine (0.0124 M), 1.5 mL 11 M NaOH, and 0.5 mL deionized water via the MIA system. They were stored in dark overnight at room temperature to complete the solubilization, while keeping the demethylation of MeHg during the course of the analysis below 1%. In a sequence, Hg^{2+} was reduced to Hg^0 by stannous chloride, and MeHg was reduced to Hg^0 by a combination of stannous chloride and cadmium chloride. Total mercury was determined in a separate step by direct addition of the stannous chloride/cadmium chloride solution to the solubilizate. In 12 analytical runs, we achieved acceptable results on the speciation of I-Hg and MeHg. In 17 analytical runs, the MeHg determinations failed according to the quality criteria set, and MeHg was calculated as T-Hg minus I-Hg. The limit of detection (LOD) of I-Hg, MeHg, and T-Hg in blood (3 × SD of the reagent blanks) was similar—about 0.12 μg/L.

Total Hg in urine was determined by inductively coupled plasma mass spectrometry (ICP-MS) using a mass spectrometer (VG PQ2 + ; VG Elemental, Winsford, Cheshire, UK) equipped with an autosampler (Gilson 222; Gilson, Villiers, France). The samples (0.50 mL) were diluted 10-fold with a solution containing EDTA (0.5 g/L), Triton X-100 (0.5 g/L),

and ammonia (5 mL/L) in Millipore water, and 100 μL of an internal standard solution containing indium, thallium, and bismuth, 50 ng of each, was added. Each sample was prepared in duplicate. A spiked urine sample was used for method calibration. The samples were introduced into the spray chamber in a segmented flow mode, using the diluting solution as a carrier/rinsing fluid. The precision, as calculated from the duplicate determinations, was 6%.

To compensate for diurnal and interindividual variation in the dilution of the urine, T-Hg was adjusted to the mean density of all samples (1.018 kg/L). The density of the urine samples was determined using a refractometer (Atago Co., Ltd., Japan).

Quality Control

Leaching tests with 0.03 M HNO_3 in the blood collection tubes (Venoject I VT-100H or II VP-050 SHL, with heparin) and urine containers (polyethylene) overnight at room temperature showed ≤ 0.09 μg Hg/L (LOD of the analytical method), which indicates negligible contamination of samples.

As one part of the analytical quality control for blood Hg, we used the reference material Seronorm trace elements in whole blood (Nycomed AS, Norway) with recommended concentrations on total Hg based on results of a couple of laboratories (Table 2). The precision of the method (CV) was 8.9 and 7.0% at T-Hg concentrations of 2.3 and 8 μg/L, respectively, and 21 and 10% at I-Hg concentrations of 0.6 and 6 μg/L, respectively. CV for MeHg was 7 and 11% at 1.8 and 2.3 μg/L, respectively. There was no systematic change over time in the results of T-Hg, I-Hg, or MeHg at any concentration (29 analytical runs). As there is no commercially available reference blood sample for control of mercury species in blood, we prepared two batches of hemolyzed cow blood spiked with MeHg (0.913 or 0.905 μg/L) and I-Hg (0.503 or 0.928 μg/L). Cow blood was chosen as it was anticipated to have low concentrations of both MeHg and I-Hg (our analyses gave 0.12 μg/L I-Hg and 0.14 μg/L total Hg; $n = 12$). Analysis of the two spiked samples in this study gave average recoveries of 101 and 108% for I-Hg at the low and high concentration ($n = 11$) and 89% for total Hg in both the spiked batches ($n = 6$). Analysis of a MeHg standard solution containing 0.4 μg Hg/L gave a recovery of 102% ($n = 23$). No signal was detected at the position of I-Hg, indicating no significant demethylation of MeHg during the course of the analysis. In addition, the sum of I-Hg and MeHg showed very good agreement with T-Hg (Fig. 1).

The accuracy of the analyses of urinary T-Hg was checked by including two samples from a laboratory intercomparison program in the analytical series (20th Intercomparison Programme for Toxicological Analysis in Occupational and Environmental Medicine, organized by the Institute and Out-Patient Clinic for Occupational, and Social and Environmental Medicine, University of Erlangen-Nuremberg, Germany). Our results were 1.3 ± 0.16 μg/L ($n = 5$) and 4.0 ± 0.18 μg/L ($n = 5$), and the reference values were 1.34 and 3.94 μg/L, respectively.

Statistical Analysis

In the statistical analysis of data, differences between groups and correlations were tested for

TABLE 2

Obtained Concentrations (μg/L) of Total Mercury (T-Hg), Inorganic Mercury (I-Hg), and Methylmercury (MeHg) in Reference Blood Samples

Batch of Seronorm trace elements in whole blood	Recommended value T-Hg	Obtained values		
		T-Hg	I-Hg	MeHg
205052	3 (2–4)[a]	2.3 ± 0.21 ($n = 27$)	0.56 ± 0.12 ($n = 27$)	1.8 ± 0.13 ($n = 11$)
203056	9 (8–9)[a]	7.9 ± 0.56 ($n = 14$)	6.0 ± 0.63 ($n = 14$)	2.3 ± 0.26 ($n = 8$)

[a] Based on the results from two laboratories.

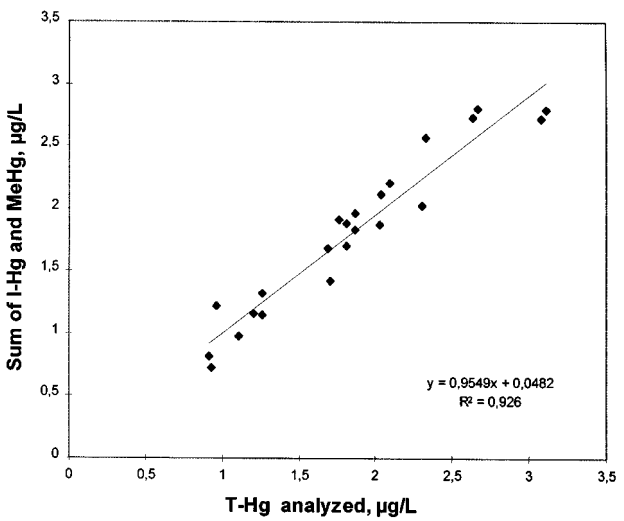

FIG. 1. Comparison of the sum of determined inorganic mercury (I-Hg) and methylmercury (MeHg) in blood and determined total mercury (T-Hg).

significance using nonparametric tests (Mann–Whitney rank sum test, Wilcoxon sign rank test, Kruskal– Wallis, Spearman (r_s)) when the data did not fulfill requirements for normal distribution. In the multiple linear regression analysis, mercury data were log transformed if required. To compensate for multiple tests in the longitudinal comparison, the criteria for significant results was set to 0.05 divided by the number of comparisons.

RESULTS

Although the measured concentrations of MeHg and I-Hg in blood in general were low, most samples were above the detection limit of the analytical method (Table 1). On average, 72% of the blood Hg in early pregnancy was in the form of MeHg. There was a substantial variation—90% of the samples ranged between 22 and 95% MeHg. Only a few women reported consumption of freshwater fish. They had higher blood MeHg than women who never ate such fish (1.3 μg/L vs 0.94 μg/L, respectively), but the difference was not statistically significant. The concentration of MeHg in blood decreased 23% during pregnancy ($P < 0.001$, paired test; Fig. 2). Also, the questionnaires indicated a decrease in the consumption of fish during pregnancy. In total, 13% of the 225 women reported that they used to have freshwater fish once a month ($n = 25$) or more often

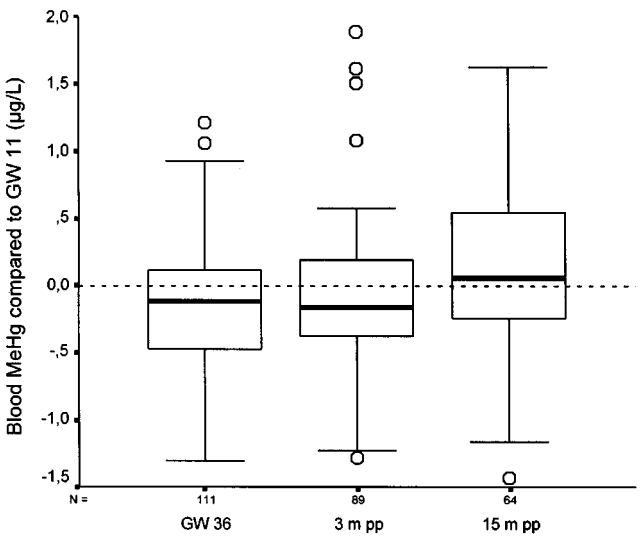

FIG. 2. Differences (paired comparisons) between blood methylmercury (MeHg) at gestational week (GW) 11 (dashed line) and that at GW 36 and at 3 and 15 months postpartum. The box plots contain 50% of all values (25th to 75th percentiles) with median values indicated. The whiskers represent the highest and lowest values, excluding outliers (○). A median value below the dashed line indicates a blood MeHg lower than that at GW 11.

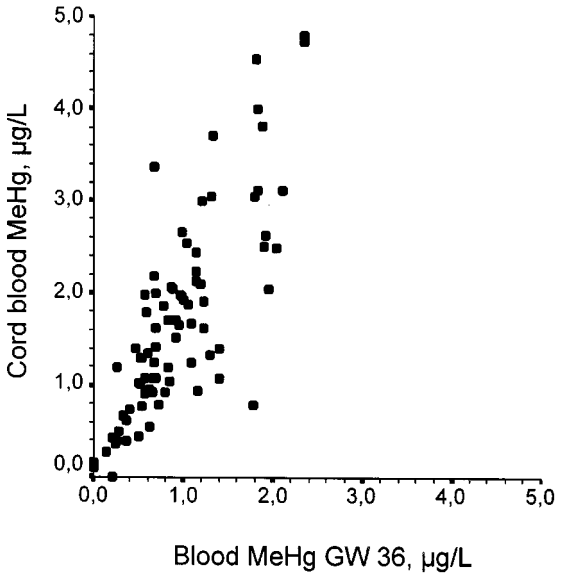

FIG. 3. Association between cord blood methylmercury (MeHg) and maternal blood MeHg.

($n = 4$) before pregnancy, whereas only 4% of 98 women reported intake of such fish during pregnancy. At 15 months after delivery, blood MeHg was slightly, but not significantly, higher than that in GW 11. The concentrations of MeHg in blood increased significantly with maternal age ($r_s = 0.30$; $P < 0.001$, GW 11) and decreased with parity (adjusted for age in multiple regression analysis, adj. $R^2 = 0.14$; $P < 0.001$). Reported consumption of freshwater fish was negatively associated with parity ($P < 0.002$), but not significantly associated with maternal age. However, in general, the consumption of seafood increased with age among women (Becker, 1994).

There was a significant correlation between cord blood MeHg and maternal blood MeHg at GW 36 ($r_s = 0.78$; $P < 0.001$; Fig. 3). On average, cord blood MeHg was 1.8 times higher than maternal blood MeHg at GW 36 ($P < 0.001$; the 5th and 95th percentiles were 0.88 and 3.1 times higher). However, linear regression analysis of cord blood MeHg against blood MeHg GW 36 (log transformed data) gave a slope of 1.5 and a significant intercept of 0.19. There was also a correlation between cord blood MeHg and maternal blood MeHg at GW 11 ($r_s = 0.69$; $P < 0.001$).

The concentration of I-Hg in blood decreased only 6% during pregnancy ($P = 0.042$; Fig. 4). After delivery, the decrease in blood I-Hg accelerated, and 15 months later I-Hg was only 61% of the concentration at GW 11 ($P < 0.001$). Also, T-Hg in urine changed very little during pregnancy, compared to the

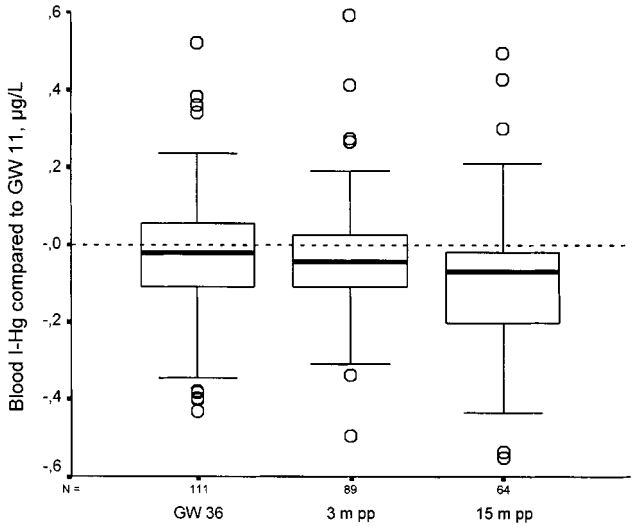

FIG. 4. Differences (paired comparisons) between blood inorganic mercury (I-Hg) at gestational week (GW) 11 (dashed line) and that at GW 36 and at 3 and 15 months postpartum. For explanation of box plots, see legend to Fig. 2. A median value below the dashed line indicates a blood I-Hg lower than that at GW 11.

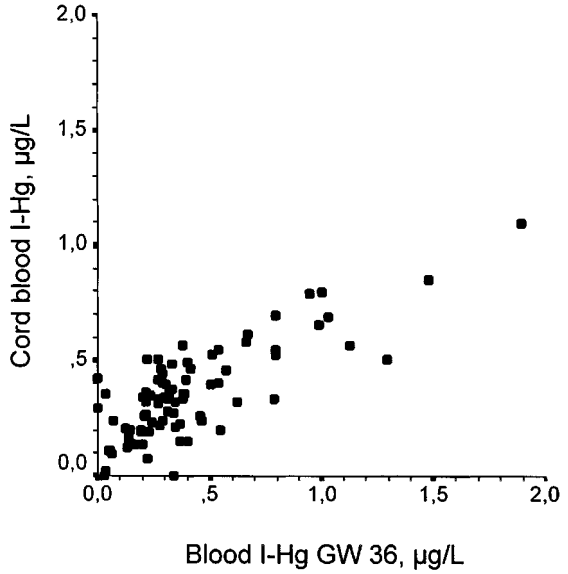

FIG. 5. Association between cord blood inorganic mercury (I-Hg) and maternal blood I-Hg.

change during the postpartum period, and at 15 months it was 78% of that at GW 11 ($P = 0.005$). Urinary T-Hg was significantly correlated with blood I-Hg ($r_s = 0.60$ in early pregnancy, $P < 0.001$, $n = 140$), but not with blood MeHg ($P = 0.66$).

There was a significant correlation between I-Hg in cord blood and I-Hg in maternal blood in late pregnancy ($r_s = 0.68$, $P < 0.001$; Fig. 5) and in early pregnancy ($r_s = 0.71$, $P < 0.001$). On average, cord blood I-Hg was 0.9 times the maternal blood I-Hg GW 36 (5th/95th 0.35/2.0) percentiles. Linear regression analysis showed a slope of only 0.47 and a significant intercept of 0.16.

Blood I-Hg was not influenced by maternal age, parity, or smoking. Based on the data on occupations in the ACU files, there were no indications of occupational exposure to Hg. Both maternal and cord blood I-Hg increased with increasing number of amalgam fillings ($P \leq 0.024$, Kruskal–Wallis; Fig. 6). Also, urinary T-Hg increased significantly with number of amalgam fillings ($P < 0.001$, Kruskal–Wallis) and women with more than 10 fillings had about twice as high T-Hg (median 1.9 µg/L) in urine as the women with no fillings (median 0.94 µg/L). Women with 1–10 fillings had median 1.5 µg/L.

DISCUSSION

The results of this study show that the improved Hg speciation method provides unique opportunities to distinguish between exposure to MeHg and exposure to Hg vapor during early development and to test for the main sources of exposure. Important findings were the large variations in concentrations of mercury species in blood and urine, although the study group was not selected with respect to high exposure, as reflected in the low average concentrations of both I-Hg and MeHg. Maternal blood MeHg

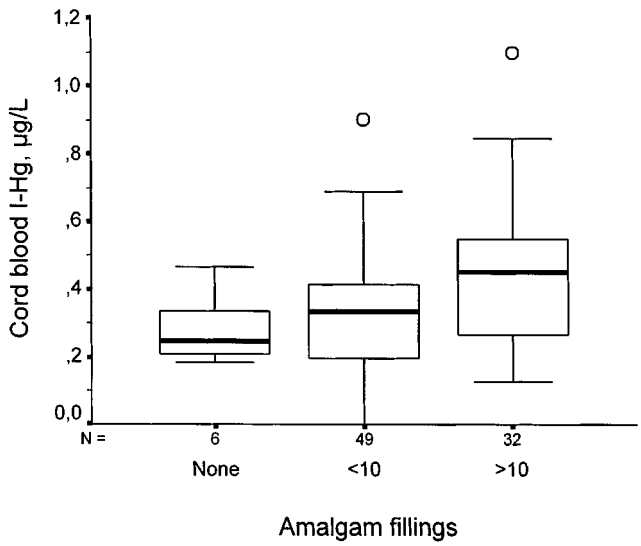

FIG. 6. Association between cord blood I-Hg and number of maternal dental amalgam fillings. For explanation of box plots, see legend to Fig. 2.

in early pregnancy ranged up to 7 μg/L. This would correspond to an average daily intake of about 9 μg Hg (as MeHg; Sherlock *et al*., 1984), which is about one third of the WHO/FAO provisional tolerable weekly intake (PTWI). This PTWI is based on neurotoxicity in adults and does not protect against developmental neurotoxicity (WHO, 1990). A recent study in the Faeroe Islands, where people have a high intake of MeHg from fish and pilot whales, showed cognitive deficits in 7-year-old children who at birth had cord blood T-Hg levels of 15–30 μg/L or more compared with those with less than 15 μg/L (Grandjean *et al*., 1997). The exact critical concentration was not determined. Considering the fact that a significant number of Swedish women certainly have a higher fish consumption than that of the urban women in the present study group (Becker, 1999) and that MeHg in cord blood generally is higher than MeHg in maternal blood (see below), it is obvious that the level for fetal MeHg toxicity may be approached. Blood Hg values exceeding 20 μg/L have been reported for people eating fish two to three times a week (Svensson *et al*., 1992). Thus, there is a need to assess the MeHg exposure in pregnant women who frequently eat fish, e.g., in families with professional or recreational fishing, which is very common in Sweden.

The decrease in blood MeHg during pregnancy could partly be explained by hemodilution and other physiological changes related to pregnancy (Hytten, 1985). However, there was probably also a decreased exposure to MeHg due to decreased fish consumption, as indicated by the results of the questionnaires.

Cord blood MeHg ranged up to 5 μg/L, with 5% exceeding 3.8 μg/L. It was almost twice as high as maternal blood MeHg in late pregnancy. This can be explained partly by the high hemoglobin level in the fetus and partly by the influence of maternal blood MeHg earlier in pregnancy. Because the half-life of MeHg in maternal blood is about 2 months (WHO, 1990), it is obvious that a high MeHg exposure before the onset of pregnancy will result in exposure of the fetus over a considerable part of its prenatal development. In addition, it seems likely that the half-life of MeHg is longer in the fetal blood than in the maternal blood. It is believed that MeHg is transported across the placenta via the neutral amino acid carriers (Kajiwara *et al*., 1996), the number of which is higher in fetal than in adult blood (Moriyama *et al*., 1990). A similar transport system for MeHg is present in the blood–brain barrier and it has been related to the structural similarities between the MeHg–cysteine complex and methionine (Aschner and Aschner, 1990). This would indicate the existence of a mainly one-way transport of MeHg in the direction of the fetus, resulting in a continuous accumulation. Thus, information about the recommended dietary restrictions during pregnancy should be given to all women of childbearing age, so that they can decrease their intake of MeHg-containing fish well in advance of conception. As fish in many other respects is excellent food, women should be advised to eat marine fish with low MeHg content.

It should be noted also that people who never eat fish might be exposed to MeHg. A total range of 1.2–2.4 μg/L (average 1.8 μg/L) blood total Hg was observed in people ($n = 13$) who never eat any kind of fish (Svensson *et al*., 1992), which is higher than could be expected from dental amalgam only. Also, they had higher Hg concentrations in red blood cells than in plasma, indicating that part of the blood Hg was MeHg, which is localized mainly in the red blood cells, whereas inorganic Hg is evenly distributed between blood cells and plasma (WHO, 1990). One possible reason is that fish powder is used as a source of protein for poultry and swine (about 1% of the feed according to the National Board of Agriculture, 1999). Obviously, there is a need to quantify the exposure to MeHg via such sources.

Despite the fact that the number of amalgam fillings was self-reported and only a rough measure of the exposure to Hg^0 from the fillings, there was a significant association between the number of amalgam fillings and the concentrations of I-Hg in blood and T-Hg in urine (mainly I-Hg). Such a relationship has previously been reported for Hg in urine, whereas total Hg in blood is highly influenced by fish consumption and is not a useful indicator of Hg exposure from dental amalgam (Langworth *et al*., 1991; Åkesson *et al*., 1991).

There was a substantial interindividual variation in I-Hg concentrations, in both blood and urine. Concentrations in the upper range (i.e., more than 1 μg/L in blood and 4 μg/L in urine) were present also in individuals with few amalgam fillings. This may be due to, e.g., enhanced release of amalgam Hg^0 because of gum chewing (Sällsten *et al*., 1996). Women with no amalgam fillings had only 0.2 μg/L I-Hg in blood, on average, with a range up to 0.4 μg/L. It is known that some MeHg is demethylated to I-Hg in the body (WHO, 1991). However, there was no correlation between MeHg and I-Hg in blood or urine, which supports indications that the demethylation takes place mainly in the tissues, where I-Hg may have a long half-life, resulting in slow release into circulating blood (Vahter *et al*., 1994).

Interestingly, there was a marked decrease in I-Hg in blood and urine during lactation, most likely related to the excretion of I-Hg in milk (Sundberg *et al*., 1998). It has been reported that 50–80% of the mercury in the milk of lactating Swedish women is in the form of I-Hg (Skerfving, 1988; Oskarsson *et al*., 1996), i.e., more than twice the I-Hg fraction in the blood. This can be explained by the low erythrocyte-to-plasma ratio for I-Hg (about 1) compared to MeHg (about 20), which favors the transfer of I-Hg from plasma to milk. Assuming that the milk I-Hg concentration is about half that in blood, it can be estimated that the women in the present study excreted about 0.15 μg I-Hg per day in milk (based on 1 L milk per day). Thus, about 10% of the Hg present in circulating blood (5 L × 0.3 μg/L) would be transferred to the milk every day.

The concentration of I-Hg in cord blood, on average 0.34 μg/L, was similar to that in maternal blood at GW 36. As experimental animal studies have shown a low transfer of Hg^{2+} to the fetus (Dencker *et al*., 1983; Dock *et al*., 1994), it seems likely that the Hg vapor from dental amalgam passes the placenta before being oxidized by catalase. In the fetal tissue it is partly oxidized and trapped. This is supported by the observed increase in cord blood I-Hg and fetal tissue Hg with increasing number of maternal dental amalgam fillings (Drasch *et al*., 1994; Lutz *et al*., 1996). The ability to oxidize Hg^0 increases with advancing fetal development (Dencker *et al*., 1983).

In conclusion, this study shows that the fetus is exposed to MeHg from maternal fish consumption and to Hg vapor from maternal dental amalgam fillings. Although the average concentrations in blood and urine are low, there are large variations. Because a considerable number of women certainly consume more fish than that reported in the present study, it is likely that the level for fetal MeHg toxicity may be approached. It is essential to decrease intake of MeHg-containing fish well in advance of pregnancy.

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