Methyl Mercury and Inorganic Mercury in Swedish Pregnant Women and in Cord Blood: Influence of Fish Consumption

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We studied exposure to methyl mercury (MeHg) in Swedish pregnant women (total mercury [T-Hg] in hair) and their fetuses (MeHg in cord blood) in relation to fish intake. The women were recruited at antenatal care clinics in late pregnancy to participate in an exposure study of environmental pollutants. Fish consumption was evaluated using food frequency questionnaires including detailed questions on fish consumption. In addition, we determined inorganic mercury (I-Hg) and selenium (Se) in cord blood. On average, the women consumed fish (all types) 6.7 times/month (range 0-25 times/month) during the year they became pregnant. They reported less consumption of freshwater fish-species that might contain high concentrations of MeHg-during than before pregnancy. T-Hg in maternal hair (median 0.35 mg/kg; range 0.07-1.5 mg/kg) was significantly associated ($R^2 = 0.53$; p < 0.001) with MeHg in cord blood (median 1.3 µg/L; range 0.10-5.7 µg/L). Both hair T-Hg and cord blood MeHg increased with increasing consumption of seafood (r = 0.41; p < 0.001 and r = 0.46; p < 0.001, respectively). Segmental hair analysis revealed that T-Hg closer to the scalp was lower and more closely correlated with MeHg in cord blood than T-Hg levels in segments corresponding to earlier in pregnancy. We found a weak association between Se (median 86 μ g/L; range 43–233 μ g/L) and MeHg in cord blood (r = 0.26; p = 0.003), but no association with fish consumption. I-Hg in cord blood (median 0.15 µg/L; range 0.03-0.53 µg/L) increased significantly with increasing number of maternal dental amalgam fillings. Key words: fetal exposure, fish consumption, human, inorganic mercury, methyl mercury, pregnancy, selenium. Environ Health Perspect 111:637-641 (2003). doi:10.1289/ehp.5618 available via http://dx.doi.org/ [Online 21 January 2003]

Because of the extensive historic release of mercury (Hg) and the continuous global transport of airborne Hg, approximately every eighth lake (i.e., around 10,000 lakes) in Sweden has fish containing more than 1 mg Hg/kg as methyl mercury (MeHg), the established European Union maximum limit for MeHg in certain predatory fish species (Johansson et al. 2001). In addition, about 50% of the lakes (i.e., 40,000 lakes) have fish containing more than 0.5 mg MeHg/kg, the European Union maximum limit for MeHg in most fish species. Even though the emission of Hg has decreased in Sweden, the Hg that has accumulated over many years in soil is slowly transported to lakes and coastal waters, where it eventually ends up as MeHg in fish. Because of the biomagnification process of MeHg, species at the top of the aquatic food chain, especially predatory fish, contain the highest levels of MeHg (de Souza Lima et al. 2000).

MeHg is highly neurotoxic, for example, via oxidative stress (Castoldi et al. 2001), and, in particular, affects neurodevelopment (NRC 2000). Therefore, the Swedish National Food Administration recommends pregnant and lactating women refrain from consuming various predatory freshwater fish species present in Swedish waters, as well as some marine fish (e.g., halibut) (SLV 2002). However, little is known about the compliance of the dietary recommendations. Our previous results within the recently established national health-related environmental monitoring program investigated MeHg exposure in urban women with low average consumption of fish (Vahter et al. 2000). Still, there were large interindividual variations in blood concentrations of MeHg. Because of limited fish consumption data, it was not possible to evaluate the main sources of MeHg exposure. The purpose of this study was to assess the exposure to MeHg in Swedish pregnant women and in their fetuses using detailed fish consumption data in combination with biomonitoring. As MeHg accounts for more than 80% of total Hg (T-Hg) in hair (Cernichiari et al. 1995), T-Hg was used to estimate the maternal exposure to MeHg (NRC 2000). To assess fetal MeHg exposure, the concentration of Hg in cord blood was speciated in MeHg and inorganic Hg (I-Hg).

We also determined selenium (Se) concentrations in cord blood to elucidate the possible associations between Se and the different Hg species during fetal development.

Se is an essential, antioxidative trace element that protects against both MeHg and I-Hg toxicity in animals (Goyer 1997) and against MeHg toxicity in human cells (Frisk et al. 2001). Se supplementation also reduces the levels of Hg in human hair (Seppänen et al. 2000).

Materials and Methods

Study participants and sampling. From January 1996 to 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 primiparas recruited from early fall 1996 onward (n = 376) were asked, when they were in late pregnancy, to participate in an exposure study of environmental pollutants (Glynn et al. 2001).

Of the 376 women approached, 131 women 20-40 years of age (median 27 years) 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. The hair sample was taken at gestational weeks 32-34. After the bundle had been tied close to the scalp end, full-length hair samples were cut as close to the scalp as possible, and placed into plastic bags. Cord blood samples were collected at birth in 10-mL Vacutainer glass tubes (Becton Dickinson, Stockholm, Sweden) and transferred to 10-mL polystyrene centrifuge tubes (Labora, Upplands Väsby, Sweden) for analysis of Hg and Se. All utensils were checked for Hg and Se contamination.

About 3 months after delivery, the women completed an extensive food frequency questionnaire reflecting their usual intake, including detailed information about fish consumption (all types of fish and seafood) as well as other foodstuffs during the year they became pregnant. The women also reported

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on their knowledge of the recommendations to avoid certain fish species and their consumption of specific freshwater fish species, potentially high in MeHg, and canned tuna during the actual pregnancy. Based on the reported fish consumption frequencies and portion sizes (75–150 g, depending on type of fish), the amount of fish consumed, namely, intake of grams of fish (fresh weight) per day (g/day), were calculated (SLV 1999). Furthermore, the women were asked to check, by using a mirror, the number and size of their dental amalgam fillings and record both the total number of fillings and their size as marks on an illustration of the upper and lower jaws.

Informed consent was obtained from the participating women, and the study was approved by the ethics committee of the Medical Faculty at Uppsala University.

Analytical methods. We analyzed T-Hg in hair, and T-Hg, I-Hg, and Se in umbilical cord blood. Hair grows approximately 1 cm per month (Cernichiari et al. 1995). For most women, 9 cm of hair from the scalp end were used to give an approximate integrated measure of the MeHg exposure during the first 7 months of pregnancy as well as 2 months before conception. Only a few hair samples were less than 9 cm long; the shortest was 3.5 cm. In addition, to study MeHg exposure during different parts of gestation as well as before pregnancy, we also analyzed T-Hg in 1- to 2cm segments (depending on available amount of hair) from a subsample (n = 15) with elevated concentrations and/or with less association with cord blood MeHg concentrations. It is in those we can expect to find variations with time. The concentrations of T-Hg in hair and T-Hg and I-Hg in cord blood were determined by alkaline solubilization/reduction and cold vapor atomic fluorescence spectrophotometry (Merlin, PSA 10.023; P.S. Analytical Ltd., Orpington, Kent, UK) as previously described for blood (Vahter et al. 2000). Hair samples weighing 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 min to complete the solubilization. Duplicate samples from all solubilizates were analyzed. MeHg in cord blood was achieved by subtracting the concentration of I-Hg from that of T-Hg, assuming that the major part of the organic Hg fraction in blood is in the form of MeHg. The concentration of Se in cord blood was determined by electrothermal atomic absorption spectrophotometry according to a method described by Alfthan (1982).

The limit of detection (LOD) $(3 \times SD \text{ of})$ the reagent blanks) in blood was $0.06-0.12 \mu g$ T-Hg/L, $0.06-0.15 \mu g$ I-Hg/L, and $12-60 \mu g$ Se/L. LOD for T-Hg in hair was 0.004-0.05mg/kg. I-Hg concentrations in a few cord blood samples were below LOD. Although these values present a larger uncertainty than those above LOD, the exact values were used in the calculations so that distributions would not be distorted.

Quality control. Results of the analytical quality controls (QCs) are presented in Table 1. Homogenized hair from the Faeroe Island was prepared, in collaboration with P Weihe and P Grandjean, to be used as QC for T-Hg in hair (hair QC). The recommended value is based on an interlaboratory comparison where T-Hg in hair has been determined by four different analytical methods at six laboratories including ours. The hair QC was analyzed in each analytical run (n = 13). Analysis of the reference material Seronorm trace elements in whole blood (Nycomed Co., Oslo, Norway) showed good agreement with recommended concentrations of T-Hg (Table 1). There are no recommended values for I-Hg in the Seronorm reference blood samples, but the obtained values for I-Hg were well in agreement with our previous results (Seronorm 404107: 0.5 ± 0.1 μ g I-Hg/L, *n* = 4; Seronorm 404108: 5.9 ± 0.5, n = 3). Repeated analysis (n = 7) of cow blood spiked with low concentrations of MeHg (0.913 or 0.905 µg/L) and I-Hg (0.503 or 0.928 µg/L) gave average recoveries of 96 and 101% for T-Hg and I-Hg, respectively. Analysis of a MeHg standard solution containing 0.4 µg Hg/L gave a recovery of 97%. We also used Seronorm 404108 to verify the accuracy of the Se analyses (Table 1). No systematic change over time could be detected in any of all the QC analyses. Overall, the analytical quality was good.

Statistics. The concentrations of T-Hg, MeHg, and I-Hg were tested for normality

and found to be not normally distributed. Logarithmic transformation of these dependent variables was used to conform to the requirement of normal distribution. We used Pearson correlation for bivariate comparisons and identification of possible predictors of the element concentrations. The variables tested were total consumption of fish/seafood, consumption of specific fish species, consumption of chicken, number of dental amalgam fillings, maternal age, and Se in cord blood. The predictors with p < 0.05 were further explored with multiple linear regression analyses whenever requirements of normally distributed residuals were met. Nonparametric tests were used for comparison between groups (Kruskal-Wallis) and for evaluation of segmental hair T-Hg analysis (Spearman's correlation and Wilcoxon test). All statistical analyses were conducted with SPSS version 10.0 for Windows (SPSS Inc, Chicago, IL, USA). Statistical significant level was set to p < 0.05.

Results

Questionnaire data. Most women (88%; n = 112) reported awareness of the dietary recommendations to refrain from consumption of certain fish during pregnancy. For 79% the information had been received at the antenatal care clinic. Other sources of information were friends, family, school, and magazines. The reported consumption of various seafoods is shown in Table 2. On average, fish and shellfish (all types) were consumed 6.7 times/month (range 0–25 times/month; 90th percentile 15 times/month; n = 127) during the year the women became pregnant. This would correspond to a mean consumption of

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QC	Analyte	Recommended value	Obtained value	Coefficient of variation (%)
Hair				
Hair-QC	T-Hg	4.9 ± 0.24 ^a	$4.8 \pm 0.1 (n = 13)$	3.2
Blood				
Seronorm 404107	T-Hg	2.2–3.3	$2.3 \pm 0.2 (n = 7)$	8.1
	I-Hg	_	$0.5 \pm 0.1 (n = 7)$	12
Seronorm 404108	T-Hg	6.7-8.4	$7.6 \pm 0.7 (n = 7)$	9.0
	I-Hg	_	$6.4 \pm 0.9 (n = 7)$	14
	Se	82-89	86 ± 10 (<i>n</i> = 6)	11

-, no recommended value is assigned by supplier.

^aInterlaboratory comparison of a hair QC sample prepared at our laboratory. The recommended value is based on analysis by four different analytical methods at six laboratories, including ours.

 Table 2. The reported consumption (times per week, month, or year) of different types of fish during the year the women got pregnant.

	Frequencies				
Type of fish	n	Median	90th Percentile	Maximum	
Deep frozen (cod, saithe)	109	1–3/month	1/week	2/week	
Fish fingers, fish balls	103	1–3/month	1/week	3–4/week	
Pickled herring	96	3/year	1–3/month	1/week	
Canned fish	98	3/year	1–3/month	2/week	
Freshwater fish	31	Never	6/year	1–3/month	
Fresh marine fish	57	Never	1–3/month	2/week	
Spawn	84	3/year	1–3/month	2/week	
Shellfish	111	6/year	1–3/month	2/week	

about 25 g/day (range 0–110 g/day). Only one woman consumed no fish at all. In total, 81% (n = 103) of the women refrained from eating freshwater fish during pregnancy, whereas 13% (n = 17) ate it 1–3 times, and 6% (n = 7) 4–8 times during the entire pregnancy. The intake of freshwater fish during the actual pregnancy (0.48 g/day) was significantly lower (p < 0.001) than that reported during the year the women became pregnant (2.2 g/day on average; n = 31).

Analytical results. The concentrations of T-Hg in hair and I-Hg, MeHg, and Se in cord blood are summarized in Table 3. MeHg in cord blood was significantly associated with T-Hg in maternal hair ($R^2 = 0.53$; n = 126) (Figure 1), and both measures increased with increasing total consumption of seafood (r = 0.41, p < 0.001, and r = 0.46, p < 0.001 for T-Hg in hair and MeHg in cord blood, respectively) (Figure 2).

In a subsample of 15 women, we compared T-Hg in four different hair segments corresponding to months 1-2 before pregnancy and months 1-3, 4-5, and 6-7 of pregnancy. The concentration of T-Hg in hair was lower in the 2-cm segment closest to the scalp (months 6-7; median [Md] = 0.62 mg/kg) than in the segments corresponding to the months 4-5 (Md = 0.79 mg/kg; p =0.002) and in the segment for the months 1-3 (Md = 0.96 mg/kg; p = 0.003). The associations between MeHg in cord blood and T-Hg in different segments were $r_s = 0.41$ (p = 0.27; n = 9) 2 months before conception; r_s = 0.59 (p = 0.02; n = 15) at 1–3 months; r_s = 0.42 (p = 0.12; n = 15) at 4–5 months; and r_s = 0.67 (p = 0.006; n = 15)at 6–7 months.

Table 3. Concentrations of T-Hg in maternal hair, and I-Hg, MeHg, and Se in cord blood.

Median

0.35

1.3

0.15

86

There was a significant association between the concentrations of Se and MeHg in cord blood (r = 0.26; p = 0.003) and an association of borderline significance between Se in cord blood and T-Hg in maternal hair (r = 0.18; p = 0.05). No significant association was found between Se concentration in cord blood and seafood consumption.

To evaluate predictors of T-Hg in hair and MeHg in cord blood, we performed multiple linear regression analyses including total consumption of seafood, consumption of chicken, Se in cord blood, and maternal age. The model with log hair T-Hg as dependent variable showed a significant association with total consumption of seafood and with maternal age adjusted (adj.; $R^2 = 0.21$; p < 0.001). However, there were no significant differences in seafood or chicken consumption between younger (20-27 years of age; average seafood consumption 6.8 times/month) and older (28-40 years of age; average seafood consumption 7.8 times/month) women during pregnancy. When log cord blood MeHg was chosen as dependent variable in the model, it showed a significant association with total consumption of seafood and chicken and with cord blood Se (adj. $R^2 = 0.27$; p <0.001). There was a significant association between consumption of seafood and chicken (r = 0.20; p = 0.02). It can be mentioned that one woman who consumed chicken 3-4 times/week but little seafood (about 13 g/day), none of which was freshwater fish, had a rather high concentration of MeHg in cord blood (2.0 μ g/L).

In the bivariate analysis, consumption of freshwater fish (included in the dietary

Minimum

0.07

0.10

0.03

43

90th Percentile

0.81

2.7

0.32

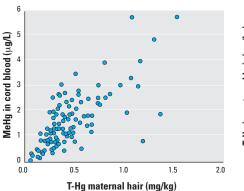
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recommendations) was not significantly associated with T-Hg in hair and MeHg in cord blood, but consumption of several fish species not included in the dietary recommendations was significantly associated with T-Hg in hair and MeHg in cord blood. For example, intake of deep-frozen fish (such as cod, saithe), pickled herring, other canned fish (such as tuna), salmon, fresh marine fish, spawn, and shellfish gave correlations (r) on the order of 0.2–0.3 (p < 0.05) with both T-Hg in hair and MeHg in cord blood. To elucidate which fish species contributed most to the levels of hair T-Hg and cord blood MeHg, we performed stepwise multiple regression analyses. In the model with log hair T-Hg as dependent variable, consumption of deep-frozen fish, pickled herring, and fresh marine fish remained significant (adj. R^2 = 0.20; p < 0.001). When log cord blood MeHg was chosen as a dependent variable, consumption of deep-frozen fish, canned fish, and shellfish remained significant (adj. R^2 = 0.16; p < 0.001).

I-Hg in cord blood increased with the number of maternal dental amalgam fillings (multiple linear regression; adj. $R^2 = 0.25$; p < 0.001) (Figure 3) but was not influenced by maternal age. There were no significant associations between I-Hg and the consumption of seafood or Se in cord blood.

Discussion

The women in the present study reported less consumption of freshwater fish—species that might contain high concentrations of MeHg—during than before pregnancy. This indicates good compliance with the recommendations issued by the Swedish National Food Administration to refrain from eating certain predatory fish species during pregnancy and lactation. The recommendations are provided in the second or third month of pregnancy as part of antenatal care, which is free for all pregnant women in Sweden. Most



п

127

130

130

131

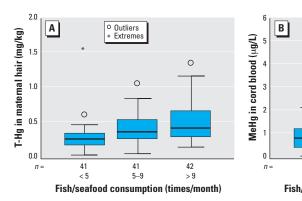
Hair T-Hg, mg/kg

Cord blood MeHg, µg/L

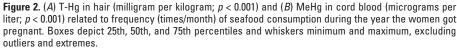
Cord blood I-Hg, µg/L

Cord blood Se, µg/L

Figure 1. Association between MeHg in cord blood (micrograms per liter) and T-Hg in maternal hair (milligram per kilogram) (linear regression: y = 0.35 + 2.73x, $R^2 = 0.53$; p < 0.001).



 $\mathbf{B}^{\mathbf{6}} = \mathbf{B}^{\mathbf{7}} = \mathbf{B}^{\mathbf{7}}$



Maximum

1.5

5.7

0.53

233

of the women (88%) reported that they had received information about the dietary recommendations. In the early 1990s, only about 30% of pregnant women in a Hg exposure study in northern Sweden reported knowledge about the recommendations (Oskarsson et al. 1994), and none of 10 antenatal care clinics in Stockholm that we interviewed in 1991 reported that they informed the visiting pregnant women about the recommendations. Obviously, information and communication have improved significantly over the last decade. Because of the neurotoxic potency of MeHg, especially during fetal development, it is essential that the general public be aware of which fish species tend to have the highest MeHg levels. Concerted attempts should be made to communicate the recommendations to all women of childbearing age so they can decrease their consumption of such fish well in advance of pregnancy. However, it is important to emphasize that fish is also a wholesome part of the diet (Olsen and Secher 2002). The total seafood consumption (almost 7 times/ month or 25 g/day, on average) in the present study was only slightly lower than that reported for Swedish women in a similar age range in a recent national food intake survey (30 g/day) (Becker 1999).

We found increasing T-Hg in hair, but not MeHg in cord blood, with age. Generally, consumption of seafood increases with age among Swedish women (Becker 1994). However, there was no difference in total seafood consumption between older or younger women during pregnancy. It can be speculated that the older women of the present study had a higher seafood intake before pregnancy and decreased their consumption of seafood during pregnancy. Therefore, increasing levels of MeHg with age will only be reflected in hair and not in cord blood.

We noted significantly lower Hg concentrations in the segment of hair corresponding to late pregnancy compared with that from early pregnancy. This fits with the decrease in the consumption of seafood during pregnancy.

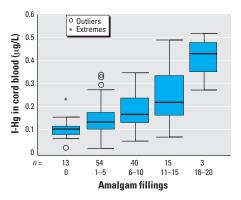


Figure 3. The relationship between I-Hg in cord blood and number of maternal dental amalgam fillings (p < 0.001).

In addition, the transport of MeHg to the growing fetus may contribute to lower maternal hair Hg concentrations in late pregnancy. The segmental hair analysis also revealed that Hg in the 2 cm of hair closest to the scalp and closest to delivery correlated somewhat more closely with cord blood MeHg than did the more distal segments. Cord blood MeHg reflects the exposure during the last months prior to birth, which overlaps with the maternal MeHg exposure in late pregnancy, assuming a half-life of MeHg in blood of approximately 2 months (NRC 2000). Hg in different segments of maternal hair is useful to study fetal MeHg exposure during various stages of fetal development. Hg in maternal hair corresponding to the whole pregnancy period is a useful biomarker of average fetal MeHg exposure.

We found that freshwater fish was rarely consumed (on average, 0.5 g/day during pregnancy), and there was no significant association with the concentrations of Hg in hair or MeHg in cord blood. In this study, we found an association between both hair Hg and cord blood MeHg with the consumption of seafood in general (all types). The most frequently consumed fish species were cod, saithe, and plaice, which generally contain less than 0.2 mg Hg/kg as MeHg (Ohlin 1993). It is difficult to clarify which types of fish contributed most to exposure, as the consumption of different types of fish covaried, namely, those women who consumed much fish in general also ate several different types of fish. It may be worth noting that two women who reported eating canned fish such as tuna, twice a week during pregnancy, had cord blood MeHg concentrations of 2.6 and 2.3 µg/L, i.e., close to the 90th percentile. Their total consumption of seafood was 26 and 36 g/day out of which 81 and 58%, respectively, consisted of canned fish. None of them consumed any freshwater fish. Because some pregnant women did consume fairly large amounts of canned tuna, which is not included in the Swedish dietary recommendations, the concentrations of MeHg in various types of canned tuna need to be investigated.

Further, our results suggest that the concentration of cord blood MeHg increased with the consumption of chicken. It can be speculated that Hg in chicken originates from fishmeal used as a source of protein in animal feed (SJV 1998). Commercial fish feed used in fish farm settings has been found to contain unexpectedly high Hg concentrations (up to 90 µg/L) (Heekyoung Choi and Cech 1998). Whether this is the case for the feed, for example, for chicken and swine, needs to be investigated.

We found fairly low median concentrations of MeHg in cord blood (1.3 μ g/L) and Hg in maternal hair (0.35 mg/kg), about the same as those reported in hair among U.S. women (mean 0.36 mg/kg) of childbearing age

with a common diet including seafood (Smith et al. 1997). However, there was quite a range in the exposure, also seen in U.S. women (Hightower 2003). Although the women were not recruited because of high fish consumption, the maximum concentrations found in this study were 5.7 µg/L in cord blood and 1.5 mg/kg in hair, which is close to the U.S. Environmental Protection Agency reference dose of a daily intake of 0.1 µg MeHg/kg body weight per day. This corresponds to 5.8 µg MeHg/L in cord blood and 1.2 mg T-Hg/kg in hair. The dose is based on a 10% benchmark dose level of 58 µg/L in cord blood and 12 mg/kg in hair, which has recently been considered scientifically justifiable by the National Research Council (NRC 2000). Thus, there seems to be a narrow margin of safety, and it is therefore important to monitor the exposure in women with generally high fish consumption, as well as continue to provide the information to pregnant women via antenatal care clinics. Continuous monitoring of MeHg concentrations in fish and human MeHg exposure is also necessary to reveal trends in exposure and risks for fetal damage. Overall, the results emphasize the need for a global reduction of Hg emissions to the environment.

The intake of Se by Swedish women, 32 µg/day, on average (Becker 1999), is below the recommended intake of 40 µg/day (55 µg/day for pregnant women) (Sandström et al. 1996). Whether this means they are less protected against MeHg toxicity is not known. In the present study, we found a weak positive association between MeHg and Se in cord blood (average 86 µg/L). Associations of MeHg and Se in blood have previously been reported for consumers of much seafood (Muckle et al. 2001; Svensson et al. 1992) as well as in pregnant women with low freshwater fish consumption and their newborns (Ask et al. 2002). The associations found in those studies were explained by the fact that fish is a major source of both MeHg and Se. However, we did not find any association between Se concentrations in cord blood and fish consumption. It should be noted that there was fairly low fish consumption and some women reported taking vitamin and mineral supplements containing Se, which is likely to distort a possible association.

We found a clear increase in fetal I-Hg exposure with increasing number of maternal dental amalgam fillings. Dental amalgam fillings have previously been shown to influence I-Hg in maternal blood (Oskarsson et al. 1996; Vahter et al. 2000), placenta (Ask et al. 2002), fetal blood (Vahter et al. 2000), and fetal tissue (Drasch et al. 1994; Lutz et al. 1996). I-Hg in cord blood (median = 0.15 μ g/L) was even lower than in our previous study of Swedish pregnant women (median = 0.34 μ g/L) (Vahter et al. 2000). Only 14% of

the women had more than 10 dental amalgam fillings, compared with 37% in the previous study, indicating that dental health status is improving and the use of amalgam as dental material is decreasing in line with the national recommendation.

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