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Associations of methylmercury and inorganic mercury between human cord blood and maternal blood: A meta-analysis and its application

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ABSTRACT

Considering the different ability of placental transfer, an assessment of the cord:maternal blood ratio for both methylmercury (MeHg) and inorganic mercury (IHg) is needed especially for interpreting the low-level prenatal exposure. In this study, we conducted a Monte Carlo-based meta-analysis to comprehensively estimate that ratio for MeHg (R_{MeHg}) and IHg (R_{IHg}). The obtained values followed log-normal distributions, with a mean (standard deviation) of 1.89 (0.98) and 1.01 (0.55) for R_{MeHg} and R_{IHg}, respectively. We also estimated the percentage of MeHg in the blood by means of THg in cord and maternal blood using the R_{MeHg} and R_{IHg}, and obtained a value very close to the measured one (relative deviation, −0.4%). In conclusion, the fetus is exposed to approximately twice as much MeHg and to the same level of IHg as in maternal blood; the introduced model provides a rough but reasonable estimate of the percentage of MeHg in the blood.

1. Introduction

Methylmercury (MeHg) in the environment is known to be originated mainly in aquatic ecosystems via methylation of Hg^{2+} by microorganisms; it accumulates in the human body primarily through fish and shellfish consumption (NRC, 2000). Recent studies have also suggested that rice is the major pathway of MeHg exposure for people in inland China (Zhang et al., 2010). MeHg is known for its carcinogenicity, genotoxicity, immunotoxicity, reproductive effects, renal toxicity, cardiovascular effects and hematological effects in humans or animals (NRC, 2000). More importantly, MeHg can readily cross the placental and blood–brain barrier, causing significant damage to the highly susceptible developing brain. Many studies have reported the adverse effects of MeHg on fetal neural development (Karagas et al., 2012; Ramirez et al., 2003; Suzuki et al., 2010), and quantitative dose–response relationship between mercury exposure and IQ was established in previous studies (Axelrad et al., 2007). There was also evidence of adverse effects of mercury on human physical development (Karagas et al., 2012). In 2001, the U.S. Environmental Protection Agency (U.S.EPA) adopted a revised oral reference dose (RfD) of 0.1 μg/kg/day based largely on an epidemiological study carried out in the Faroe Islands, which was derived from a benchmark dose of 58 μg/L mercury in fetal cord blood (NRC, 2000; U.S.EPA, 2001, 2003). Residents in the Faroe Islands were exposed to high levels of mercury as a result of the consumption of large amounts of whale meat and reached a mean concentration of 23 μg/L in cord blood; the mercury was assumed to be primarily in the form of MeHg (Clarkson and Magos, 2006; Grandjean et al., 1992). In the assessment, the U.S.EPA used a one-compartment model to describe the linear relationship between mercury concentration in maternal blood and the maternal intake dose. The U.S.EPA also estimated a cross-study cord:maternal blood mercury ratio of 1.7, but did not apply it to the derivation of the RfD (U.S.EPA, 2001, 2003). Instead, it was considered to be part of the overall adjusting factor of 3 for pharmacokinetic variability and uncertainty in the final estimate of the maternal dose. To analyze the contribution of variability in the cord:maternal ratio to the overall variability in the toxicokinetics of the one-compartment model, Stern and Smith (2003) estimated the ratio distributions for total mercury (THg) and MeHg by means of a meta-analysis of data from previous

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publications. In addition, Stern (2005) conducted a probabilistic estimate of maternal RfD for MeHg using the ratio distribution for THg. The U.S.EPA RfD and Stern's probabilistic RfD distribution both provide important information on risk assessment regarding prenatal MeHg exposure.

However, recent studies on human mercury exposure (e.g. Ding et al., 2013; Guo et al., 2013; Kozikowska et al., 2013) have been mostly focused on THg without mercury speciation, while the RfD for MeHg was still directly used for purposes of health risk assessment. It is believed that MeHg is the primary form of exposure for populations with a blood THg level higher than 2 μg/L (Brune et al., 1991; Stern and Smith, 2003). Thus, placental transfer of inorganic mercury (IHg) was assumed to be negligible for such populations. However, Hg^{2+} is actually also an important form of mercury in the human body, especially in people with limited or no fish consumption. There are three main sources of Hg^{2+} in the human body. The first source is the gastrointestinal absorption. Studies in man (Hattula and Rahola, 1975; Rahola et al., 1973) have found that about 7% of Hg^{2+} in the food can be absorbed from the intestines. The second source is the oxidization of Hg^0. Hg^0 released from dental amalgam or presented in the ambient atmosphere can be absorbed and oxidized in the body, subsequently presenting as Hg^{2+} (Clarkson and Magos, 2006). On average, 75% of the inhaled mercury vapor will be retained in the body (Hursh et al., 1976). The third source of Hg^{2+} is the demethylation of MeHg. A small proportion of MeHg in the body can be demethylated into Hg^{2+} by intestinal microflora, phagocytic cells (Suda et al., 1992, 1993) and liver microsomes (Suda and Hirayama, 1992). Unsurprisingly, some studies (e.g. Ong et al., 1993; Ou et al., submitted for publication; Butler Walker et al., 2006) have reported a relatively low percentage of MeHg in the blood of certain populations. For such populations, the percentage of MeHg in the blood is very informative and necessary when evaluating mercury exposure, because the toxicokinetics and toxicity of mercury species in the human body are significantly different (Clarkson and Magos, 2006). However, the mechanisms of toxic effects of IHg and MeHg are similar, and the high-affinity binding of Hg^{2+} to sulfhydryl or thiol groups is believed to be primarily responsible (ATSDR, 1999). Undeniably, IHg can cross the placenta to a certain extent (Ong et al., 1993; Ou et al., submitted for publication; Butler Walker et al., 2006), and potentially cause damage to the fetus. Even though kidneys are the main targets of toxicity of Hg^{2+}, it can also cause stomatitis, gastroenteritis and autoimmune diseases (Clarkson and Magos, 2006). *In vitro* studies have even found evidence of the detrimental effects on hormone synthesis, even at low Hg^{2+} concentrations (Knazicka et al., 2013).

In this context, if the dominant pathway of mercury exposure is not clear, it seems more reasonable to take MeHg as well as IHg into account when evaluating prenatal mercury exposure, instead of roughly considering THg as MeHg. Thus, we conducted an assessment of the cord:maternal blood ratio for both MeHg (R_{MeHg}) and IHg (R_{IHg}) using a meta-like analysis of previous studies to explore the relationship between the concentrations in cord and maternal blood. Theoretically, the cord:maternal blood ratio for THg (R_{THg}) is controlled by the R_{MeHg}, R_{IHg} and the percentage of MeHg. Therefore, when R_{MeHg} and R_{IHg} were obtained, we made an attempt to estimate the percentage of MeHg in the maternal blood using R_{THg}. In this article, we describe two separately yielded distributions of the R_{MeHg} and R_{IHg}, and a practical model that can be used to estimate the percentage of MeHg in the maternal blood.

2. Materials and methods

2.1. Data acquisition and included studies

To collect data for the meta-analysis, we conducted a search of the peer-reviewed literature published in scientific journals using a Web of Science search for the following keywords: "mercury", "methylmercury", "methyl mercury", "blood", and "cord blood" as well as a review of references therein. Considering the purpose of this analysis, the selected studies should be relevant to the ratio assessment of MeHg or IHg, and have acceptable scientific quality. The selected studies were also required to have sufficient data to provide statistically meaningful results and be comparable across the studies. Additionally, we referred to the selection criteria in the study of Stern and Smith (2003). Together, the following criteria were used to select the studies for the meta-analysis: a) the cord:maternal paired sample size must be >10. This somewhat arbitrary sample size was considered as the minimum needed to derive a reasonable distribution of mercury concentration (Stern and Smith, 2003); b) MeHg and IHg must be measured in whole blood. Mercury concentrations measured in blood components are not comparable with those measured in whole blood, since the concentration of mercury species is different between blood components (U.S.EPA, 1997; WHO, 1990). In any event, most of the studies on prenatal exposure have only reported mercury concentration in whole blood; c) studies must report the R_{MeHg} and/or R_{IHg}, or else the data must be expressed with a central tendency and variability (i.e. mean and SD, geomean and geoSD, or percentiles), as well as the correlation coefficient between the cord and maternal blood, so that we could estimate the ratio distribution; and d) the correlation coefficient between the cord and maternal blood concentrations must be ≥0.3 for MeHg and IHg. Mercury in the cord and maternal blood are well known to be pharmacokinetically linked, and according to Stern and Smith (2003), the lack of such correlation suggests either abnormal components of mercury species in maternal blood or imprecision in the analytical procedure. The correlation is the premise of this assessment. Since the reported blood IHg in the literature tended to have a larger variability than MeHg, and the correlation between cord maternal blood for IHg was not as strong as that for MeHg, here we have used the departure value of 0.3 rather than the stricter value of 0.4 used for MeHg and THg by Stern and Smith (2003). Any study that failed to meet one of the criteria was excluded. Besides, the study of Ask et al. (2002) was excluded because the same data set on blood mercury was used as in another included study (Vahter et al., 2000). Details of the excluded studies were not shown in this paper due to the large number.

Finally, we obtained 10 studies involving MeHg and 5 involving IHg that met all of the selection criteria. These studies represented at least eight and five geographically and ethnically distinct populations for MeHg and IHg, respectively. Four studies (Ou et al., submitted for publication; Ursinyova et al., 2012; Vahter et al., 2000; Butler Walker et al., 2006) had reported ratio distributions calculated using individual cord-maternal blood paired samples for MeHg, and two had reported these distributions for IHg (Ou et al., submitted for publication; Butler Walker et al., 2006). The SD value for R_{MeHg} in the study by Ursinyova et al. (2012) was not shown in the published paper but was obtained by personal communications. Two studies regarding IHg (Morrissette et al., 2004; Ong et al., 1993) did not report the correlation coefficients. However, in order to obtain more than just three studies for the meta-analysis of IHg, we did not exclude them and used a calculated correlation coefficient (a sample size-weighted value calculated from the other three studies) for them in the Monte Carlo simulation. Details of all of the selected studies are presented in Table 1.

2.2. Estimation of ratios

Ratio estimation for individual studies was aimed to obtain both the central tendency and variability of R_{MeHg} and/or R_{IHg}. In light of the results of previous studies (Ou et al., submitted for publication; Stern and Smith, 2003), R_{MeHg} and R_{IHg} were assumed to be log-normally distributed in individual studies. The ratio distributions used in the meta-analysis were acquired in one of the following two ways: a) as reported, when the ratio distributions were available in the literature; and b) using Monte Carlo simulation, when mercury concentrations in the maternal and cord blood and their correlation coefficients were available. Briefly, in the Monte Carlo simulation, raw data with a central tendency and the variability in mercury concentrations in both cord and maternal blood were fitted into two correlated log-normal distributions. Then, the simulated paired samples that were constrained by the reported correlation coefficient were randomly drawn from these two distributions; a ratio was then calculated for each pair. For studies that had reported the ratios, we also simulated the ratio distributions when the required data were available, and validated the simulation method by comparing the reported and simulated values. To minimize the variability caused by simulation, we calculated the result for every individual study by averaging five simulations with 5000 iterations each. Monte Carlo simulation was carried out using the Crystal Ball software (version 7.3.1, Oracle Crystall Ball, Oracle USA, Inc.).

In the meta-analysis, we assumed that each study yielded a sample of the underlying common ratio distribution to all populations for both MeHg and IHg in accordance with Stern and Smith (2003). In this way, the reliability of each study was decided by its sample size compared to the total sample size of all studies and thus the number of sampling times for each study was in proportion to its sample size. The meta-analysis based on this sampling method was defined as n-weighted. The total number of iterations for the meta-analysis in this study was 10,000. Alternatively, we also conducted a meta-analysis to estimate the ratio distribution with an equal weight to each study which was defined as unweighted, considering the possibility that an underlying common distribution may not exist. In this way,

Table 1
Studies that were included in the meta-analysis.

Study	Mecury species	Population		N[a]	Maternal blood (mean ± SD)	Cord blood (mean ± SD)	Cord-maternal correlation
Ong et al., 1993	IHg	Singapore		30	10.1 ± 5.7 μg/L	10.2 ± 5.8 μg/L	0.73[b]
Vahter et al., 2000	IHg	Sweden		79	0.43 ± 0.41 μg/L[c]	0.37 ± 0.20 μg/L[c]	0.68
Butler Walker et al., 2006	IHg	Arctic Canada	*Caucasian*	96	0.54 ± 0.38 μg/L	0.52 ± 0.36 μg/L	0.64
			Dene/Metis	63	0.68 ± 0.54 μg/L	0.54 ± 0.33 μg/L	0.73
			Inuit	107	1.09 ± 0.84 μg/L	1.23 ± 1.00 μg/L	0.89
Morrissette et al., 2004	IHg	United States		92	0.30 ± 0.26 μg/L[c]	0.23 ± 0.20 μg/L[c]	0.73[b]
Ou et al., submitted for publication	IHg	China		42	1.14 ± 0.68 μg/L	0.84 ± 0.45 μg/L	0.359
Ong et al., 1993	MeHg	Singapore		29	5.46 ± 4.59 μg/L	8.82 ± 5.39 μg/L	0.44
Vahter et al., 2000	MeHg	Sweden		79	0.97 ± 0.54 μg/L	1.75 ± 1.05 μg/L	0.78
Butler Walker et al., 2006	MeHg	Arctic Canada	*Caucasian*	87	0.76 ± 0.78 μg/L	1.27 ± 1.35 μg/L	0.72
			Dene/Metis	73	1.05 ± 0.90 μg/L	1.67 ± 1.67 μg/L	0.74
			Inuit	125	4.32 ± 4.72 μg/L	9.73 ± 10.27 μg/L	0.94
Tsuchiya et al., 1984	MeHg	Japan		221	9 ± 5 μg/L	14 ± 9 μg/L	0.59
Hansen et al., 1990	MeHg	Greenland Inuit		37	38.1 ± 22 μg/L	80.2 ± 52.2 μg/L	0.8
Nishima et al., 1977	MeHg	Japan		48	6 ± 3 ng/g	13 ± 6 ng/g	0.74
Sakamoto et al., 2007	MeHg	Japan		115	5.33 ± 2.61 ng/g[c]	10.79 ± 6.14 ng/g[c]	0.888
Morrissette et al., 2004	MeHg	United States		92	0.29 ± 0.27 μg/L[c]	0.49 ± 0.43 μg/L[c]	0.72
Ou et al., submitted for publication	MeHg	China		42	1.11 ± 0.47 μg/L	2.11 ± 0.91 μg/L	0.678
Ursinyova et al., 2012	MeHg	Slovakia		75	0.22 μg/L (ND-1.17 μg/L)[d]	0.32 μg/L (0.10–1.56 μg/L)[d]	0.645

NA not available; ND not detectable.
[a] Number of reported maternal and cord pairs.
[b] Calculated correlation coefficient.
[c] Estimated values using Crystal Ball with reported distributions.
[d] Median (range).

the number of sampling times for every study was the same. A comparison of the results from these two meta-analyses was conducted to acquire information regarding the sensitivity of data selection.

2.3. Estimation of MeHg percentage and validation

For an individual, the cord:maternal ratio for THg can be calculated using the following equation:

$$R_{THg} = P_{m.MeHg} \times R_{MeHg} + (1 - P_{m.MeHg}) \times R_{IHg}$$

Where R_{THg}, R_{MeHg} and R_{IHg} are the cord:maternal blood ratios for THg, MeHg and IHg, respectively; $P_{m.MeHg}$ is the percentage of MeHg in the maternal blood.

Thus, the percentage of MeHg in the maternal blood can be calculated as follows:

$$P_{m.MeHg} = (R_{THg} - R_{IHg})/(R_{MeHg} - R_{IHg})$$

For a given population, as long as we can obtain the R_{MeHg} and R_{IHg} distributions, and there exists a significant difference between them, theoretically we are able to estimate the percentage of MeHg in the maternal or cord blood using the R_{THg} by means of Monte Carlo simulation. Unfortunately, since the three variables (i.e. R_{THg}, R_{MeHg} and R_{IHg}) are mutually correlated and constrained, we were unable to put those estimated distributions into the model by Monte Carlo sampling, and could only use a single value to represent each distribution. In this way, variations of the ratios were ignored. Data from our manuscript under review (Ou et al., submitted for publication) were used to validate the model, since R_{THg}, R_{MeHg} and R_{IHg} were available for individual subjects. We chose a certain value (e.g. central tendency) from each ratio distribution for the model. To validate the model as well as identify the effects of the selected values, we conducted a sensitivity analysis by calculating the MeHg percentages in the maternal blood using the mean of R_{THg} (1.31) with different values of R_{MeHg} and R_{IHg}; the results were compared with the mean of the measured percentages (49.6%). R_{MeHg} or R_{IHg} was chosen using percentiles of the distribution obtained from the *n*-weighted meta-analysis with the other one serving as a constant (the median of its distribution).

3. Results and discussion

Table 2 presents all of the estimated cord:maternal blood ratios obtained using the Monte Carlo simulation and the available reported values. Means of the estimated ratios varied between 0.90–1.24 and 1.69–2.39 for IHg and MeHg, respectively. No significant correlation was observed across studies between the ratio of MeHg or IHg and the concentration in cord or maternal blood. The relative deviations of means and SDs between the reported and estimated values were 2–17% and −6–11% for IHg, and −0.5–14% and −30–33% for MeHg, respectively. The largest deviations were from the study of Butler Walker et al. (2006), but the reasons for that discrepancy were unclear since no data for individuals were available. The estimated ratios in other studies showed quite close agreement with their reported values, and the relative deviations were −4–8% (Ou et al., submitted for publication; Vahter et al., 2000). In total, this consistency suggested we had obtained a reasonable central tendency and variability regarding R_{MeHg} and R_{IHg} for individual studies using the Monte Carlo simulation.

Table 2
The calculated (the average of five simulations of 5000 trials each) and available reported cord:maternal blood ratios for MeHg and IHg.

Study	Mercury species	Calculated ratio			Reported ratio	
		Mean	SD	CV	Mean	SD
Ong et al., 1993	IHg	1.09	0.42	0.39	NA	NA
Vahter et al., 2000	IHg	1.24	0.74	0.59	NA	NA
Butler Walker et al., 2006	IHg					
Caucasian		1.11	0.62	0.56	0.95	0.56
Dene/Metis		0.96	0.47	0.49	0.94	0.50
Inuit		1.16	0.38	0.32	1.10	0.36
Ou et al., submitted for publication	IHg	0.90	0.58	0.64	0.83	0.60
Morrissette et al., 2004	IHg	0.90	0.48	0.54	NA	NA
Ong et al., 1993	MeHg	2.29	1.75	0.77	NA	NA
Vahter et al., 2000	MeHg	1.88	0.68	0.36	1.89	0.71
Butler Walker et al., 2006	MeHg					
Caucasian		1.99	1.37	0.69	1.75	1.03
Dene/Metis		1.73	1.04	0.60	1.56	0.92
Inuit		2.39	0.71	0.30	2.11	1.02
Tsuchiya et al., 1984	MeHg	1.69	0.89	0.53	NA	NA
Hansen et al., 1990	MeHg	2.15	0.77	0.36	NA	NA
Nishima et al., 1977	MeHg	2.31	0.76	0.33	NA	NA
Sakamoto et al., 2007	MeHg	2.01	0.47	0.23	NA	NA
Morrissette et al., 2004	MeHg	1.95	1.14	0.58	NA	NA
Ou et al., submitted for publication	MeHg	2.00	0.65	0.33	1.91	0.66
Ursinyova et al., 2012	MeHg	NA	NA	NA	1.80	1.27

SD Standard deviation.
CV Coefficient of variation (mean/SD).
NA Not available.

Table 3
The cord:maternal blood ratios for MeHg and IHg derived from meta-analyses (calculated values are the average of five simulations).

	MeHg studies		IHg studies	
	n-Weighted meta-analysis	Unweighted meta-analysis	*n*-Weighted meta-analysis	Unweighted meta-analysis
Mean	1.89	1.96	1.01	1.01
SD	0.98	1.04	0.55	0.55
CV	0.52	0.53	0.54	0.54
25th percentile	1.24	1.29	0.65	0.63
50th percentile	1.72	1.79	0.91	0.90
75th percentile	2.32	2.38	1.25	1.25
90th percentile	3.04	3.13	1.67	1.67
95th percentile	3.63	3.73	1.99	2.01

R_{MeHg} and R_{IHg} distributions derived from the meta-analyses are presented in Table 3. The unweighted meta-analysis resulted in slightly larger mean and SD values for MeHg than the *n*-weighted meta-analysis, while both yielded very close mean and SD values for IHg. In accordance with a previous study regarding THg and MeHg (Stern and Smith, 2003), the meta-analysis derived distributions for both MeHg and IHg in the present study fitted well with log-normal distributions. A comparison of the estimated MeHg percentages in maternal blood with the mean of the measured values (49.6%) is presented in Table 4. The estimated MeHg percentage using the medians of R_{MeHg} and R_{IHg} distributions was very close to the measured percentage, with only a relative deviation of −0.4%. The model output decreased with the increase of either of the ratio values. The estimated MeHg percentage varied more dramatically when the R_{IHg} was fixed which indicates the output was more sensitive to R_{MeHg}.

The R_{MeHg} obtained in this meta-analysis was greater than the value of 1.0 assumed by the U.S. National Research Council (NRC, 2000), indicating that the MeHg concentration is significantly elevated in cord blood during pregnancy. MeHg bound to thiol-groups in neutral amino acid carriers (e.g. cysteine) can be readily transported across the placenta to the fetus (Kajiwara et al., 1996). Specifically, the elevated MeHg concentration in cord blood can mainly be explained as follows: a) MeHg exists in the blood primarily bound to hemoglobin, which is more concentrated in the cord blood (Doi et al., 1984); b) absence or reduced affinity of the neutral amino acid carrier on the fetal side of the placenta leads to a one-way placental transfer of MeHg to cord blood (Stern and Smith, 2003), and additionally the fetus appears to have little capacity to demethylate MeHg (Dock et al., 1994; Nordenhill et al., 1995), which results in a longer half-live of MeHg in cord blood; and c) MeHg in maternal blood decreases during pregnancy due to hemodilution or other physiological changes (Tan and Tan, 2013). Because the half-life of MeHg in cord blood is longer, the MeHg level in cord blood is influenced by the higher maternal blood MeHg concentration in earlier pregnancy. Even though we used a more comprehensive data set in this meta-analysis than Stern and Smith (2003), the R_{MeHg} distribution obtained was very close to their value, showing that both data sets were possibly sufficient to represent the human population. In any event, the significantly elevated MeHg concentration in the cord blood should be of great concern considering the high susceptibility of the developing fetus; the ratio distribution for MeHg obtained in the present study can be used in future studies to describe the relationship between MeHg concentration in the cord and maternal blood.

Table 4
Estimated MeHg percentages in the maternal blood using selected MeHg and IHg ratio percentiles and a comparison with the measured value (49.6%).

Percentiles	R_{MeHg}	R_{IHg}	Percentage[a]	Relative deviation[a]	Percentage[b]	Relative deviation[b]
30th	1.30	0.68	102.6%	106.8%	60.6%	22.1%
40th	1.48	0.78	70.2%	41.5%	56.4%	13.7%
50th	1.72	0.91	49.4%	−0.4%	49.4%	−0.4%
60th	1.90	1.01	40.4%	−18.5%	42.3%	−14.8%
70th	2.17	1.16	31.7%	−36.0%	26.8%	−46.0%

Relative deviation was calculated by (estimated percentage-49.6%) / 49.6%.
[a] calculated with $R_{IHg} = 0.91$.
[b] calculated with $R_{MeHg} = 1.72$.

The ratio distribution for IHg obtained in the present study validated the placental transfer of Hg^{2+}, at least in humans, even at low concentrations. The level of Hg^{2+} in the cord blood is almost as high as in the maternal blood. In contrast, animal studies have suggested that Hg^{2+} seems only to be transferred to the fetus via the placenta at high concentrations (Dencker et al., 1983; Dock et al., 1994); thus, Vahter et al. (2000) and Ask et al. (2002) considered Hg^0 as the primary form of IHg in the cord blood that passes the placenta before oxidization. However, we obtained a R_{IHg} of approximately 1.0 for all of the five included studies on different IHg levels, with only Vahter et al. (2000) reporting a notable percentage of subjects using dental amalgam but no special pathway of Hg^0 exposure reported in other studies. Whether or not mercury vapor from amalgam is oxidized, or to what extent, before crossing the placenta is still unclear. However, we did obtain the highest mean value of the IHg ratio for the study by Vahter et al. (2000) among the five included studies; this was in accordance with previous findings on animals that Hg^0 from amalgam can readily cross the placenta (Pamphlett and Kum-Jew, 2001; Takahashi et al., 2001; Warfvinge, 2000).

Even though the placental transfer exists, both MeHg and IHg accumulate in the placenta (Ask et al., 2002; Ou et al., submitted for publication); however, whether or not mercury in the placenta causes any adverse effects in humans is not clear. A recent study (Abdelouahab et al., 2010) found that maternal mercury was negatively correlated with placenta monoamine oxidase (MAO) activity in subjects with low MAO activity, which can potentially influence further brain development in the fetus (Beyrouty et al., 2006). *In vitro* studies have found that Hg^{2+} can affect placental activities, such as amino acid transfer, placental oxygen consumption (Urbach et al., 1992), hormonal secretion, and enzyme activity (Boadi et al., 1992a, 1992b). Animal studies have also reported the effects of MeHg in the placenta regarding the activity of selenoenzymes (Watanabe et al., 1999), which would presumably decrease the protection for the fetus from the oxidative stress produced during the MeHg metabolism (Yee and Choi, 1996) and lead to neurotoxicity (Farina et al., 2011).

With regard to the utterly different toxicokinetics and toxicity of MeHg and IHg in the fetus and the significantly different cord:-maternal blood ratios, it is necessary to differentiate these two ratios when interpreting prenatal mercury exposure. Stern and Smith (2003) reported a significant difference between the ratios for MeHg and THg in a similar meta-analysis even though the

authors tried to minimize the effect of IHg by selecting studies on populations with relatively higher mercury exposure, and thus, to a certain degree, their results confirmed the important role of IHg in the placental transfer of total mercury. The differentiation is necessary especially for low-level exposed populations with limited or no fish consumption and relatively low MeHg percentage, so that R_{THg} can reflect the percentage of mercury species in blood. For example, Ou et al., submitted for publication reported a small R_{THg} (1.30 ± 0.44), in accordance with a small percentage of MeHg in the maternal blood (49.6%). Butler Walker et al. (2006) also reported small THg ratios for the *Caucasian* and *Dene/Metis* groups (1.34 ± 0.58 and 1.26 ± 0.46, respectively), but a relatively larger ratio for *Inuit* group (1.73 ± 0.45). The higher placental transfer of THg indicates a larger proportion of MeHg in the maternal blood. Roughly estimated from the given concentration values, the percentage of MeHg in the maternal blood of the *Inuit* group was about three times higher than that of the *Caucasian* and *Dene/Metis* groups. Further, even for the relatively high-level dosed populations, the THg ratio can also be an informative parameter since it is not always true that IHg can be ignored in such populations. For instance, Ong et al. (1993) reported a THg level of 15.8 ± 6.9 μg/L in the maternal blood of ordinary pregnant residents in Singapore, but the percentage of MeHg was surprisingly small, which was confirmed by the small estimated THg ratio (1.28 ± 0.50).

The close agreement between the estimated and reported values of two studies regarding IHg (Ou et al., submitted for publication; Butler Walker et al., 2006) and three studies regarding MeHg (Ou et al., submitted for publication; Vahter et al., 2000; Butler Walker et al., 2006) indicated that ratio distributions can be effectively obtained using Monte Carlo simulation. The reported mean and SD were almost the same as the estimated ones for IHg in the studies of Ou et al., submitted for publication and Butler Walker et al. (2006, *Dene/Metis* and *Inuit group*), and for MeHg in the studies of Ou et al., submitted for publication and Vahter et al. (2000). But other estimated values showed a discrepancy of up to about 17% and 30% in the mean and SD, respectively. This was presumably due to a discrepancy in the raw data and the distributions used in the Monte Carlo sampling. However, more specific reasons are not clear to us. In addition, the estimated means ($n = 9$) of R_{MeHg} and R_{IHg} were slightly higher than the reported ones, and the difference was statistically significant. If this was a bias caused by the simulation method, the yielded ratios should have been slightly overestimated.

The small difference between the *n*-weighted and unweighted results for MeHg indicated the potential influence of study selection on the results of the meta-analysis. However, we obtained almost the same distribution for the MeHg ratio (1.89 ± 0.98) as Stern and Smith (2003), even though we included five more recent studies (Morrissette et al., 2004; Ou et al., submitted for publication; Sakamoto et al., 2007; Ursinyova et al., 2012; Butler Walker et al., 2006) in the meta-analysis. This close agreement in turn confirmed the accuracy of the R_{MeHg} estimation. There was almost no difference between the *n*-weighted and unweighted result for IHg. This implied that the variance in the IHg ratios of different populations is small, and the estimated distribution should be very close to the real one. However, this was potentially affected by the fact that we used a calculated correlation coefficient from three studies for the other two.

Once R_{MeHg} and R_{IHg} are treated as constants, the percentage of MeHg is the only variable controlling R_{THg}. Data from our manuscript under review (Ou et al., submitted for publication) showed a significant correlation between the ratios for THg and MeHg percentages in the maternal blood (Spearman's $r = 0.561$; $p = 0.000$; $n = 41$). Unfortunately, we had to ignore all variations in the distributions, since we could not realize their mutual correlations in a randomized sampling. In the validation study, as expected, ratios for THg and MeHg were significantly correlated ($r = 0.373$; $p = 0.023$; $n = 37$), and this was also observed for THg and IHg ($r = 0.705$; $p = 0.000$; $n = 37$). As a compromise, we suggest using median values of R_{MeHg} and R_{IHg} distributions in the model, since it resulted in a very close estimated value to the measured one in the model validation. The method of using a single value to represent each distribution in the model is similar to the method that the U.S.EPA used to derive the RfD from the one-compartment model. In that deviation, only the central tendency of each parameter was used, and an uncertainty factor for the variability of all parameters was applied. Even so, we do not recommend estimation for individual subjects, because the notable variation in individual THg ratios caused by the ignored variation in R_{MeHg} and R_{IHg} in the model would lead to some abnormal results; specifically, when the function "$P_{m.MeHg} = (R_{THg} - R_{IHg}) / (R_{MeHg} - R_{IHg})$" is used, THg ratios smaller than 1.01 or larger than 1.89 would lead to a calculated percentage <0 or >1, respectively. However, if R_{MeHg} and R_{IHg} distributions are used in the model for an individual subject, we can estimate a probabilistic distribution of the MeHg percentage in the blood. After all, the model provides a rough but reasonable estimation of the mean MeHg percentage in the maternal blood by means of the mean THg ratio, and is more practical for a certain population with a small variation in the MeHg percentage in individuals. In this way, we are able to acquire information regarding how much mercury is in the methyl form, even when only THg concentrations were measured in the maternal and cord blood, and thus more effectively interpret prenatal mercury exposure and the health risk. Only one validation study may not be quite sufficient, and we suggest using data from other appropriate studies to validate or modify this approach in the future.

4. Conclusion

The placental barrier hardly exists for both MeHg and IHg, with the concentration of MeHg in the cord blood being about twice that in the maternal blood, and the IHg level being about the same as that in maternal blood. The introduced model provides a rough but reasonable estimate of the percentage of MeHg in the maternal blood via the R_{THg} for a population. In future studies on prenatal mercury exposure, the placental transfer of maternal IHg to the fetus as well as that of MeHg should be considered, especially for populations with relatively low MeHg exposure.

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