An Assessment of the Cord Blood:Maternal Blood Methylmercury Ratio: Implications for Risk Assessment

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In the current U.S. Environmental Protection Agency reference dose (RfD) for methylmercury, the one-compartment pharmacokinetic model is used to convert fetal cord blood mercury (Hg) concentration to a maternal intake dose. This requires a ratio relating cord blood Hg concentration to maternal blood Hg concentration. No formal analysis of either the central tendency or variability of this ratio has been done. This variability contributes to the overall variability in the dose estimate. A ratio of 1.0 is implicitly used in the model, but an uncertainty factor adjustment is applied to the central tendency estimate of dose to address variability in that estimate. Thus, incorporation of the cord:maternal ratio and its variability into the estimate of intake dose could result in a significant change in the value of the RfD. We analyzed studies providing data on the cord:maternal blood Hg ratio and conducted a Monte Carlo–based meta-analysis of 10 studies meeting all inclusion criteria to generate a comprehensive estimate of the central tendency and variability of the ratio. This analysis results in a recommended central tendency estimate of 1.7, a coefficient of variation of 0.56, and a 95th percentile of 3.4. By analogy to the impact of the similar hair:blood Hg ratio on the overall variability in the dose estimate, incorporation of the cord:maternal ratio may support a 3-fold uncertainty factor adjustment to the central tendency estimate of dose to account for pharmacokinetic variability. Whether the information generated in this analysis is sufficient to warrant a revision to the RfD will depend on the outcome of a comprehensive reanalysis of the entire one-compartment model. We are currently engaged in such an analysis. *Key words:* **blood, cord blood, fetus, maternal, mercury, methylmercury, ratio, reference dose, RfD.** *Environ Health Perspect* **111:1465–1470 (2003). doi:10.1289/ehp.6187 available via** *http://dx.doi.org/* **[Online 19 May 2003]**

The U.S. Environmental Protection Agency (U.S. EPA) recently revised its reference dose (RfD) for methylmercury (MeHg; U.S. EPA 2001, 2003). The revision is based largely on analyses described in a 2000 report by the National Research Council (NRC 2000), a body of the National Academy of Sciences. Although the revision does not reflect a change in the numerical value of the RfD (it remains 0.1 μg/kg/day), it does represent a change in the basis. The revised RfD is now based largely on epidemiologic data from a study of fetal MeHg exposure resulting from maternal intake of whale meat and fish for a cohort of children from the Faroe Islands (NRC 2000; U.S. EPA 2001, 2003). Benchmark dose analyses were performed on a battery of neuropsychologic tests of cognitive function administered at 7 years of age to identify a point of departure for RfD derivation. The primary measure of exposure was total mercury (Hg) levels in umbilical cord blood (i.e., μg Hg/L blood). The RfD, however, is, by definition, a dose (i.e., micrograms MeHg per kilogram body weight per day)—in this case the maternal intake dose of MeHg. To derive an RfD from a benchmark dose based on cord blood data, it is necessary to estimate an empirical factor that relates the cord blood Hg concentration to a corresponding maternal blood Hg concentration; the latter can then be used in the one-compartment pharmacokinetic model employed by the U.S. EPA to estimate an associated steady-state dose. This exercise is analogous to the need to relate hair Hg levels to maternal blood levels, as was done in the derivation of the prior RfD (U.S. EPA 1997).

The relationship between cord blood and maternal blood can be expressed as the ratio Hg_c:Hg_m, where c and m designate cord blood and maternal blood, respectively. This cord blood:maternal blood Hg concentration ratio (henceforth, the cord:maternal ratio), as well as the other factors in the one-compartment pharmacokinetic model, describes a property whose values differ among individuals in a heterogeneous population. For example, Vahter et al. (2000) found individual ratios ranging from 0.4 to 4.9; Dennis and Fehr (1975) found ratios ranging from 0.6 to 5.7; and Fujita and Takabatake (1977) found ratios of 0.8–4.4. The cord:maternal ratio can therefore be described in terms of its central tendency estimate and the variability about that central tendency. Although there have been some efforts to estimate a central tendency value of the cord:maternal ratio, little effort has gone into describing its variability. Based on a partial review of the published literature for several populations, the NRC report concluded that the central tendency for cord:maternal ratio was 1.2–1.3 at most. On the basis of a more comprehensive review of 21 studies reporting cord-blood and maternal-blood data, the U.S. EPA (2001, 2003) estimated a cross-study central estimate for the cord:maternal ratio of 1.7. The U.S. EPA (2001, 2003) estimated a 5th–95th percentile range in the ratio of 3.5. In revising the RfD, however, the U.S. EPA chose not to make a numerical adjustment in the dose conversion based on a revised estimate of the central tendency of the ratio, implicitly applying a ratio of 1.0. Instead, the agency chose to consider such differences as additional aspects of toxicokinetic variability and uncertainty to be included in the determination of the overall uncertainty factor for the estimate of maternal dose (U.S. EPA 2001, 2003). The NRC (2000) recommended that the overall variability in the model relating maternal blood Hg to the maternal MeHg dose be accounted for by applying a factor of 2 for toxicokinetic variability to the central tendency estimate of maternal dose (Clewell et al. 1999; NRC 2000; Stern 1997; Stern et al. 2002; Swartout and Rice 2000). The U.S. EPA (2001, 2003) applied an overall factor of 3 for toxicokinetic variability to the central tendency estimate of maternal dose, in contrast to the NRC's factor of 2, apparently, in part, to account for the added variability associated with the cord:maternal ratio.

A formal probabilistic analysis of the contribution of variability in the cord:maternal ratio to overall variability in toxicokinetics as estimated with the one-compartment pharmacokinetic model has yet to be done. The first step to undertaking such an analysis is to generate a statistical distribution describing both the central tendency and population variability about the central tendency of this ratio. In this article, we describe such a distribution derived from a formal meta-like analysis of the published studies. Importantly, because the relationships between the RfD and the maternal intake dose, and between the maternal intake dose and the fetal blood concentration, are all assumed to be linear, a significant departure from the assumption of central tendency value for cord:maternal ratio of 1.0 would have an

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equally significant effect on the estimate of a maternal dose. It might also have a similar effect on the RfD, depending on the outcome of a probabilistic analysis.

Materials and Methods

Criteria for selection of studies. We conducted a search of the peer-reviewed literature to identify reports providing data on Hg and/or MeHg concentration in maternal and fetal cord blood. In conducting an assessment that employs multiple independent sources of data, several basic requirements must be met: The studies should all be of a minimum acceptable overall scientific quality; the studies should all contain data that are relevant to the assessment; the data should be sufficiently robust to support statistically meaningful conclusions; and the data should be collected and expressed in a manner that allows comparability across studies. To ensure that these requirements were met, we reviewed the reports according to the following specific *a priori* criteria:

a) If data are reported in terms of total Hg, the mean concentration in the sample population must be > 2.0 ppb to minimize significant contributions to the cord:maternal ratio from background sources of inorganic Hg unrelated to MeHg. Populations with little or no fish consumption appear to have characteristic blood Hg concentrations of about 2 ppb or less (Brune et al. 1991). In the absence of significant fish consumption, total Hg concentration in blood will likely reflect a significant contribution from inorganic Hg from the diet and/or from dental amalgams.

b) The total sample size must be > 10. Although this value is somewhat arbitrary, it can be viewed as an approximate minimum value consistent with deriving a reasonable estimate of the mean and standard deviation (SD) of a population distribution.

c) The correlation (r) between maternal and cord blood Hg (or MeHg) concentration must be ≥ 0.4. The underlying premise of this assessment is that the MeHg-derived cord and maternal blood Hg concentrations both originate from maternal MeHg, and both concentrations are pharmacokinetically linked. This implies that a reasonable correlation should exist between these two parameters. The lack of such a correlation in a study suggests either that other (i.e., non-MeHg) sources of Hg incapable of readily crossing the placenta significantly contribute to the maternal blood Hg concentration or that the analytical procedure employed in that study was insufficiently precise.

d) The Hg (or MeHg) concentration must be measured in whole blood. The pharmacokinetic model predicts concentration in whole blood. Concentrations of Hg in individual blood components cannot readily be recombined in a manner that provides useful information on the variability in concentration in the whole blood.

e) At a minimum, studies must provide data on the mean and SD of the concentration of Hg or MeHg in maternal and cord blood, and the correlation coefficient relating the two parameters.

Estimation of ratio parameters. The goal of this analysis is to estimate not only the central tendency of the cord:maternal ratio but also the distribution of the ratio. It was therefore necessary to use the reported or simulated values of individual paired cord and maternal concentrations rather than the summary mean cord and maternal concentrations from studies. Given the non-normal and correlated distribution of cord and maternal blood Hg concentrations, the ratio of mean cord and maternal values will not yield a reliable estimate of the true mean of the distribution of the ratio. Therefore, the parameters of statistical distributions for the cord:maternal ratio were estimated in one of three ways: *a*) as reported, if statistics were provided for the ratio; *b*) by calculation, when cord and maternal Hg concentration data were reported for each mother–child pair; and *c*) by simulation when only summary statistics and the correlation coefficient were reported for the cord and maternal blood Hg levels. Only one study reported the mean and SD of the cord:maternal ratio (Soria et al. 1992). For three studies (Dennis and Fehr 1975; Fujita and Takabatake 1977; Vahter et al. 2000), cord and maternal Hg concentrations were available for each mother–child pair and could therefore be used to calculate the ratio. The data corresponding to Vahter et al. (2000) were supplied by the authors. Dennis and Fehr (1975) presented data for the northern Saskatchewan cohort as a scatter plot (their Figure 1). The figure was scanned as a JPEG file using ArcMap to obtain numerical values for each cord and maternal pair (Dennis and Fehr 1975).

Most of the studies meeting the selection criteria only reported the mean, SD, and correlation coefficient for maternal and child blood Hg levels. In such cases, the joint distribution of maternal and child blood Hg levels was simulated using Monte Carlo sampling. In brief, this approach draws simulated paired samples of cord blood and maternal blood Hg from prespecified statistical distributions of each parameter. The statistical distributions were initially assumed to be log-normal in shape based on examination of those data sets where raw data were available. The mean and SD of each distribution were specified by the respective reports, as was the correlation coefficient. The samples of cord and maternal blood Hg concentration were randomly drawn from their respective distribution, except that the relationship between the cord and maternal values in each pair was constrained by the reported correlation coefficient. A ratio value was calculated for each sampled maternal–cord data pair, and the overall estimate from each study was calculated from the simulated ratio values across all samples. Monte Carlo simulation was carried out using @RISK, version 3.5.2 (Palisade Corp., Newfield, NY). Because the distributional parameters reported for the sample populations were assumed to provide a reasonable estimate of the underlying population, the number of simulation samples of each distribution was not constrained by the original sample size but was selected so as to give a reasonable simulation of the maternal, cord, and ratio distributions.

For the analysis of the individual studies, the distributions were sampled with 5,000

Table 1. Studies included.

Study	Population	Sample size	Mercury species analyzed	Cord blood parameters (mean ± 1 SD)	Maternal blood parameters (mean ± 1 SD)	Cord–maternal correlation
Lauwreys et al. 1978	Belgium	468	Total Hg	1.42 ± 0.85 mg/100 mL	1.26 ± 0.69 mg/100 mL	0.62
Vahter et al. 2000	Solna, Sweden	79[a]	MeHg	1.75 ± 1.05 mg/L	0.97 ± 0.54 mg/L	0.77
Tsuchiya et al. 1984	Nagoya City, Japan	221	MeHg	14 ± 9 mg/L	9 ± 5 mg/L	0.59
Bjerregaard and Hansen 2000	Greenland Inuit	178	Total Hg	35.6 ± 32.1 mg/L	16.8 ± 13.6 mg/L	0.84
Hansen et al. 1990	Greenland Inuit	37	MeHg	80.2 ± 52.2 mg/L	38.1 ± 22 mg/L	0.8
Nishima et al. 1977	Tokyo, Japan	48	MeHg	13 ± 6 ng/g	6 ± 3 ng/g	0.74
Ong et al. 1993	Singapore	29	MeHg	8.82 ± 5.39 mg/L	5.46 ± 4.59 mg/L	0.44
Soong et al. 1991	Taiwan	85	Total Hg	28.8 ± 26.46[b] mg/L	19.4 ± 13.83[b] mg/L	0.75
Soria et al. 1992	Seville, Spain	18[c]	Total Hg	5.25 ± 2.83 mg/L	4.97 ± 1.87 mg/L	0.53
Dennis and Fehr 1975	Northern Saskatchewan	41[d]	Total Hg	26.66 ± 23.41 mg/L	15.33 ± 14.24 mg/L	0.88

[a]Matched samples for maternal blood at 36 weeks. [b]SD calculated from reported SE. [c]Cord venous blood. [d]Forty-three mother–child pairs are documented, but only 41 observations were generated from the scanned figure.

iterations because we found this to give a reproducible estimate of the ratio parameters. Empirical distributions and distributions generated by sampling were assessed for log-normality using BestFit, version 2.0d (Palisade Corp.). Additional statistical analysis was carried out using Statistica, release 5.5A (Statsoft Inc., Tulsa, OK). For the comparison of ratio values from studies measuring MeHg only, and studies measuring total Hg only, the number of sampling iterations of studies for which raw data were not available was equal to the number of paired observations reported for that study

Validation studies. We investigated the validity of the assumptions and methods employed in the simulation of the cord:maternal ratio and its statistical parameters using four studies that provided direct information on the cord:maternal ratio. Three of these studies (Dennis and Fehr 1975; Soria et al. 1992; Vahter et al. 2000) met the selection criteria. A fourth study not meeting the selection criteria, by Fujita and Takabatake (1977), was nonetheless used to assess the simulation approach. We compared the reported or calculated values of the ratios from these four studies with the values estimated using the Monte Carlo simulation approach. This comparison addressed both the assumption of the log-normal shape of the distributions and the simulation methodology. In addition, we investigated the assumption of log-normality in the study by Vahter et al. (2000) directly by comparing the empirical distribution of the cord and maternal blood Hg concentrations to the best-fitting log-normal distribution based on maximum likelihood estimation.

Meta-analysis. To employ information about the cord:maternal blood Hg ratio in a risk-based probabilistic assessment of the MeHg RfD, it is necessary to derive a summary expression of the population distribution of the ratio. Our primary approach for combining the data from all included studies to provide such a summary expression was based on the assumption that there is an underlying distribution of ratio values common to all populations, and that each study yields a sample of that common distribution. Under this assumption, the reliability of an estimate from any given study is largely a function of the sample size (n) of that study. Each study is therefore sampled in proportion to its sample size (n-weighted). As an alternative approach, we also considered the possibility that different populations do not necessarily have the same underlying distribution for the cord:maternal blood Hg ratio, and that each study therefore yields a ratio distribution specific to the population under study. Under this assumption, each study is an independent observation, and all studies are therefore given equal weight. Also inherent in this approach is the notion that the available data represent a random sample from among the various populations contributing to the heterogeneous target population for the RfD. For meta-analysis using this approach, the total number of sampling iterations was equal to 5,000 times the number of pooled studies in each analysis. Meta-analyses for the total Hg-only and MeHg-only studies were conducted to examine the possibility that the type of Hg included in the measurements (i.e., MeHg only vs. total Hg) influenced the resulting ratio. Meta-analyses for these subsets of studies were carried out in the same manner as the analyses of the total set of pooled samples.

Table 2. Studies excluded.

Study	Reasons for exclusion
Ramirez et al. 2000	Small sample: $n = 5$ for paired samples No correlation reported
Sandborgh-Englund et al. 2001	Small sample. Data as reported in figure and table disagree: $n = 7$ (figure), $n = 4$ (table)
Suzuki et al. 1984	Small sample: $n = 7$
Baglan et al. 1974	Hg concentration reported on a dry-weight basis
Fujita and Takabatake 1977	$r = 0.13$ (with outliers); $r = -0.65$ without outliers. Correlation is unstable
Pitkin et al. 1976	$r = 0.31$. Only 36% of samples had detectable Hg in both cord and maternal blood Ratio values not recoverable due to inclusion of nondetects values as 0 in summary data
Yang et al. 1997	Small sample: $n = 9$
Kuhnert et al. 1981	Hg concentration reported separately for erythrocytes and plasma; individual hematocrit data not reported
Kuntz et al. 1982	$r = 0.19$ Cord and maternal Hg concentration < 2.0 ppb
Sikorski et al. 1989	$r = 0.36$ Data reported as geometric means and confidence intervals; data cannot be consistently converted to arithmetic parameters
Truska et al. 1989	Hg concentration reported separately for erythrocytes and plasma; individual hematocrit data not reported
Muckle et al. 2001	Inconsistent statistical reporting

Results

Our search of the scientific literature yielded 22 studies providing data on maternal and cord blood Hg concentrations. Ten studies met all criteria for evaluation of the cord:maternal Hg ratio (Table 1). These studies address at least eight geographically distinct populations and several different ethnic groups. Reasons for exclusion of the remaining studies are presented in Table 2. The most common reasons for excluding studies were samples < 10 and correlations between cord blood or maternal blood < 0.4.

The data from Vahter et al.'s (2000) study were chosen to examine the assumption of log-normality because the study provides the largest data set among studies providing direct information on individual cord and maternal concentrations. The raw data (Vahter M. Personal communication) for both maternal and cord blood Hg concentrations were fitted to their maximum-likelihood log-normal distributions using curve-fitting software. The results assessed using the Kolmogorov-Smirnov goodness-of-fit test ($Hg_m - D = 0.066$, $p > 0.15$; $Hg_c - D = 0.071$, $p > 0.15$; where D is equal to the maximum vertical difference between the empirical and theoretical distributions) indicate a close fit and are consistent with the assumption of log-normality that we applied to both cord and maternal blood Hg concentrations in the Monte Carlo simulation procedure. We obtained similar results from the other studies providing individual concentration data (Dennis and Fehr 1975; Fujita and Takabatake 1977; Soria et al. 1992).

Table 3 presents the cord:maternal blood Hg ratios calculated or estimated from each of the included studies. Also presented are the SDs and coefficients of variation (CV) of each ratio. The CV (also referred to as the relative SD) is defined as SD/mean and provides a basis for comparing the extent of the variability in the ratio across different studies. These results are presented graphically in Figure 1. The ratios for each of the included studies were > 1.0. The mean ratio was 1.9, the smallest value was 1.1, and five of the 10 studies had ratios > 2.0. With one exception, the CVs of the ratios fell in a relatively narrow range of 0.34–0.56. Based on examination of scatter plots of the ratios from each of the studies to either the maternal or cord blood Hg concentrations for the same studies, there was no obvious correlation across studies between the cord:maternal ratio and either of the blood concentrations.

Table 4 presents a comparison of the cord:maternal ratio values estimated using the Monte Carlo simulation approach to the reported or calculated ratio values for the four studies providing this information. Note that the study of Fujita and Takabatake (1977) was not among those included because of the small

cord–maternal correlation coefficient. For three of the studies, the agreement between the observed and predicted ratio parameters was quite close. For Dennis and Fehr's (1975) study, the estimate of the mean ratio was also in close agreement, whereas the estimated SD was 35% smaller than the value calculated directly from the data. The reason for this discrepancy is not clear to us. These comparisons suggest that the simulation approach can provide a reasonable estimate of the ratio and its associated parameters for those studies providing only summary statistical data for cord and maternal Hg concentrations.

We compared the ratios generated from studies in which MeHg was measured (n = 5, mean = 1.94) with the ratios generated from studies in which total Hg was measured (n = 5, mean = 1.53). The difference was significantly different ($p < 0.0001$) based on t-test as well as analysis of variance controlling for independent interstudy differences. Meta-analyses were therefore conducted for all studies combined as well as for MeHg and total Hg studies separately. Table 5 presents the statistical parameters of the ratio distributions generated by the two meta-analysis approaches described in "Materials and Methods." In general, the unweighted analyses yield a larger mean ratio than do the n-weighted analyses, and the MeHg studies yield a larger mean ratio than do the total Hg studies. To help apply the results of these analyses in the derivation of the MeHg RfD, it would be useful to summarize the distributions in terms of simple parametric distributions. We previously investigated the fit of Vahter et al.'s (2000) maternal and cord Hg distributions using a goodness-of-fit test. However, given the large number of simulation samples resulting from the meta-analyses, goodness-of-fit tests will have large power to identify as statistically significant even small divergences from the ideal maximum likelihood fit. Thus they are of limited use for determining the practical utility of the best-fit parametric distributions. We therefore investigated the log-normal fit of the meta-analyses data using a graphical approach. Figures 2 and 3 present the normal probability plots of the log-transformed meta-analysis simulation data for all studies combined. For the data between the 0.5th and 95.5th percentiles of the distribution, the maximum-likelihood log-normal distributions provide a close fit to the simulated data. Thus, for the primary (n-weighted) meta-analysis, the maximum-likelihood estimate gives a log-normal distribution with a mean of 1.7 and an SD of 0.9, and for the alternative (unweighted) analysis, the ratio can be described as a log-normal distribution with a mean of 1.9 and an SD of 1.1.

Discussion

This analysis among widely differing populations indicates that the ratio of MeHg or MeHg-derived Hg in fetal cord blood to maternal blood, at least at the time of delivery, is greater than the value of 1.0 originally assumed in the NRC (2000) and U.S. EPA (2001, 2003) assessments. Doi et al. (1984) discussed possible reasons for the increased concentration of MeHg in cord blood relative to maternal blood. They suggested that the binding of MeHg to hemoglobin is the key factor in determining the relative MeHg concentration in the blood, with the greater concentration in the cord blood resulting from the larger hematocrit and greater hemoglobin concentration in the newborn. They found no evidence for a significantly greater MeHg binding capacity in fetal hemoglobin than in adult hemoglobin. In addition to maternal hematocrit decreasing during pregnancy, maternal plasma albumin concentration also decreases during pregnancy. This would appear to favor increasing concentrations of unbound xenobiotics. At the same time, the concentration of albumin in fetal plasma increases, providing increased opportunity for binding and retention of xenobiotics, presumably including MeHg (Manson 1986). Fetal-specific serum albumin proteins, such as α-fetoprotein (Deutsch 1991), may also lead to greater inherent affinity of fetal blood for MeHg compared with maternal blood. To date, however, the differential binding of MeHg to fetal and adult serum proteins does not appear to have been investigated. Finally, although MeHg passes freely across the placenta from mother to fetus because of the binding of the cysteine–MeHg complex by the neutral amino acid carrier (Kajiwara et al. 1996, 1997; Mokrzan et al. 1995), absence or reduced affinity of an analogous carrier on the fetal side of the placenta could also result in MeHg accumulation in fetal blood.

Two of the available studies contained all necessary information for assessing the cord:maternal Hg ratio but were excluded from the analysis because they narrowly failed to meet the *a priori* sample size requirement of 10. The mean cord:mean maternal MeHg ratio in Yang et al.'s (1997) study (non-occupationally exposed group, n = 9) was 1.67, and that in Suzuki et al.'s (1984) study (n = 7) was 1.73. These values are quite close to the means generated in the meta-analyses. Therefore, omission of these studies from the formal analysis had little effect on the results of the analysis. Two of the excluded studies that reported correlation coefficients only slightly smaller than the *a priori* requirement of 0.4 also had methodologic and/or reporting problems that created significant difficulties with their inclusion in the formal analysis. In Pitkin et al.'s (1976) study (r = 0.31, summary mean cord:maternal ratio = 1.2), only 36% of the samples had detectable levels of Hg in both cord and maternal blood. The statistical parameters for the study population were reported by the authors assuming that Hg concentration below the detection limit was 0 μg/L, thus precluding accurate reconstruction of the ratio and its parameters. In the Sikorski et al. (1989) study (r = 0.36, summary mean ratio = 1.0), means, SDs, and confidence intervals were reported on the logarithmic scale. Conversion to the arithmetic scale was uncertain because of the reporting of asymmetrical log confidence intervals. The two studies with low correlation coefficients [Fujita and Takabatake (1977), r = 0.13; Kuntz et al. (1982), r = 0.19] failed to meet other inclusion criteria or had significant

Table 3. Cord:maternal Hg ratios for studies meeting the inclusion criteria (calculated values are the average of five simulations of 5,000 iterations each).

Study (meeting selection criteria)	Hg_c:Hg_m (cord blood:maternal blood Hg ratio)	SD	CV
Lauwreys et al. 1978[a]	1.23	0.60	0.49
Vahter et al. 2000[b]	1.89	0.71	0.38
Tsuchiya et al. 1984	1.70	0.92	0.54
Bjerregaard and Hansen 2000	2.21	0.98	0.44
Hansen et al. 1990	2.18	0.81	0.37
Nishima et al. 1977	2.32	0.79	0.34
Ong et al. 1993	2.30	1.85	0.80
Soong et al. 1991	1.54	0.86	0.56
Soria et al. 1992[b]	1.09	0.50	0.46
Dennis and Fehr 1975[b]	2.09	1.17	0.56
Mean	1.86	—	0.49

[a]Based on values as reported in units of μg/100 mL. [b]Ratio calculated on the basis of reported raw data, not on sampled distributions.

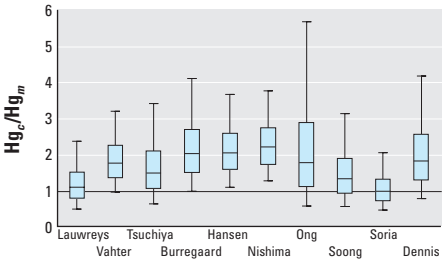

Figure 1. Percentiles of cord:maternal Hg ratios for studies meeting the inclusion criteria. Box plot: box, 25th–75th percentiles; center line, median value; whiskers, 5th/95th percentiles. Names on x-axis refer to references cited in Table 1.

methodologic problems (Table 2). Thus, no study was excluded solely on the basis of its correlation coefficient.

Three of the four studies that were available for evaluating the accuracy of the Monte Carlo simulation approach indicated that this approach resulted in a close agreement with both the reported mean and SD. For the fourth study, the estimate of the SD was biased low despite a good fit of both the maternal and cord blood Hg concentrations to log-normal distributions. Although the reasons for this are not clear to us, if there is indeed a bias in the simulation approach leading to an underestimation of the SD, the estimates of the upper percentiles of the ratio (e.g., 90th, 95th percentiles) will likewise be underestimated.

The mean cord:maternal ratio estimated from the pooled MeHg-only studies was significantly larger than the mean ratio calculated from the pooled total Hg-only studies. Because total Hg and MeHg concentration measurements differ by the concentration of inorganic Hg, this suggests the possibility that the type of Hg measurement could influence the parameters of the ratio. There are three possible sources of inorganic Hg in blood: nonspecific sources of ionic Hg (Hg^{2+}) in the diet, release of elemental Hg vapor (Hg^0) from dental amalgams, and metabolism of MeHg to ionic Hg. Ideally, we would want to consider only the inorganic Hg arising from metabolism of MeHg. Ionic Hg does not readily cross the placenta (Khayat and Dencker 1982), and the fetus appears to have little capacity to metabolize MeHg to ionic Hg (Dock et al. 1994; Nordenhill et al. 1995). Dietary inorganic Hg is not likely to contribute significantly to maternal blood inorganic Hg except in populations with frequent consumption of marine mammals that have relatively high concentrations of inorganic Hg from metabolism of MeHg. The Agency for Toxic Substances and Disease Registry (ATSDR 1999) estimated that dietary inorganic Hg accounts for about 2% of the Hg retained in the body in the general population. Therefore, for most populations, essentially all of the inorganic Hg that should be included in the ratio will be found on the maternal side. This conclusion is consistent with our observation that the cord:maternal ratio calculated from MeHg-only studies is larger than the ratio calculated from total Hg-only studies, and suggests that the MeHg-only ratio may overestimate the true ratio. However, Hg^0 from amalgams readily crosses the placenta (Pamphlett and Kum-Jew 2001; Takahashi et al. 2001; Warfvinge 2000) and can therefore result in an overestimate of the MeHg-derived Hg concentration in both cord and maternal blood when total Hg studies are considered.

Because the extent of dental amalgam use is likely to differ among individuals, populations, and ethnic groups, the effect of dental amalgam Hg on the cord:maternal ratio estimated from total Hg studies is uncertain. It is not clear, however, that the observed difference in the cord:maternal ratio between MeHg-only and total Hg-only studies in fact derives from the influence of inorganic Hg. Both Hansen et al. (1990) and Bjerregaard and Hansen (2000) studied Inuit in western Greenland. The former study measured MeHg, and the latter total Hg. The ratios from both studies were nearly identical (Table 3). Dennis and Fehr (1975) measured total Hg in two populations, one in northern Saskatchewan, and one in southern Saskatchewan (the data for only the northern group were available for our analysis). The summary mean ratio in the former was 1.8 and 1.1 in the latter. The northern group ate more fish and had blood concentrations two to four times higher than the southern group, but the two groups presumably did not have significantly different inorganic Hg exposures. In light of this uncertainty, we have presented the

Table 4. Comparison of reported and calculated ratios for studies reporting ratio data.

Study	Reported mean Hg_c:Hg_m	Reported SD Hg_c:Hg_m	Calculated mean Hg_c:Hg_m	Calculated SD Hg_c:Hg_m
Vahter et al. 2000	1.91	0.76	1.89	0.71
Soria et al. 1992	1.11	0.51	1.09	0.50
Fujita and Takabatake 1977[a]	1.4	0.9	1.47	1.11
Dennis and Fehr 1975	2.09	1.17	1.92	0.76

[a]Did not meet selection criteria due to weak maternal–cord Hg correlation.

Table 5. Ratios derived from meta-analyses (calculated values are the average of five simulations).

	All studies combined		MeHg studies only		Total Hg studies only	
	n-Weighted meta-analysis	Unweighted meta-analysis	*n*-Weighted meta-analysis	Unweighted meta-analysis	*n*-Weighted meta-analysis	Unweighted meta-analysis
Mean	1.65	1.85	1.89	2.08	1.51	1.60
SD	0.93	1.07	0.98	1.11	0.85	0.87
CV	0.56	0.58	0.52	0.53	0.56	0.54
25th percentile	1.00	1.13	1.23	1.38	0.92	0.98
50th percentile	1.45	1.65	1.71	1.88	1.32	1.41
75th percentile	2.07	2.32	2.34	2.52	1.90	2.01
90th percentile	2.81	3.10	3.07	3.30	2.61	2.72
95th percentile	3.37	3.71	3.63	3.93	3.14	3.24

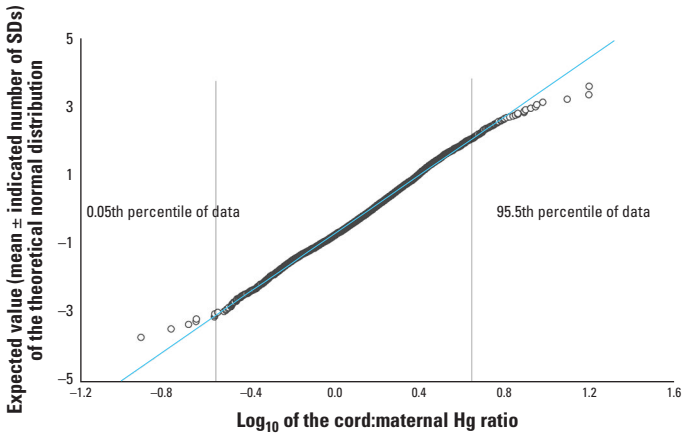

Figure 2. Normal probability plot of log-transformed *n*-weighted meta-analysis simulation data. This figure displays the fit of the meta-analysis simulation data (after logarithmic transformation) to the theoretical normal distribution (the blue line). Deviations of the data points from the blue line indicate deviations from true log-normality.

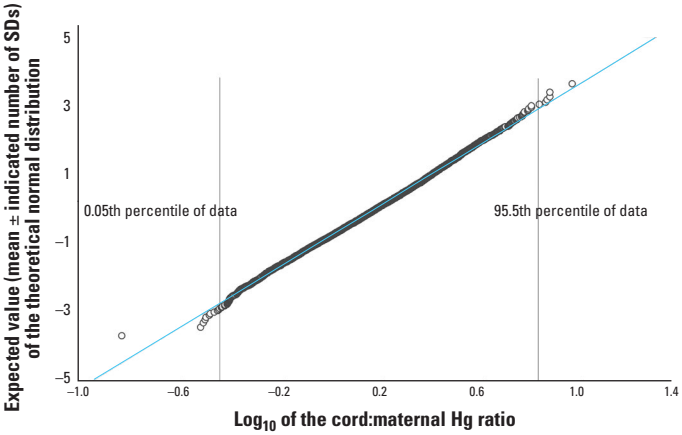

Figure 3. Normal probability plot of log-transformed unweighted meta-analysis simulation data. This figure displays the fit of the meta-analysis simulation data (after logarithmic transformation) to the theoretical normal distribution (the blue line). Deviations of the data points from the blue line indicate deviations from true log-normality.

ratios and their distributions calculated based on all included studies as well as on MeHg-only and total Hg-only studies. The differences among these values are relatively small, and the potential influence of inorganic Hg is modest. In light of the various uncertainties, we recommend that the ratio values from the analysis of all studies (Table 5, *n*-weighted) be used for purposes of risk assessment.

Recall that prior estimates of overall variability in the one-compartment pharmacokinetic model by the NRC (2000) and Stern et al. (2002) concluded that applying an uncertainty factor of 2 to the central tendency estimate of dose could essentially account for the variability in dose corresponding to a given maternal blood concentration. That conclusion, however, was specific to maternal blood and did not consider the variability around the central tendency estimate of the cord:maternal blood Hg ratio. These same sources concluded that applying an uncertainty factor of 3 to the central tendency estimate of dose could account for the overall variability in the dose corresponding to a given maternal hair concentration. The additional overall variability in the latter case results from the additional pharmacokinetic variability inherent in the empirical ratio relating maternal hair Hg concentration to maternal blood Hg concentration. From the standpoint of overall variability in the one-compartment model, that ratio is analogous to the cord blood:maternal blood ratio derived in the present analysis. Interestingly, the CV describing the variability in the hair:blood ratio is similar to that derived here for the cord:maternal ratio. We have not yet completed a comprehensive reanalysis of dose estimation using the one-compartment model (including the cord:maternal ratio) in the context of the MeHg RfD derivation. However, by comparison with the variability in the model with the hair:blood ratio included, incorporation of the cord:maternal ratio in the model appears to support the use of a full 3-fold uncertainty factor for toxicokinetic variability alone. Whether, ultimately, a revision will be needed to the RfD for neurologic developmental effects as a result of the present analysis will depend on the results of a formal comprehensive reanalysis. We are currently engaged in such an analysis.

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