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# Evaluation of the Uncertainty in an Oral Reference Dose for Methylmercury Due to Interindividual Variability in Pharmacokinetics

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An analysis of the uncertainty in guidelines for the ingestion of methylmercury (MeHg) due to human pharmacokinetic variability was conducted using a physiologically based pharmacokinetic (PBPK) model that describes MeHg kinetics in the pregnant human and fetus. Two alternative derivations of an ingestion guideline for MeHg were considered: the U.S. Environmental Protection Agency reference dose (RfD) of 0.1 μg/kg/day derived from studies of an Iraqi grain poisoning episode, and the Agency for Toxic Substances and Disease Registry chronic oral minimal risk level (MRL) of 0.5 μg/kg/day based on studies of a fish-eating population in the Seychelles Islands. Calculation of an ingestion guideline for MeHg from either of these epidemiological studies requires calculation of a dose conversion factor (DCF) relating a hair mercury concentration to a chronic MeHg ingestion rate. To evaluate the uncertainty in this DCF across the population of U.S. women of child-bearing age, Monte Carlo analyses were performed in which distributions for each of the parameters in the PBPK model were randomly sampled 1000 times. The 1st and 5th percentiles of the resulting distribution of DCFs were a factor of 1.8 and 1.5 below the median, respectively. This estimate of variability is consistent with, but somewhat less than, previous analyses performed with empirical, one-compartment pharmacokinetic models. The use of a consistent factor in both guidelines of 1.5 for pharmacokinetic variability in the DCF, and keeping all other aspects of the derivations unchanged, would result in an RfD of 0.2 μg/kg/day and an MRL of 0.3 μg/kg/day.

**KEY WORDS:** MeHg; pharmacokinetics; PBPK model; variability; risk assessment.

## INTRODUCTION

The current reference dose (RfD) for MeHg (MeHg) developed by the U.S. Environmental Protection Agency (USEPA)[(1)] is based on a retrospective study of an acute poisoning incident in Iraq in which grain contaminated with a MeHg fungicide was inadvertently used in the baking of bread.[(2)] The exposures, which were relatively high, but lasted only a few months, were associated with neurological effects in both adults (primarily paresthesia) and infants (late walking, late talking). In particular, neurodevelopmental effects were observed in the offspring of mothers exposed to MeHg who themselves were asymptomatic. The USEPA derived an RfD of 0.1 μg/kg/day based on a NOAEL of 11 ppm mercury in maternal hair estimated by benchmark dose modeling of the combined neurological endpoints re-

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ported for children exposed *in utero*. Due to the acute nature of the exposure, the maternal hair concentrations used in this analysis were the peak concentrations achieved during pregnancy. This RfD included an uncertainty factor of 10, consisting of a factor of 3 to consider pharmacokinetic variability and a factor of 3 for database limitations (lack of data on multigeneration effects or possible long-term sequelae of perinatal exposure). The USEPA[1] also conducted a Monte Carlo uncertainty analysis which estimated the impact of pharmacokinetic variability on the determination of the ingestion rate associated with the NOAEL hair concentration, but they did not explicitly use the results of this analysis in the derivation of the RfD.

A more recent study conducted on a population in the Seychelles Islands[3,4] was selected as the critical study for a chronic oral minimal risk level (MRL) by the Agency for Toxic Substances and Disease Registry (ATSDR). The exposures to MeHg in this population resulted from chronic, multigenerational ingestion of contaminated fish. This prospective study was carefully conducted and analyzed, included a large cohort of mother–infant pairs, and was relatively free of confounding factors. The results of this study were essentially negative, and a daily ingestion rate of 0.5 μg/kg/day, derived from the median of the distribution of maternal hair mercury concentrations in the studied population (5.9 ppm), was proposed by ATSDR as the chronic oral MRL for MeHg.[5] In the case of this chronically exposed population, the average maternal hair concentration during pregnancy was used, although the authors found no evidence of significant temporal variation. Due to the large size of the study population, no uncertainty factor was considered necessary in the derivation of the MRL.

In their risk assessments for MeHg, both the USEPA[1] and ATSDR[5] employed empirical, one-compartment pharmacokinetic models to describe the relationship between hair concentration and ingestion rate of MeHg. Parameters in these models were chosen on the basis of empirical data regarding the kinetics and partitioning of MeHg in human subjects. Based on the selected parameter values, the agencies then calculated "best estimates" of an average daily ingestion rate which would produce a given hair concentration. The USEPA[1] also evaluated the uncertainty in this dose conversion factor (DCF) resulting from the potential variability in the pharmacokinetics of MeHg across a population, and used the results of this analysis to support their application of an uncertainty factor of 3 to address this concern in their derivation of the RfD. A more recent analysis by Stern[6] also evaluated the pharmacokinetic uncertainty in the RfD. The analysis described in this paper differs from these previous analyses in that instead of an empirical, one-compartment pharmacokinetic model, a physiologically based pharmacokinetic (PBPK) model was used. The resulting estimate of the variability in the relationship between ingestion rate and hair concentration (i.e., the DCF) in a population of U.S. women of child-bearing age was then used to define a reasonable uncertainty factor for pharmacokinetic variability which could be applied consistently in both of the guidelines.

## METHODS

### PBPK Model

The structure of the PBPK model of MeHg in the human used in this analysis (Fig. 1) has been described previously.[7] For the present study, the model was reparameterized specifically for U.S. women of child-bearing age. Enterohepatic recirculation of MeHg is described by the excretion of MeHg in the bile ($k_b$) and its subsequent reabsorption into the gut tissue ($k_r$). Oral absorption is modeled as zeroth-order stomach emptying ($k_0$) followed by intestinal absorption ($k_r$). The transport of MeHg and its conversion to inorganic mercury ($k_i$) in the model is described by linear processes. Distribution in the blood is assumed to be plasma-flow-limited, with the exception of transport across the placenta ($k_{fe}$), blood–brain barrier ($k_{br}$), and red cell membranes ($k_{rbc}$ and $k_{rbcf}$), which are considered to be diffusion-limited. The most important excretion mechanisms for mercury are excretion in hair ($k_h$) and conversion of MeHg to inorganic mercury by the gut flora ($k_d$), with subsequent excretion of inorganic mercury in the feces ($k_f$). Urinary excretion ($k_u$) only becomes important at the higher experimental doses used in animals, in which cases renal damage often occurs. Following the approach of Farris *et al.*,[8] loss of hair ($k_l$) and (in the case of rodents) reingestion of hair by preening ($k_{lr}$) are also described.

The fetal portion of the model consists of four compartments which grow during the time of gestation: plasma, RBCs, brain, and the remaining fetal tissue. Increases in maternal tissue during pregnancy are also described in the case of plasma, RBCs, richly perfused tissues (representing changes in the uterus

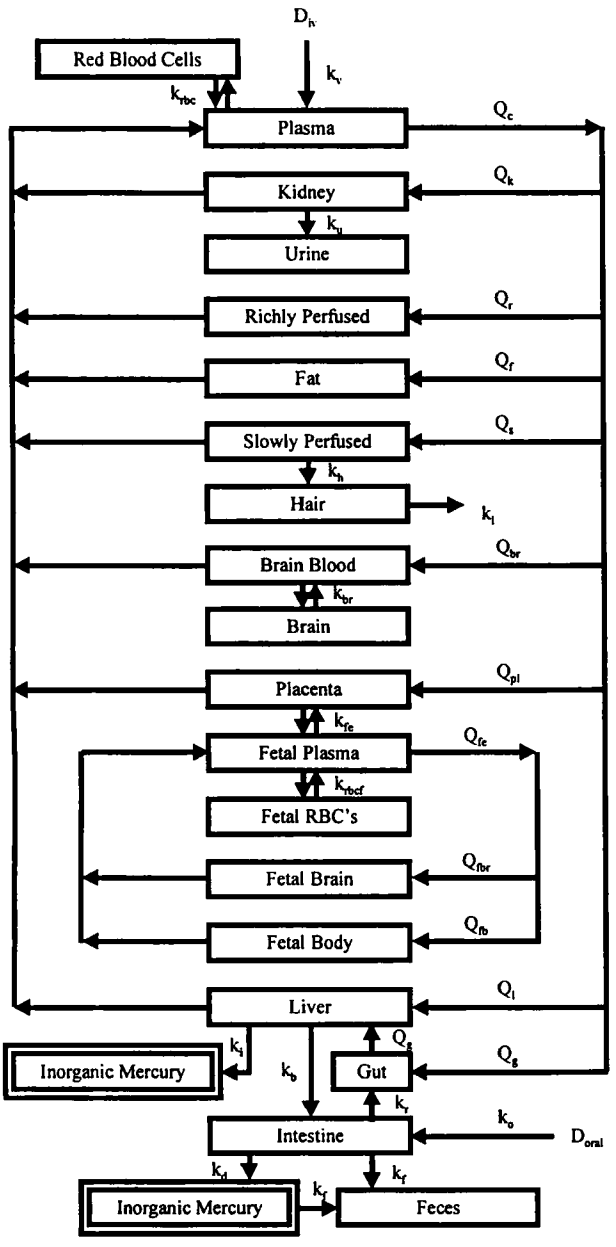


**Fig. 1.** Physiologically based pharmacokinetic model for MeHg used in this analysis. Abbreviations are defined in Table I.

and mammary glands), fat, and fluid. The time course for these physiological changes during pregnancy and gestation was taken from Hytten and Leitch.[9] Maternal dietary intake in the model also increases over the course of pregnancy, based on data for U.S. women.[9] The prepregnancy tissue volumes ($V$) and blood flows ($Q$) in the model are standard values taken from Brown *et al.*[10] and ICRP,[11] while the tissue/blood partition coefficients ($P$) were based on tissue mercury data from Berlin *et al.*,[12] Kitamura *et al.*,[13] Kawasaki *et al.*,[14] and Vahter *et al.*[15] Tissue volumes are scaled in the model in proportion to body weight, while blood flows and kinetic parameters (in the form of clearances) are scaled in proportion to body weight raised to the three-fourths power.[16] The kinetic parameters in the model were estimated either from the physiological literature or by simultaneously fitting data from a number of MeHg pharmacokinetic studies for a variety of dosing scenarios in both monkeys[17-23] and humans.[24-28]

The resulting model is able to describe accurately both the uptake and clearance of MeHg in hair and blood for human volunteers ingesting various diets of MeHg in fish.[7] For the analysis described here, the model was run at a constant daily dietary intake of MeHg (1 $\mu$g/kg/day) until steady state was achieved in all maternal tissues. At this point (600 days into the exposure) the pregnancy was initiated and the dosing was continued until conception, at which time the average and peak maternal hair concentrations during pregnancy were calculated.

## Monte Carlo Analysis

In order to provide an estimate of the distribution of ingestion rates in a population that could be associated with a given hair level, probability distributions for each of the model parameters were determined from the literature and used in a Monte Carlo analysis to generate a distribution of DCFs. The parameter distributions used in the Monte Carlo analysis, expressed as means and coefficients of variation (CV = standard deviation/mean), are defined in Table I. In most cases, the means of the distributions are the parameter values identified during the development and validation of the model described above. The exceptions are those for which data more relevant to the specific population of interest (U.S. women of child-bearing age) were available. The distribution of body weights was obtained from the NHANES III database,[29] and includes only women of child-bearing age (14–45 years, inclusive) in the United States. Data from the human physiological literature were used to estimate variability for the plasma flows[30-35] and tissue volumes,[11,29,36-40] as well as the critical kinetic parameters, e.g., hair excretion rate constant $k_{hi}$,[11,41-45] and fecal excretion rate constant, $k_{fi}$.[11,46] The variability of tissue/blood partition coefficients was estimated from autopsy data.[47] In

the case of the hair/blood partition coefficient, a global distribution was estimated (see Appendix) from data reported in nine independent studies.[24,48–55] Normal distributions were used for plasma flows and tissue volumes, while lognormal distributions were used for partition coefficients and kinetic parameters. To avoid physiologically implausible values, most distributions were truncated at three standard deviations above and below the mean, and normal distributions were also truncated at 1% of the mean (to avoid negative or zero values). In the case of body weights, the extreme values were obtained directly from the NHANES III database.

To perform the Monte Carlo simulation, the probability distributions for each of the PBPK model parameters were repeatedly sampled, and the PBPK model was run using each chosen set of parameter values. Random sampling was performed with the Latin hypercube method, which provides a thorough coverage of the distributions using fewer iterations than the standard Monte Carlo method. It was found that 1000 iterations were adequate to ensure the reproducibility of the mean and standard deviation of the output distributions as well as the 1st through 99th percentiles. The output of the Monte Carlo simulation was a distribution of hair concentrations (peak and average during pregnancy) associated with an ingestion rate of 1 $\mu$g/kg/day. To obtain the ingestion rate distributions, the output distributions were inverted (to produce a distribution of DCFs in $\mu$g/kg/day/ppm) and multiplied by the NOAEL hair concentration.

In conjunction with the Monte Carlo analysis, sensitivity analysis was performed by two different methods. First, analytical sensitivity coefficients, defined as the ratio of the percentage change in the DCF to the percentage change in a model input parameter that produced it, were obtain by varying each of the parameters in turn by 1% and noting the resulting change in the DCF predicted by the PBPK model. Second, analysis of the correlation of the DCF with each of the input parameters was performed on the results of the Monte Carlo analysis. The analytical sensitivity coefficients most accurately represent the functional relationship of the output to the specific inputs under the conditions being modeled. The correlation coefficients, on the other hand, document the impact of interactions between the parameters during the Monte Carlo analysis.

In their Monte Carlo analysis for MeHg, the USEPA[1] considered three correlations between parameters: blood volume with body weight, fraction of MeHg in blood with body weight, and hair/blood partition with elimination half-life. The first and last of these correlations result naturally from the PBPK model's physiological structure, but the second does not. We reviewed the study[27] which was cited as evidence for the correlation between the fraction of MeHg in blood and body weight in the USEPA[1] analysis. It appears that the observed correlation actually reflects a higher ratio of men to women in the groups with the larger average body weights. Men have relatively less fat per kilogram body weight than women, and fat has a much lower partition for MeHg than the other tissues. Thus the negative correlation between fraction of MeHg in the blood and body weight observed by the USEPA[1] can be understood physiologically as a positive correlation between fraction of MeHg in blood and fraction of fat in the body. Therefore, the correlation of fat content and body weight in adult females[56] was included in the Monte Carlo analysis. There was no evidence that any of the other key (high-sensitivity) input parameters in the PBPK model were significantly correlated. Of course, the structure of the model itself provides many physiological contraints on the parameters (e.g., sum of tissue blood flows equal to cardiac output, tissue/plasma partitions related to tissue/blood partitions by the hematocrit, etc.). A more complete description of the derivation of the parameter distributions and the details of the Monte Carlo analyses is available from the authors.[57]

## RESULTS

To validate the selection of parameter distributions for the Monte Carlo analysis, the distribution of biological half-lives of MeHg predicted by the model was compared with half-lives published in the literature. Reported half-lives from four studies of controlled exposures to MeHg[25,27,28,49] ranged from 32 to 70 days, with a pooled mean of approximately 49 days (SD 7.45), while half-lives obtained from patients during the Iraqi grain poisoning incident[58] averaged 72 days (SD 27.9). In the study conducted by Sherlock *et al.*,[27] the mean half-life in males was 49.7 days (SD 7.47; $n$ = 14), while the mean half-life for women was 54.2 days (SD 3.62; $n$ = 6). The distribution of half-lives for women of child-bearing age output by the PBPK Monte Carlo analysis had a mean of 61 days, with a standard deviation of 35 days. Thus the central tendency of the distribution for this model output is reasonably consistent with

observations, but the Monte Carlo analysis tends to somewhat overestimate the variability observed in most studies.

The result of the Monte Carlo analysis of hair concentrations is shown in Fig. 2, which portrays the distribution of ingestion rates associated with the BMDL of 11 ppm MeHg in maternal hair calculated by USEPA from the Iraqi study. The geometric mean (GM) for the distribution of daily ingestion rates in this case was 0.84 $\mu$g/kg/day with a geometric standard deviation (GSD) of 1.33, and the percentiles for the daily ingestion rate are shown in Table II. The similar distribution of dietary MeHg ingestion rates corresponding to the hair mercury concentration identified by ATSDR as the NOAEL for the Seychelles study, 5.9 ppm, is shown in Table III. The GM and GSD for this distribution are 0.45 $\mu$g/kg/day and 1.33, respectively. In both cases, the ratio of the 5th percentile of the distribution to the median is approximately 1.5, while the ratio of the 1st percentile to the median is approximately 1.8.

Several additional Monte Carlo analyses were performed to investigate the sensitivity of the resulting distribution to the approach used for the analysis. In the first alternative case, the explicit treatment of the correlation between the fractional fat volume and body weight was removed. In the second alternative case, only the seven parameters with the greatest sensitivities were varied, and all the rest of the parameters in the model were fixed at their preferred (mean) values. In the third alternative case, all of the parameter distributions were changed to lognormal instead of the mix of normal and lognormal shown in Table I. In all three cases, the resulting mean, standard distribution, and 5th through 90th percentiles in the distribution of ingestion rates were within 1% of the values obtained in the primary analysis. Somewhat greater differences were observed in the 1st percentile as well as in the 95th and 99th percentiles of the distribution.

The results of the sensitivity analyses are shown in Table IV. The parameters identified as most significant for relating dietary ingestion to hair concentration by the analytical sensitivity analysis were the hair excretion rate constant ($k_{hi}$), hair/blood partition coefficient (PHB), the body weight (BW), gut tissue/blood partition coefficient (PG), fecal excretion rate constant ($k_{fi}$), fractional fat volume (VFC), and fractional slowly perfused tissue volume (VSC). The same parameters were also identified as significant by correlation analysis, with the exception of VSC. The most sensitive parameters for predictions of half-life were similar to those for hair concentration, with the addition of the partition coefficient for slowly perfused tissue/blood.

## DISCUSSION

### Variability Versus Uncertainty

In performing a Monte Carlo analysis it is important to distinguish uncertainty from variability. As it relates to the impact of pharmacokinetics in risk assessment, uncertainty can be defined as the possible error in estimating the "true" value of a parameter for a representative ("average") person. Variability, on the other hand, should only be considered to represent true interindividual differences. Understood in these terms, uncertainty is a defect (lack of certainty) which can typically be reduced by experimentation, and variability is a fact of life which must be considered regardless of the risk assessment methodology used. Unfortunately, in practice it is often difficult to differentiate the contributions of variability and uncertainty to the observed variation in the reported measurements of a particular parameter.[59] The parameter distributions used in the Monte Carlo analysis described here were chosen to represent interindividual variability; however, where there was doubt regarding whether differences between studies represented experimental uncertainty or population variability the conservative position was taken that the differences should be assumed to reflect interindividual variability.

For example, it is likely that the study-to-study differences in the means of reported evaluations of the hair/blood partition coefficient for MeHg reflect experimental bias due to differences in the analytical methodologies used rather than to real differences in the populations. If the differences in reported means were indeed due to experimental bias, the study means could have been combined in an unweighted fashion, and a coefficient of variation could have been separately determined from one or more of the larger studies. A narrower distribution would have been obtained if the studies were combined in this way. However, the assumption of experimental bias cannot be supported by comparison data, so the hair/blood partition distribution was calculated assuming all of the reported measurements represented estimates of a single global distribution.

The large standard deviation produced by the Monte Carlo analysis for half-life probably reflects

**Table I.** Parameter Distributions Used in the Monte Carlo Analysis

| Parameters | | Mean | CV | Upper bound | Lower bound | Distribution |
|---|---|---|---|---|---|---|
| Plasma flows (fraction of cardiac output) | | | | | | |
| QCC | Cardiac output (L/hr scaled by $BW^{3/4}$) | 20.0 | 0.22 | 33.2 | 6.8 | Normal |
| QBrBC | Brain | 0.114 | 0.30 | 0.217 | 0.011 | Normal |
| QFC | Fat | 0.052 | 0.30 | 0.099 | 0.0052 | |
| QGC | Gut | 0.181 | 0.33 | 0.360 | 0.002 | Normal |
| QKC | Kidney | 0.175 | 0.30 | 0.333 | 0.018 | Normal |
| QLC | Liver | 0.046 | 0.32 | 0.090 | 0.01 | Normal |
| QRC | Richly perfused tissues | 0.183 | 0.30 | 0.348 | 0.018 | Normal |
| QSC | Slowly perfused tissues | 0.249 | 0.30 | 0.473 | 0.025 | Normal |
| QP1M | Placenta (L/hr scaled by $BW^{3/4}$) | 58.5 | 0.35 | 119.9 | 10.0 | Normal |
| QFeC | Fetal (L/hr scaled by $BW^{3/4}$) | 54.0 | 0.30 | 102.6 | 10.0 | Normal |
| Tissue volume (fraction of body weight) | | | | | | |
| BW | Body weight (kg) | 67.77 | 0.26 | 139.9 | 30.81 | Lognormal |
| VBrC | Brain | 0.02 | 0.30 | 0.038 | 0.002 | Normal |
| VBrBC | Brain plasma | 0.007 | 0.30 | 0.013 | 7.0*e*-4 | Normal |
| VFC | Fat | 0.273 | 0.24 | 0.47 | 0.076 | |
| VGC | Gut | 0.017 | 0.15 | 0.025 | 0.009 | Normal |
| VHC | Hair | 0.002 | 0.50 | 0.005 | 1.0*e*-4 | Normal |
| VIC | Intestine | 0.014 | 0.30 | 0.027 | 0.001 | Normal |
| VKC | Kidney | 0.004 | 0.30 | 0.008 | 4.0*e*-4 | Normal |
| VLC | Liver | 0.026 | 0.25 | 0.046 | 0.006 | Normal |
| VPC | Plasma | 0.024 | 0.14 | 0.058 | 0.024 | Normal |
| VRBCC | Red blood cells | 0.024 | 0.25 | 0.046 | 0.006 | Normal |
| VRC | Richly perfused tissues | 0.10 | 0.30 | 0.190 | 0.01 | Normal |
| VSC | Slowly perfused tissues | 0.35 | 0.16 | 0.52 | 0.18 | Normal |
| VRem | Remainder (nonperfused) | 0.122 | 0.30 | 0.23 | 0.012 | Normal |
| Partition coefficients for MeHg | | | | | | |
| PBr | Brain/blood | 3.0 | 0.30 | 6.93 | 1.19 | Lognormal |
| PBrB | Brain blood/plasma | 1.0 | 0.30 | 2.31 | 0.397 | Lognormal |
| PF | Fat/blood | 0.15 | 0.30 | 0.347 | 0.060 | |
| PFe | Fetal plasma/placenta | 2.0 | 0.30 | 4.62 | 0.794 | Lognormal |
| PG | Gut/blood | 1.0 | 0.70 | 5.45 | 0.123 | Lognormal |
| PHB | Hair/blood | 248.7 | 0.70 | 1361.7 | 30.4 | Lognormal |
| PK | Kidney/blood | 4.0 | 0.30 | 9.24 | 1.59 | Lognormal |
| PLiv | Liver/blood | 5.0 | 0.30 | 11.6 | 1.99 | Lognormal |
| PP1 | Placenta/blood | 2.0 | 0.30 | 4.62 | 0.794 | Lognormal |
| PRBC | Red blood cell/plasma | 12.0 | 0.30 | 27.7 | 4.76 | Lognormal |
| PRBCFe | RBC/plasma for fetus | 14.0 | 0.30 | 32.4 | 5.56 | Lognormal |
| PR | Richly perfused tissues/blood | 1.0 | 0.30 | 2.31 | 0.397 | Lognormal |
| PS | Slowly perfused tissues/blood | 2.0 | 0.30 | 4.62 | 0.794 | Lognormal |
| Kinetic parameters (L/hr scaled by $BW^{3/4}$) | | | | | | |
| $k_{brici}$ | Incorporation of inorganic Hg in brain | 5.0*e*-5 | 0 | | | |
| $k_{brili}$ | Loss of inorganic Hg from brain | 0.001 | 0 | | | |
| $k_{brini}$ | Brain MeHg to inorganic Hg | 1.2*e*-5 | 0.30 | 2.77*e*-5 | 4.76*e*-6 | Lognormal |
| $k_{bi}$ | Biliary clearance of MeHg | 0.0001 | 0.30 | 2.31*e*-4 | 3.97*e*-5 | Lognormal |
| $k_{bri}$ | Brain uptake | 0.01 | 0.30 | 0.0231 | 3.97*e*-3 | Lognormal |
| $k_{di}$ | MeHg to inorganic Hg in intestine | 0.0001 | 0.30 | 2.31*e*-4 | 3.97*e*-5 | Lognormal |
| $k_{fi}$ | Fecal excretion | 0.0002 | 0.36 | 5.36*e*-4 | 6.60*e*-5 | Lognormal |
| $k_{hi}$ | Excretion into hair | 7.0*e*-6 | 0.25 | 1.42*e*-5 | 3.25*e*-6 | Lognormal |
| $k_{ii}$ | Conversion to inorganic Hg | 1.0*e*-5 | 0.30 | 2.31*e*-5 | 3.97*e*-6 | Lognormal |
| $k_{rbci}$ | RBC/plasma diffusion | 1.5 | 0.30 | 3.47 | 0.596 | Lognormal |
| $k_{ri}$ | Intestinal reabsorption | 0.005 | 0.30 | 0.012 | 1.99*e*-3 | Lognormal |
| Fetal kinetic parameters (L/hr) | | | | | | |
| $k_{fe}$ | Placenta/embryo diffusion | 1.0 | 0.50 | 3.69 | 0.217 | Lognormal |
| $k_{rbcfe}$ | Fetal RBC/plasma diffusion | 100.0 | 0.50 | 369.0 | 21.7 | Lognormal |

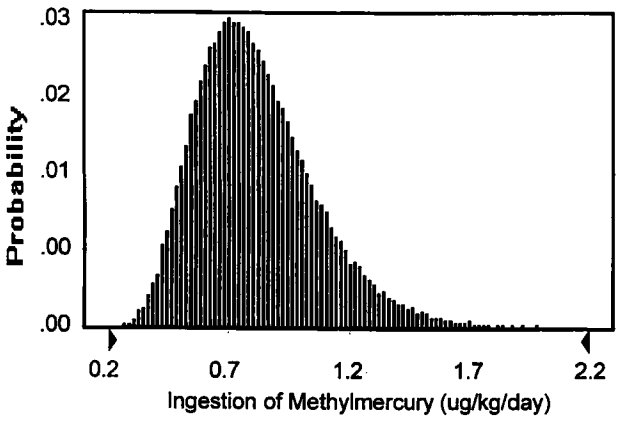


**Fig. 2.** Distribution of daily ingestion rates in the U.S. population of women of child-bearing age associated with the NOAEL for MeHg in hair derived by USEPA from the study of the Iraqi poisoning incident.

the fact that the distributions calculated for the input parameters do indeed reflect not only interindividual variability, but also uncertainty regarding the true value of the parameters. The performance of the PBPK model with respect to half-life is a reliable indicator of its ability to estimate the variability in the DCF, because the sensitivities of these two outputs (half-life and hair concentration) to the various input parameters were very similar. Thus the result for half-life provides some assurance that the variability of ingestion rates predicted by the Monte Carlo analysis would also provide a conservative (broad) estimate.

**Table II.** Percentiles in the Distribution of Ingestion Rates of MeHg Associated with the NOAEL in the Iraqi Population Identified by Benchmark Dose Modeling (11 ppm)

| Percentile | Rate ($\mu$g/kg/day) |
|---|---|
| 1% | 0.45 |
| 5% | 0.54 |
| 10% | 0.60 |
| 25% | 0.69 |
| 50% | 0.83 |
| 75% | 1.00 |
| 90% | 1.20 |
| 95% | 1.36 |
| 99% | 1.73 |

**Table III.** Percentiles of the Distribution of Ingestion Rates of MeHg Associated with the Median Hair Concentration in the Seychelles Population (5.9 ppm)

| Percentile | Rate ($\mu$g/kg/day) |
|---|---|
| 1% | 0.24 |
| 5% | 0.29 |
| 10% | 0.32 |
| 25% | 0.37 |
| 50% | 0.44 |
| 75% | 0.53 |
| 90% | 0.65 |
| 95% | 0.73 |
| 99% | 0.93 |

## Comparison with Results from Empirical One-Compartment Models

The variability in ingestion rates predicted by this PBPK analysis is comparable to the results of two previous compartmental analyses.[1,6] In the PBPK analysis, the ratio of the ingestion rate at the 5th percentile in the distribution to the median ingestion rate is 0.66. The same ratio in the compartmental analyses is approximately 0.5. The agreement of these three analyses is encouraging since they not only reflect different choices for the data underlying the distributions of common parameters (hair/blood partition coefficient, body weight), but also are to a large extent based on different types of parameters. For example, one of the important input parameters in the compartmental models is the apparent half-life

**Table IV.** Parameter Sensitivity

| Parameter | Analytical sensitivity coefficient | Pearson correlation coefficient |
|---|---|---|
| BWF | 0.24 | 0.19 |
| $k_{fi}$ | −0.13 | −0.23 |
| $k_{hi}$ | −0.77 | −0.66 |
| PG | −0.13 | −0.32 |
| PHB | 0.22 | 0.42 |
| VFC | 0.08 | 0.15 |
| VPC | 0.02 | −0.13 |
| VRBCC | 0.02 | −0.13 |
| VRemain | 0.03 | −0.13 |
| VSC | 0.09 | 0.01 |

[a] Parameters were only included in this table if they had an analytical sensitivity coefficient or Pearson correlation greater than 0.1 in absolute value.

for MeHg in the blood. In the case of the PBPK model, on the other hand, the half-life is actually one of the outputs of the model, and is predicted on the basis of the more fundamental physiological, partitioning, and kinetic parameters.

The somewhat greater variability predicted by the empirical one-compartment analyses probably results primarily from the inability of the empirical approach to represent the functional relationships between parameters. In the analysis performed by Stern[6] only one relationship was described explicitly: blood volume with body weight, while in the USEPA[1] analysis three correlations between parameters were considered: blood volume with body weight, fraction of MeHg in blood with body weight, and hair/blood partition with elimination half-life. In the PBPK model each of these relationships, along with many others, is defined functionally within the structure of the model and interacts with the parameter selections during the Monte Carlo analysis. For example, the USEPA[1] estimated a correlation of −0.5 for hair/blood partition with half-life; the correlation observed in the PBPK Monte Carlo analysis was −0.66. In the case of the PBPK analysis, however, the variability in the half-life was not used as one of the inputs to determine the variability of DCFs for hair, rather it was an output predicted in parallel with the DCFs.

The use of a PBPK model in place of an empirical compartmental description in an analysis of variability provides several benefits. The principal benefit is the structural framework the model provides, which defines the functional relationship among the physiological, chemical, and pharmacokinetic factors determining the uptake, disposition, and clearance of MeHg in an individual. In the empirical approach it is necessary to combine parameters which are primary determinants of kinetic behavior, such as body weight and hair/blood partition, with parameters which are empirical measures of the kinetics resulting, in part, from these primary determinants, such as the fraction of MeHg body burden in the blood and the half-life for its excretion. In this case, the latter two parameters reflect the results of complex underlying processes, and are functionally dependent on the former two parameters. However, in the compartmental description any functional relationship between the parameters must be determined empirically, an approach which is often hindered by the lack of adequate data.[1]

While an empirical compartmental analysis provides a useful means for summarizing and generalizing kinetic information, its use in extrapolation or uncertainty/variability analysis must be carefully considered. An example of the potential shortcomings of an empirical modeling approach is the importance in the one-compartment models of the blood volume. In contrast, in the PBPK model there is very little sensitivity to the plasma and RBC volumes. Indeed, there is no biological reason to expect a significant dependence of MeHg pharmacokinetics on the volume of the blood. The appearance of blood volume in the equation for the one-compartment description is an artifact of the simplified model structure. The basic description of the one-compartment model is

$$\frac{V\,dC}{dt} = d * BW * A * f - b * V * C$$

where $C$ is the concentration of MeHg in blood ($\mu$g/L), or $C$ = concentration in hair divided by hair/blood partition ($P_{HB}$), $d$ is the daily dietary intake ($\mu$g MeHg/kg/day), BW is the body weight (kg), $A$ is the absorption factor (unitless), $f$ is the fraction of daily intake taken up by the blood (unitless), $b$ is the elimination constant (days$^{-1}$), and $V$ is the volume of blood in the body (L).

At steady state, $dC/dt = 0$ and the equation used by USEPA and ATSDR can be derived. Note that in this description the role of the blood volume is to calculate an apparent extrinsic clearance ($b * V$). From a biological viewpoint, it is this clearance (fecal, hair, etc.) which varies among individuals, and the separation into half-life and blood volume components is an analytical convenience. While this simplification makes no difference in terms of capturing steady-state behavior, it unfortunately imputes an unwarranted influence to a physiological factor (blood volume) which in itself is not actually an important determinant of MeHg kinetics.

## CONCLUSIONS

On the basis of the results of this analysis, the use of an uncertainty factor of 3 for pharmacokinetic variability in the USEPA RfD appears to be more than is necessary. The 1st and 5th percentiles of the distribution of DCFs calculated in this analysis were a factor of 1.8 and 1.5 below the median, respectively. The other factor of 3, for database limitations constitutes a judgment which specifically addresses limitations in the Iraqi study, and is not affected by this analysis. In deriving the MRL, on the other hand, ATSDR did not apply any uncertainty factor. Indeed,

it can reasonably be assumed that the impact of pharmacokinetic variability on the dose–response relationship within an exposed population is adequately reflected in the results of an epidemiological study on a large population such as the Seychelles study cohort. Moreover, many of the concerns regarding limitations in the Iraqi study are addressed by the multigenerational nature of the exposure in the Seychelles and the prospective study design. However, the impact of pharmacokinetic variability in the U.S. population of women of child-bearing age must still be considered, since in deriving the MRL it was necessary to use a pharmacokinetic model to calculate an ingestion rate associated with the hair concentration derived from the epidemiological cohort.

The results of the present analysis of pharmacokinetic variability can be used to provide a consistent adjustment for pharmacokinetic variability in these two guidelines by selecting an ingestion rate which would be associated with a hair concentration equal to or lower than the desired level for 95% of the population, rather than for an average person. Specifically, if the ratio of the 5th and 50th percentiles (1.5) is used in both cases as a measure of the pharmacokinetic variability in an ingestion rate for U.S. women of child-bearing age associated with a hair mercury concentration derived from an epidemiological study, and if the USEPA's uncertainty factor of 3 for database inadequacies is retained in the case of the RfD, the result would be an RfD of 0.2 $\mu$g/kg/day and an MRL of 0.3 $\mu$g/kg/day. Derived in this way, both guidelines would represent reasonable estimates of an acceptable daily MeHg ingestion rate in a population of U.S. women of child-bearing age.

The sensitivity analyses performed in this study suggest that the most important determinants of pharmacokinetic variability for MeHg are the hair/blood partition, body weight, and hair growth rate. The first two parameters have been the subject of much greater attention than the third. The hair growth rate used in the PBPK model is in units of liters per hour. Thus it represents a composite of linear hair growth rate, hair diameter, hair follicle density, and body surface area covered with hair. Of course, the values of these components vary not only among individuals, but also among different areas of skin on the same individual. More data are needed on the potential for racial or ethnic differences in both hair parameters, since they could lead to significantly different pharmacokinetic susceptibility to the effects of MeHg across populations.

## APPENDIX. CALCULATION OF GLOBAL DISTRIBUTION FOR HAIR-TO-BLOOD RATIOS

A global mean and standard deviation for the MeHg hair-to-blood ratio were computed from nine independent studies.[24,48–55] In one case,[53] the hair-to-blood ratio of MeHg was defined by the sample size $n$, the estimated mean $\bar{x}$, and the estimated standard deviation $s$. However, in most of the studies only the linear coefficient, sample size, and the standard error from a regression analysis of the blood concentrations of MeHg to the hair concentrations of MeHg were available. In a few cases, the correlation coefficient $R$ or its square was given instead of the standard error. In the cases where the linear regression coefficient, sample size, and either the standard error or the correlation coefficient were given, the mean and standard deviation of the hair to blood ratio were determined based on the formulas given below.

Given the following definition of the regression equation:

$$\text{HairConc} = h$$

$$\text{Bloodconc} = b$$

$$h = a \times b + e$$

then $\hat{a}$ is the estimate of $a$, $n$ is the sample size, and $\text{se}(\hat{a})$ is the standard error. When $R$ or $R^2$ was given instead of the standard error, $\text{se}(\hat{a})$ was determined as

$$\text{se}(\hat{a}) = \frac{\hat{a} \times \left(\frac{1}{R^2} - 1\right)}{n - 2}$$

From this, we determined the following:

$$\hat{a} = \frac{\sum b_i \times h_i}{\sum b_i^2} \approx \text{mean of } \frac{h}{b} = \bar{x}$$

$$\text{Var}\, h = \sigma^2 = \text{Var}\, e^2$$

$$\text{Var}\, a = \frac{\sigma^2}{\sum b_i^2}$$

$$\text{Var}\left(\frac{h}{b} \,\middle|\, b\right) = \frac{\sigma^2}{b^2}$$

Then the standard deviation of $h/b$, the hair-to-blood ratio, is computed from

$$\text{Var}\left(\frac{h}{b}\right) = E\left(\text{Var}\left(\frac{h}{b}\,\middle|\, b\right)\right) + \text{Var}\left(E\left(\frac{h}{b}\,\middle|\, b\right)\right)$$

$$= \sigma^2 \times E\left(\frac{1}{b^2}\right) + 0$$

$$= \sigma^2 \times E\left(\frac{1}{b^2}\right)$$

If we use the approximation

$$E\left(\frac{1}{b^2}\right) \approx \frac{n}{\sum (b_i)^2}$$

then

$$\text{Var}\left(\frac{h}{b}\right) = n \times \text{Var}\,\hat{a}$$

$$s \text{ of } \left(\frac{h}{b}\right) = \sqrt{n} \times \text{se}(\hat{a})$$

Once the means and standard deviations were computed for all the studies, the means and standard deviations were combined into a global mean and standard deviation using the equations

$$\text{Var}\, a = \frac{\sigma^2}{\sum b_i^2}$$

$$\text{Var}\left(\frac{h}{b}\,\middle|\, b\right) = \frac{\sigma^2}{b^2}$$

Then the standard deviation of $h/b$, the hair-to-blood ratio, is computed from

$$\text{Var}\left(\frac{h}{b}\right) = E\left(\text{Var}\left(\frac{h}{b}\,\middle|\, b\right)\right) + \text{Var}\left(E\left(\frac{h}{b|b}\right)\right)$$

$$= \sigma^2 \times E\left(\frac{1}{b^2}\right) + 0$$

$$= \sigma^2 \times E\left(\frac{1}{b_2}\right)$$

If we use the approximation

$$E\left(\frac{1}{b^2}\right) \approx \frac{n}{\sum (b_i)^2}$$

then

$$\text{Var}\left(\frac{h}{b}\right) = n \times \text{Var}\,\hat{a}$$

$$s \text{ of } \left(\frac{h}{b}\right) = \sqrt{n} \times \text{se}(\hat{a})$$

Once the means and standard deviations were computed for all the studies, the means and standard deviations were combined into a global mean and standard deviation as follows. Given $n_i$, $\bar{x}_i$, and $s_i^2$ for each study $i$, where

$$s_i^2 = \frac{1}{n_i - 1} \sum_j (x_{ij} - \bar{x}_i)^2$$

$$N = \sum_i n_i$$

We obtain

$$\text{Global mean} = \bar{x} = \frac{1}{N} \sum_i \sum_j x_{ij} = \frac{1}{N} \sum_i (n_i \times \bar{x}_i)$$

$$\text{Global } s^2 = \frac{1}{N-1} \sum_i \sum_j (x_{ij} - \bar{x}_i + \bar{x}_i - \bar{x})^2$$

$$= \frac{1}{N-1} \sum_i \left\{ \sum_j [(x_{ij} - \bar{x}_i)^2 + n_i(\bar{x}_i - \bar{x})^2] \right\}$$

$$= \frac{1}{N-1} \left[ \sum_i (n_i - 1) s_i^2 + \sum_i n_i (\bar{x}_i - \bar{x})^2 \right]$$

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