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Reference Dose for Methylmercury

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EXECUTIVE SUMMARY

In 1997, U.S. EPA issued the *Mercury Study Report to Congress* (MSRC*).* Among the assessments in the MSRC was a state-of-the-science evaluation of the health effects of methylmercury. There has been considerable discussion within the scientific community regarding the level of exposure to methylmercury that is likely to be without appreciable risk of adverse health effects. Congress directed EPA through the House Appropriations Report for FY99 to contract with the National Research Council to evaluate the body of data on the health effects of methylmercury, with particular emphasis on new data since the 1997 *Mercury Study Report to Congress*, and provide recommendations regarding issues relevant to the derivation of an appropriate RfD for methylmercury. EPA has thoroughly reviewed this document, and concurs with the NRC findings and recommendations. The NRC document is used as the basis for the current EPA derivation of the RfD for methylmercury.

Methylmercury is a highly toxic substance; there are a number of adverse health effects associated with methylmercury exposure. Most extensive are the data for neurotoxicity, particularly in developing organisms. Therefore the brain is considered to be the most sensitive target organ for which there are data suitable for derivation of an RfD. The NRC considered three epidemiological longitudinal developmental studies suitable for quantitative risk assessment: the Seychelles Islands, the Faroe Islands, and New Zealand. The Seychelles study has yielded no evidence of impairment related to methylmercury exposure, while both the other studies have found dose-related adverse effects on a number of neuropsychological endpoints. The Faroe Islands study is the larger of the latter two studies, and has been extensively peer-reviewed. Therefore, the Faroe Islands study is used for derivation of the RfD. A benchmark dose analysis was chosen as the most appropriate method of quantifying the dose-effect relationship, using the lower limit on a 5% effect level obtained by applying a K power model (K $ 1) to dose-response data based on Hg in cord blood. The Boston Naming Test was chosen as the critical endpoint. This is the endpoint that yields the second lowest BMDL (bench mark dose lower limit), but is judged to be more reliable than the endpoint that yields the lowest BMDL. The BMDL is 58 ppb Hg in cord blood for the Boston Naming Test from the Faroe Islands study. Dose conversion is based on a one-compartment model similar to that used in the *Mercury Study Report to Congress.*

With regard to uncertainty factors, EPA discusses several sources of variability and uncertainty and chooses an uncertainty factor of 10. This is based on a factor of 3 for pharmacokinetic inter-individual variability, and an additional factor of 3 for data gaps with regard to possible long-term sequelae for neurotoxic effects, cardiovascular effects, uncertainty concerning the relationship between cord and maternal blood mercury concentrations, and reproductive effects.

The RfD derived in this assessment is 0.1 ug/kg per day. This is the same as the RfD derived by EPA in 1995 based on an earlier study of a poisoning episode in Iraq, in

which data on adverse neurological effects in infants was used as the point of departure for derivation of the RfD.

1. BACKGROUND

EPA has published two reference doses (RfDs) for methylmercury that represented the Agency consensus at that time. The original RfD of 0.3 µg/kg/day was determined in 1985. The current RfD of 0.1 µg/kg/day was established as the Agency consensus estimate in 1995. While EPA was developing the *Mercury Study Report to Congress* (MSRC) (U.S. EPA, 1997), it became apparent that considerable new data on the health effects of methylmercury in humans were emerging. Among these data sources were large studies of seafood-consuming populations in the Seychelles and Faroe Islands. Smaller scale studies were being reported on effects in populations around the U.S. Great Lakes and in the Amazon basin. Publications also included novel statistical approaches and applications of physiologically based pharmacokinetic (PBPK) models.

In 1997 the MSRC was undergoing final review; at that time many of the new data had either not been published in the peer-reviewed press or not been subjected to rigorous review. EPA decided that it was premature to make a change in the 1995 methylmercury RfD for the MSRC. This decision was in accordance with the advice of the Science Advisory Board (SAB). Since 1997 the field of methylmercury toxicology and assessment has expanded dramatically. This document presents a revised RfD that considers data from the human studies published in the 1990s, recent evaluations of health and pharmacokinetic data, and recent statistical and modeling approaches to assessing those data.

The following sections include brief descriptions of the previously published EPA RfDs as well as descriptions of some of the evaluation processes that took place at the end of the 1990s.

1.1 Other RfDs Published by EPA

1.1.1 1985 RfD

A hazard identification and dose-response assessment was proposed for methylmercury in 1980 (U.S. EPA, 1980). This assessment was reviewed and consensus was achieved by the EPA RfD/RfC (reference concentration) Work Group on December 2, 1985. This RfD was published on EPA's Integrated Risk Information System (IRIS) in 1986. The critical effects were multiple central nervous system (CNS) effects, including ataxia and paresthesia in populations of humans exposed to methylmercury through consumption of contaminated grain (summarized by Clarkson et al., 1976; Nordberg and Strangert, 1976; and WHO, 1976).

The RfD for methylmercury was determined to be 3×10^{-4} mg/kg-day (0.3 µg/kg/day), based on a lowest observed adverse effect level (LOAEL) of 0.003 mg/kg-day (corresponding to 200 µg/L blood concentration) and an uncertainty factor of 10 to adjust the LOAEL to what is expected to be a no observed adverse effect level (NOAEL). An additional uncertainty factor of 10 for sensitive individuals for chronic

exposure was not deemed necessary, as the adverse effects were seen in what was regarded as a sensitive group of individuals: adults who consumed methylmercury-contaminated grain.

The RfD/RfC Work Group ascribed medium confidence to the choice of study, the database, and the RfD. The blood levels associated with the LOAEL were well supported by more recent data, but neither the chosen studies nor supporting database described a NOAEL. Medium confidence generally indicates that new data may change the assessment of the RfD.

1.1.2 1995 RfD

After publication of the RfD of 0.3 µg/kg/day, questions were raised as to its validity; some of these questions were in formal submissions requesting a change on the IRIS entry. In particular it was asked whether the RfD based on effects in exposed adults was protective against developmental effects. Subsequent to the RfD publication, the effects in Iraqi children of *in utero* exposure to methylmercury were reported by Marsh et al. (1987). The RfD/RfC Work Group discussed the methylmercury RfD in 1992 and again in 1994. Consensus on a revised RfD was reached in January 1995. Detailed description of the RfD derivation can be found in Volume V of the MSRC (U.S. EPA, 1997).

Marsh et al. (1987) was chosen as the most appropriate study for determination of an RfD protective of a putative sensitive subpopulation, namely infants born to mothers exposed to methylmercury during gestation. The data collected by Marsh et al. (1987) summarize clinical neurologic signs of 81 mother-and-child pairs. Maternal hair mercury concentrations were collected as the exposure metric. Concentrations ranging from 1 to 674 ppm mercury were determined from X-ray fluorescent spectrometric analysis of selected regions of maternal scalp. These were correlated with clinical signs observed in the affected members of the mother-child pairs. The hair concentration at a hypothetical NOAEL for developmental effects was determined by application of a benchmark dose (BMD) approach (see subsequent section for discussion of methods and data used). The analysis used the combined incidence of all neurological effects in children exposed *in utero* as reported in the Marsh et al. (1987) study. A Weibull model for extra risk was used to determine the BMD; in current terminology, this was a benchmark dose limit (BMDL) (95% lower confidence limit) on the dose corresponding to a 10% risk level. This level was calculated to be 11 ppm mercury in maternal hair (11 mg/kg hair). A description of BMD determination, choice of model, and issues on grouping of data is on pages 6-25 to 6-31 of Volume V of the MSRC.

The BMD of 11 ppm maternal hair mercury was converted to an exposure level of 44 Fg mercury/L blood using a 250:1 ratio:

$$11 \text{ mg/kg hair} / 250 = 44 \text{ Fg/L blood}$$

To obtain a daily dietary intake value of methylmercury corresponding to a specific blood concentration, factors of absorption rate, elimination rate constant, total blood volume, and percentage of total mercury present in circulating blood were taken into account. Calculation was by the following equation, based on the assumptions that steady-state conditions exist and that first-order kinetics for mercury are being followed.

$$d\ \mu g/day = \frac{C \times b \times V}{A \times f}$$

where:

d	=	daily dietary intake (expressed as µg of methylmercury)
c	=	concentration in blood (expressed as 44 µg/L)
b	=	elimination constant (expressed as 0.014 $days^{-1}$)
V	=	volume of blood in the body (expressed as 5 L)
A	=	absorption factor (expressed as a unitless decimal fraction of 0.95)
f	=	fraction of daily intake taken up by blood (unitless, 0.05)

Solving for d gives the daily dietary intake of mercury that results in a blood mercury concentration of 44 µg/L. To convert this to daily ingested dose (µg/kg-day), a body weight of 60 kg was assumed and included in the equation denominator:

$$d = \frac{c \times b \times V}{A \times f \times bw}$$

$$d = \frac{44\ \mu g/L \times 0.014\ days^{-1} \times 5\ L}{0.95 \times 0.05 \times 60\ kg}$$

$$d = 1.1\ \mu g/kg\text{-}day$$

The dose d (1.1 µg/kg-day) is the total daily quantity of methylmercury that is ingested by a 60 kg individual to maintain a blood concentration of 44 µg/L or a hair concentration of 11 ppm. The rationales for use of the hair:blood ratio and specific values for equation parameters can be found on pages 6-21 to 6-25 of Volume V of the MSRC.

A composite uncertainty factor of 10 was used. This uncertainty factor was applied for variability in the human population, in particular the wide variation in biological half-life of methylmercury and the variation that occurs in the hair-to-blood ratio for mercury. In addition, the factor accounts for lack of a two-generation reproductive study and lack of data for possible chronic manifestations of the adult effects (e.g., paresthesia observed during gestation). The default value of 1 was used for the modifying factor.

The RfD was calculated using the following equation:

$$RfD = \frac{BMD}{UF \times MF}$$

$$= \frac{1.1\ \mu g/kg/day}{10}$$

$$= 1 \times 10^{-4}\ mg/kg/day$$

or 0.1 µg/kg/day.

Confidence in the supporting database and in the RfD were considered medium by the RfD/RfC Work Group. The MSRC (U.S. EPA, 1997) says the following:

> The principal study (Marsh et al. 1987) is a detailed report of human exposures with quantitation of methylmercury by analysis of specimens from affected mother-child pairs. A strength of this study is that the quantitative data are from the affected population and quantitation is based upon biological specimens obtained from affected individuals. A threshold was not easily defined; extended application of modeling techniques was needed to define the lower end of the dose-response curve. This may indicate high variability of response to methylmercury in the human mother-child pairs or misclassification in assigning pairs to the cohort.

Further discussion of areas of uncertainty and variability are on pages 6-31 to 6-51 of Volume V of the MSRC (U.S. EPA, 1997). A quantitative analysis of uncertainty in an RfD based on the Iraqi data is found in Appendix D of Volume V, and additional discussions of areas of uncertainty are in Volume VII, Risk Characterization, of the MSRC (U.S. EPA, 1997).

1.2 Risk Assessments Done by Other Groups

Quantitative estimates of hazards of oral exposure to methyl mercury exposure have been considered by the Food and Drug Administration (FDA), Agency for Toxic Substances and Disease Registry (ATSDR), and other countries (WHO/IPCS), among others.

1.2.1 Food and Drug Administration

In 1969, in response to the poisonings in Minamata Bay and Niigata, Japan, the U.S. FDA proposed an administrative guideline of 0.5 ppm for mercury in fish and shellfish moving in interstate commerce. This limit was converted to an action level in 1974 (Federal Register 39, 42738, December 6, 1974) and increased to 1.0 ppm in 1979 (Federal Register 44; 3990, January 19, 1979) in recognition that exposure to mercury was less than originally considered. In 1984, the 1.0 ppm action level was

covered from a mercury standard to one based on methyl mercury (Federal Register 49; November 19, 1984).

The action level takes into consideration the tolerable daily intake (TDI) for methyl mercury, as well as information on seafood consumption and associated exposure to methyl mercury. The TDI is the amount of methyl mercury that can be consumed daily over a long period of time with a reasonable certainty of no harm. U.S. FDA established a TDI based on a weekly tolerance of 0.3 mg of total mercury per person, of which no more than 0.2 mg should be present as methyl mercury. These amounts are equivalent to 5 and 3.3 ug, respectively, per kilogram of body weight. Using the values of merthylmercury, this tolerable level would correspond to approximately 230 ug/week for a 70 kg person or 33 ug/person/day. The TDI was calculated from data developed in part by Swedish studies of Japanese individuals poisoned in the episode of Niigata which resulted from the consumption of contaminated fish and shellfish and the consideration of other studies of fish-eating populations.

Based on observations from the poisoning event later in Iraq, U.S. FDA has acknowledged that the fetus may be more sensitive than adults to the effects of mercury (Federal Register 44; 3990, January 19, 179; Cordle and Tollefson, 1984, U.S. FDA Consumer, September, 1994). In recognition of these concerns, U.S. FDA has provided advice to pregnant women and women of child-bearing age to limit their consumption of fish known to have high levels of mercury (U.S. FDA Consumer, 1994). U.S. FDA believes, however, that given existing patterns of fish consumption, few women (less than 1%) eating such high mercury fish will experience slight reductions in the margin of safety. However, due to the uncertainties associated with the Iraqi study, U.S. FDA has chosen not to use the Iraqi study as a basis for revising its action level. Instead, the U.S. FDA has chosen to wait for findings of prospective studies of fish-eating populations in the Seychelles Islands.

1.2.2 World Health Organization

The International Programme on Chemical Safety (IPCS) of the World Health Organization published a criteria document on mercury (WHO 1990). In that document, it was stated that “a daily intake of 3 to 7 ug Hg/kg body weight would cause adverse effects of the nervous system, manifested as an approximately 5% increase in the incidence of paraesthesias”. The IPCS expert group also concluded that developmental effects in offspring (motor retardation or signs of CNS toxicity) could be detected as increases over background incidence at maternal hair levels of 10-20 ppm mercury. These levels of concern were based on evaluation of data including the human poisoning incident in Iraq.

1.2.3 ATSDR

In 1998, ATSDR used the Seychelle Islands study (Davidson et al. 1998) as the starting point for estimating a minimal risk level for exposure to MeHg (ATSDR 1999). In this study, the investigators examined the correlation between subtle neurological effects and low-dose chronic exposure to MeHg. No correlation between Hg concentrations and neurological effects was seen. ATSDR determined a minimal risk level of 0.3 ug/kg per day, based on a dose of 1.3 ug;/kg per day, which reflects the average concentration of the upper quintile of the exposed population but does not necessarily correspond to a no-observed-adverse-effect level (NOAEL). The agency used two uncertainty factors of 1.5 each to account for pharmacokinetic and pharmacodynamic variability within the human population. A modifying factor of 1.5 was applied to account for the possibility that domain-specific tests used in the Faroe Island study might have allowed detection of subtle neurological effects that were not evaluated in the Seychelle cohort. Although the conventional risk assessment approach is to multiply uncertainty factors, the agency summed these factors to develop an overall safety factor of 4.5.

1.3 National Academy of Sciences Review

Congress directed EPA, through the House Appropriations Report for FY99, to contract with the National Research Council (NRC, a body of the National Academy of Sciences) to evaluate the body of data on the health effects of methylmercury, with particular emphasis on new data since the publication of the MSRC. NRC was asked to provide recommendations regarding issues relevant to the derivation of an appropriate RfD for methylmercury.

The NRC empaneled a group of scientific experts who held public meetings at which there were presentations from methylmercury researchers, government agencies, trade organizations, public interest groups, and concerned citizens. The panel evaluated the scientific basis for risk assessments done by EPA and other groups as well as new data and findings available since publication of the MSRC. The committee was not charged with developing an RfD as an alternative to the EPA assessment, but rather provided scientific guidance that would inform such an assessment. The NRC report, *Toxicological Effects of Methylmercury*, was released to the public on July 11, 2000. Conclusions of that report are summarized below.

The report concludes that methylmercury is a highly toxic substance; a number of adverse health effects are associated with methylmercury exposure identified in humans and in animal studies. Most extensive are the data for neurotoxicity, particularly in developing organisms. The nervous system is considered by the NRC Committee to be the most sensitive target organ for which there are data suitable for derivation of an RfD. The committee also concludes on the basis of data from humans and from animal studies that exposure to methylmercury can have adverse effects on the developing and adult cardiovascular system. They note that some research demonstrated adverse cardiovascular effects at or below levels associated with effects

on the developing nervous system. The NRC also cites evidence of low-dose methylmercury effects on the immune and reproductive systems.

The NRC report presents some conclusions on the public health implications of methylmercury exposure; one conclusion is quoted below:

> The committee's margin-of-exposure analysis based on estimates of MeHg exposure in the U.S. population indicates that the risk of adverse effects from current MeHg exposure in the majority of the population is low. However, individuals with high MeHg exposure from frequent fish consumption might have little or no margin of safety (i.e., exposures of high-end consumers are close to those with observable adverse effects). The population at highest risk is the children of women who consumed large amounts of fish and seafood during pregnancy. The committee concludes that the risk to that population is likely to be sufficient to result in an increase in the number of children who have to struggle to keep up in school and who might require remedial classes or special education.

The NRC report gives an evaluation of the 1995 EPA RfD. Their conclusion is as follows:

> On the basis of its evaluation, the committee's consensus is that the value of EPA's current RfD for MeHg, 0.1 µg/kg/day, is a scientifically justifiable level for the protection of public health. However, the committee recommends that the Iraqi study no longer be used as the scientific basis of the RfD.

The NRC report made several recommendations on the appropriate basis for a revised RfD. The Committee thoroughly reviewed three epidemiological longitudinal developmental studies: the Seychelles Islands, the Faroe Islands, and New Zealand. (For descriptions of these studies see pages x-xx of the draft criteria document for methylmercury. Very brief summaries are in Section 2.1 of this document.) The Seychelles study yielded scant evidence of impairment related to *in utero* methylmercury exposure through 5.5 years of age, whereas the other two studies found dose-related effects on a number of neuropsychological endpoints. The Faroe Islands study is the larger of the latter two studies and has been extensively peer-reviewed. NRC recommends use of data from the Faroe Islands study for derivation of the RfD.

NRC recommends BMD analysis as the most appropriate method of quantifying the dose-effect relationship. They recommend the lower limit on a 5% effect level obtained by applying a K-power model (K$1) to dose-response data based on Hg in cord blood. NRC noted that for the Faroe Islands data the results of the K-power model under this constraint are equivalent to a linear model.

NRC recommends use of the Boston Naming Test as the critical endpoint. This endpoint yields the second-lowest BMDL but was judged by the Committee to be more reliable than the endpoint that yields the lowest BMDL. The BMDL for the Boston Naming Test from the Faroe Islands study is 58 ppb Hg in cord blood.

NRC described alternative dose conversion processes using a one-compartment model similar to that used in the MSRC.

In their discussion of uncertainty factors, NRC reviews several sources of variability and uncertainty and recommends that an uncertainty factor of at least 10 be used. NRC recommends a factor of 2 to 3 for biological variability in dose estimation. They also recommend an additional factor to account for data gaps relating to possible long-term neurological effects not evident in childhood, as well as possible effects on the immune and cardiovascular systems.

1.4 Revised RfD

This document presents the derivation of a revised RfD. The development of this RfD considered the NRC recommendations and followed them for the most part. The following sections describe each choice and provide the rationale.

2. CHOICE OF CRITICAL STUDY AND ENDPOINT

NRC concluded, and EPA agrees, that the data from human studies showing developmental neurotoxicity are the most appropriate basis for the RfD. NRC concluded that human studies on methylmercury carcinogenicity are inconclusive and that the renal tumors observed in mice were found only when animals were exposed at or above the maximally tolerated dose (MTD). In the MSRC, EPA noted that if one applied the principles of the revisions to the Risk Assessment Guidelines for Carcinogenicity, the following conclusions would be reached:

> Methylmercury is not likely to be a human carcinogen under conditions of exposure generally encountered in the environment. Data in humans were inadequate; interpretation is limited by inappropriate study design and incomplete descriptions of methodology. Dietary exposure in two strains of mice resulted in increased renal adenomas and adenocarcinomas. Tumors were observed only in dose groups experiencing profound nephrotoxicity. Studies in rats exposed to an MTD showed no increased tumor incidence. Several studies show that methylmercury can cause chromosomal damage in somatic cells. While evidence is good for chromosomal effects, it does not appear that methylmercury is a point mutagen. The mode of action in renal tumor induction is likely to be related to reparative changes in the tissues. Human exposure is likely to be from consumption of contaminated foods, especially fish. It is expected that exposure, even in groups consuming large amounts of fish from contaminated sources, will be to levels far below those likely to cause the tissue damage associated with tumor formation in animals (U.S. EPA, 1997).

NRC concluded that human data, as well as results of animal tests, indicate the cardiovascular system is a sensitive target for methylmercury effects. This is particularly true for developing organisms. Their report also cites animal and *in vitro* data linking methylmercury exposure to immunotoxic and reproductive effects. It is clear, however, that at the current time the human data set on developmental neurotoxicity is the most extensive, best reviewed, and most thoroughly evaluated. The RfD will thus rely on those data. It is expected that an RfD based on developmental neurotoxicity will be protective against adverse effects likely to occur at higher levels of mercury exposure. Following NRC's recommendation, EPA's choice of critical study was limited to those developmental studies of populations experiencing long-term, low-dose exposure. Only those studies are summarized in subsequent sections of this document.

2.1 Summary of Available Data

This section gives brief summaries of studies on the developing central nervous system that were described by NRC. This section follows the format used by the NRC report; studies are grouped into subsections by endpoint and chronologically within subsection. Section 2.1.1 describes the evidence for effects of methylmercury on neurological status; Section 2.1.2 describes the effects on attainment of developmental milestones during infancy; Section 2.1.3 describes other effects during infancy and early childhood; Section 2.1.4 presents evidence for cognitive deficits during childhood (schoold age); and Section 2.1.5 describes sensory and other effects of methylmercury.

For more detailed study descriptions refer to the MSRC.

2.1.1 Status on Neurological Examination

2.1.1.1 Cree population—McKeown-Eyssen et al. (1983)

McKeown-Eyssen et al. (1983) studied a population of 234 12- to 30-month-old Cree children for whom prenatal methylmercury exposure was estimated on the basis of maternal hair samples. The subjects lived in four communities in northern Quebec. Hair samples were collected on 28% of the mothers during pregnancy; prenatal exposure for the rest of the cohort was estimated from hair segments assumed to date from the time the study child was *in utero*. No child was judged to have any abnormal physical findings. Overall, 3.5% (4) of the boys and 4.1% (5) of the girls were considered to have abnormal neurological findings. The most frequent abnormality (observed in 11.4% [13] of the boys and 12.2% [14] of the girls) involved tendon reflexes. Abnormalities of muscle tone or reflexes in boys were the only neurological finding for which there was a statistically significant association with prenatal methylmercury exposure, either before or after adjustment for confounding. The risk of an abnormality of tone or reflexes increased seven times with each 10 ppm increase in maternal hair mercury. When exposure was categorized, the prevalence of tone or reflex abnormality did not increase in a clear dose-response manner across categories. In girls, incoordination was negatively associated with prenatal methylmercury exposure. The authors noted that these mild, isolated neurologic findings were different from those described in previous reports of neurologic abnormalities after prenatal exposure to higher levels of methylmercury.

2.1.1.2 Mancora, Peru—Marsh et al. (1995)

Neurological examination was done on 194 children in Mancora, Peru. Although the study was conducted in the early 1980s, it was not published until 1995 (Marsh et al., 1995). Fish consumption was the primary route of MeHg exposure, and maternal hair was used as the index of exposure (geometric mean 7.05 ppm; range 0.9 to 28.5 ppm). Comparison of peak and mean hair-mercury concentration suggested that the women's exposure was at steady state because of stability in their fish-consumption patterns. Maternal hair samples and data on child neurological status were available for 131 children. Several elements of the study design are not described: the size of the eligible population from which the 131 children were sampled, the specific elements of the neurological assessment conducted, and the ages at which the children were examined. Frequencies are reported for the following endpoints: tone decreased, tone increased, limb weakness, reflexes decreased, reflexes increased, Babinski's sign, primitive reflexes, and ataxia. No endpoint was significantly associated with either mean or peak maternal hair mercury.

2.1.1.3 SCDS Pilot Study—Myers et al. (1995)

In the cross-sectional or pilot study of the SCDS (Myers et al., 1995), 789 infants and children between the ages of 5 and 109 weeks were evaluated by a pediatric neurologist. Mean maternal hair mercury in the cohort was 6.1 ppm (range 0.6 to 36.4 ppm). The endpoints assessed were mental status, attention, social interactions, vocalizations, behavior, coordination, postures and movements, cranial nerves, muscle strength and tone, primitive and deep tendon reflexes, plantar responses, and age-appropriate abilities such as rolling, sitting, pulling to stand, walking, and running. The statistical analyses focused on three endpoints chosen on the basis of their apparent sensitivity to prenatal methylmercury exposure in the Iraq and Cree studies: overall neurological examination, increased muscle tone, and deep tendon reflexes in the extremities. There was no association between maternal hair mercury and questionable and abnormal results. The frequency of those results ranged from 16.5% in the group with Hg at 0 to 3 ppm, to 11.7% in the group with Hg at more than 12 ppm. The frequencies of abnormalities of limb tone or deep tendon reflexes were about 8%; there was no dose-dependent variation in frequency of either endpoint.

2.1.1.4 SCDS Main Study—Myers et al. (1995b)

The main cohort of the SCDS consisted of 779 mother-infant pairs, representing approximately 50% of all live births during the period of recruitment. The final sample size was 740. When the infants were 6.5 months old, a pediatric neurologist administered essentially the same neurological examination that had been used in the pilot phase; testing was blinded as to child's exposure. A total of 3.4% (25) of the children had overall neurological scores considered abnormal or questionable; this frequency was too low to permit statistical analysis of the overall neurological examination. The frequency of abnormalities was 2% for both limb tone and abnormal deep tendon reflexes. Questionable limb tone was identified in approximately 20% of the children, and questionable deep tendon reflexes in approximately 15%. Although such findings were not considered pathological, they were combined with abnormal findings for statistical analyses. The frequency of abnormal and questionable findings for limb tone or deep tendon reflexes was not significantly associated with maternal hair mercury concentrations.

2.1.1.5 Faroes Population—Dahl et al. (1996)

A functional neurological exam was part of a general physical examination administered to a cohort of 7-year-old children from the Faroe Islands. Of 1,386 infants eligible at recruitment, cord-blood and maternal hair samples were obtained from 1,022 singleton births (75%), and 917 children were examined (66%) (Grandjean et al., 1992). The mean cord-blood concentration was 22.9 µg/L; the mean maternal hair mercury concentration was 4.3 ppm. The examination focused on motor coordination and perceptual-motor performance (Dahl et al., 1996). Results were scored as automatic, questionable, or poor. There was no association between cord-blood mercury and the number of tests on which a child's performance was considered automatic or performed optimally. On the tests of reciprocal motor coordination, simultaneous finger

movement, and finger opposition, fewer than 60% of the children achieved a score of automatic for optimal performance. On the finger opposition test, children with questionable and poor performance (425 children) had a significantly higher mean cord-blood Hg concentration than children with automatic performance (465 children) (23.9 versus 21.8 µg/L, p = 0.04) (Grandjean et al., 1997).

2.1.1.6 Faroes Population—Steurwald et al. (2000)

A cohort of 182 singleton, full-term infants born in the Faroe Islands was recruited for evaluation of the associations between neurological function at 2 weeks of age and various dietary contaminants and nutrients. The cohort represented 64% of all births in the study area. The primary outcome variable was the neurological optimality score (NOS). Two subscores were generated (muscle tone and reflexes) and a variety of thyroid-function indices were also assessed. Data were collected on maternal hair mercury, cord whole-blood mercury, and cord serum mercury. Measurements were also taken of 18 pesticides or metabolites and 28 polychlorinated biphenyl (PCB) congeners in maternal serum. Maternal hair mercury concentrations were not significantly associated with NOS score, but there was a significant inverse relationship between NOS scores and cord whole-blood mercury. The mean mercury concentration was 20.4 µg/L (range 1.9 to 102 µg/L). Based on NOS score, a tenfold increase in cord-blood mercury was associated with the equivalent of a 3-week reduction in gestational age. Adjustments for total PCBs and fatty acid concentrations had no effect on results, and selenium was not an effect modifier. Muscle-tone and reflexes subscores were not significantly associated with any exposure biomarker.

2.1.1.7 Cordier and Garel (1999)

Cordier and Garel (1999) studied a cohort of Amerind children from a gold-mining area in French Guiana. Median maternal hair concentration was 6.6 ppm with a range of 2.9 to 17.8 ppm; 35% of maternal hair mercury levels were greater than 10 ppm. Neurologic examination included the following: neuromotor examination of the upper an lower limbs, body axis, deep reflexes, and postural reactions; neuromotor functions; neurosensory examination; and cranial growth. The authors report that for children greater than 2 years of age, increased reflexes were found with greater incidence as a function of maternal hair mercury; the effect was greater in boys than in girls. When 10 children were retested 9 months later by a different examiner, only 3 were found to have the increased reflex response. The authors commented that this poor reproducibility makes the reflex response difficult to interpret.

2.1.1.8 Conclusions

There is some evidence that neurological status in children is associated with low-dose *in utero* exposure: (1) an increased incidence (not dose dependent) of tone or reflex anomalies in boys associated with increased maternal hair mercury (McKeown-

Eyssen et al., 1983); (2) an inverse association between newborn neurological optimality score and cord-blood mercury in Faroese children (Steurwald et al., 2000); (3) a statistically significant increase in the mean cord-blood mercury of 7-year-old Faroese children who performed less than optimally on a finger opposition test, compared with Faroese children with normal performance (Grandjean et al., 1997); (4) the association of increased reflexes with increasing maternal hair mercury in a group of children aged 9 months to 6 years in French Guiana (Cordier and Garel, 1999). NRC notes that a particular limitation of the use of neurological status is the categorical nature of the response; in other words, the subject has either an abnormal response or a normal response. This may have been a factor in the evaluation of results from the SCDS. The number of abnormal responses in this population was very low; thus there was reduced statistical power for hypothesis testing.

2.1.2 Age at Achievement of Developmental Milestones

2.1.2.1 SCDS—Myers et al. (1997) and Axtell et al. (1998)

The association between achievement of developmental milestones and prenatal methylmercury exposure was evaluated in the main cohort of the SCDS (Myers et al., 1997). Data were available for 738 of the 779 children enrolled. The mean average age for walking was 10.7 months for girls and 10.6 months for boys; for talking it was 10.5 months for girls and 11.0 months for boys. The mean age at which a child was considered to talk was not significantly associated with maternal hair mercury in any of the regression models used. In regressions stratified by child sex, a positive association was found between age at walking and maternal hair mercury in boys only. The interaction between mercury and sex was not statistically significant in the analyses of the complete cohort. The authors considered the magnitude of the delay in boys' walking to be clinically insignificant; a 10 ppm increase in maternal hair mercury was associated with approximately a 2-week delay. This association in boys was not significant when four statistical outliers were excluded from the analysis. Authors concluded that the hockey-stick models provided no evidence of a threshold for developmental delay, as the fitted curves were essentially flat.

Axtell et al. (1998) reanalyzed the milestone data, applying semiparametric generalized additive models that are less restrictive than the approaches used by Myers et al. (1997). Their major finding was that the association between age at walking and maternal hair mercury in boys was nonlinear. In their modeled estimates, walking was delayed as maternal hair concentrations increased from 0 to 7 ppm but was observed at a slightly earlier age as mercury concentration increased beyond 7 ppm. The size of the effect associated with the increase from 0 to 7 ppm was very small, corresponding to a delay of less than 1 day in the achievement of walking. Because of the contradictory nature of the dose-response relationships above and below 7 ppm, the authors expressed a doubt that the association found below 7 ppm reflected a causal effect of mercury exposure on age at walking.

2.1.2.2 Mancora, Peru—Marsh et al. (1995)

Data on developmental milestones were collected in the Peruvian study conducted by Marsh et al. (1995). The study was conducted prospectively and data were apparently collected in an ongoing manner over the course of a mother's visits to a postnatal clinic. Regression analyses, including analyses stratified by child sex, did not reveal any significant associations between maternal hair mercury concentrations and the ages at which children sat, stood, walked, or talked. The rates of developmental retardation, especially in speech (13 of 131), were substantial. Children's birthweight, height, and head circumference were unrelated to maternal hair mercury concentrations.

2.1.2.3 Faroes population—Grandjean et al. (1995)

Ages at achievement of motor development milestones were investigated in a 21-month birth cohort (1,022 infants born in 1986-1987) of children in the Faroe Islands. Complete data were available for 583 children. Three motor-development milestones commonly achieved between 5 and 12 months of age were selected for analysis: "sits without support," "creeps," and "gets up into standing position with support." There was no significant association between age at achievement and either cord-blood or maternal hair mercury for any of the three milestones. For all three, however, the authors reported a significant inverse association between age at achievement and the child's hair mercury concentration at 12 months. Children's hair mercury was interpreted as an index of postnatal exposure to methylmercury. Breast-feeding was associated with both increased hair mercury concentrations and more rapid achievement of milestones. Therefore, the authors concluded that the inverse association reflected residual confounding by duration of breast-feeding.

2.1.2.4 Conclusions

The recent human studies provide little evidence of an association between maternal hair mercury below 30 ppm and delayed developmental milestones. The NRC report noted that in the SCDS, mean age of walking was higher in the part of the population born to mothers with higher hair mercury. The association was for male children only and it was not dose related. In the Faroese population, there was a negative association for maternal hair mercury and three developmental milestones. The study authors attributed this to higher mercury exposure in the breast-fed population and the salutary effect of breast milk on development. The NRC report commented on the reported developmental delays in the Iraqi population, which has been the subject of much discussion as to the degree of uncertainty in the estimates (see also MSRC Volumes V and VII). NRC cites analyses by Cox et al. (1995) and Crump et al. (1995), which indicate that the earlier estimates of the Iraqi threshold for late walking were too low. The threshold for late walking appears highly dependent on assumptions on background incidence, the definition of delayed walking, and the effect of a small number of influential data points.

2.1.3 Infant and Preschool Development

2.1.3.1 Cree population—McKeown-Eyssen et al. (1983)

In the study of a Cree population, the Denver Developmental Screening Test (DDST) was administered to the 12- to 30-month-old children in the cohort (n = 234). Scores were reported as the percentage of items passed on each subscale as well as on the entire test. The authors did not provide estimates of significance of association between test scores and maternal hair mercury concentrations; they concluded that there was no significant association indicative of an adverse effect of methylmercury before or after adjustment for confounding variables.

2.1.3.2 New Zealand population—Kjellstrom et al. (1986)

Kjellstrom et al. (1986) studied a cohort of New Zealand children for whom prenatal MeHg exposure was estimated on the basis of maternal hair samples as well as dietary questionnaires collected during the period when the study child was *in utero*. Exposure information was collected on nearly 11,000 women; the study focused on 935 women who reported eating fish more than three times per week during pregnancy. Seventy-three women had hair mercury concentrations greater than 6 ppm. The 74 children of those women were designated as the high-mercury group. Efforts were made to match each child in the high-Hg group with a reference child on the basis of maternal ethnicity, hospital of birth, maternal age, and child age. In the followup evaluations at 4 years of age, a total of 38 exposed and 36 reference children were tested; this data set included 30 completely matched pairs. Fifty-two percent of the children in the high-mercury group had an abnormal or questionable DDST score compared with 17% of the children in the control group ($p < 0.05$). That result corresponds to an odds ratio of 5.3. Results were similar when pairs that were poorly matched on ethnicity were excluded.

2.1.3.3 SCDS pilot study—Myers et al. (1995)

In the SCDS cross-sectional study, a revised version (DDST-R) of the DDST was administered to 789 children between the ages of 1 and 25 months. No association was found between maternal hair mercury concentration during pregnancy (mean 6.6 ppm) and DDST-R results when normal and questionable examinations were combined. The prevalence of abnormal findings was so low (three children <1%) that the statistical analysis was not meaningful. When abnormal and questionable results were grouped (in 65 children, 8%), high maternal hair mercury concentrations were significantly associated with poor outcomes ($p = 0.04$, one-tailed test). That result was largely attributable to the higher frequency of abnormal and questionable results among children in the highest maternal hair mercury category (greater than 12 ppm), by contrast to the frequency of approximately 7% among children in each of the other four groups (0-3, 3-6, 6-9, and 9-12 ppm).

2.1.3.4 SCDS main study—Myers et al. (1995)

In the main SCDS study, the DDST-R was administered to a cohort of 740 children at age 6.5 months. The frequency of examinations considered to be abnormal or questionable was very low, precluding meaningful statistical analysis of the DDST-R data. They also administered the Fagan Test of Infant Intelligence, an assessment of visual-recognition memory or novelty preference. Results were not related to maternal hair mercury concentrations.

2.1.3.5 SCDS main cohort at 19 and 29 months—Davidson et al. (1995)

The Bayley Scales of Infant Development (BSID) were administered to children in the SCDS cohort at ages 19 and 29 months. In addition, at 29 months, six items of the Infant Behavior Record, a rating scale, were completed by the examiner. There are two primary scores on the BSID: the mental development index (MDI) and psychomotor development index (PDI). At both ages, MDI scores were similar to the expected mean for U.S. children. At both ages, however, the Seychellois SCDS children performed markedly better on PDI than the expected mean for U.S. children. There was no association between MDI scores at 19 or 29 months with maternal hair mercury concentration during pregnancy. Similar results were obtained in a secondary analysis that included only children with the lowest or highest maternal hair mercury concentrations. Assessments of perceptual skills at 19 months were not associated with mercury exposure. Scores on that test at 29 months could not be evaluated because of a pronounced ceiling effect; that is, there were so many high scores on the test that no difference would be detectable. Likelihood of a PDI score below the median was not significantly associated with maternal hair mercury concentration in the full logistic regression model, but was associated with this exposure index in a model that included limited covariates.

2.1.3.6 Conclusions

There is some indication of low-dose mercury effects in very young children, but there are difficulties in the measurement of such effects. The DDST was administered to four study populations. When abnormal and questionable results were combined, there was a significant association with increasing maternal hair mercury in the New Zealand cohort and in the SCDS cross-sectional study (but not the main study). The NRC report comments on the bases for the different findings: age at examination, different rates of abnormal and questionable scores, and the possibility that test items or criteria for judging scores differed among studies. NRC offered the general conclusion that screening tests such as the DDST are not useful in neurobehavioral toxicology studies; such tests are insufficiently sensitive to variations in the range of normal performance.

The NRC panel opined that the BSID is the best currently available instrument for infant assessment and is useful for measurement of prenatal exposures to

neurotoxicants. In the SCDS main study there was no significant association between young children's scores on the BSID and maternal hair mercury. At 19 and 29 months, the Seychellois children scored higher than the means for U.S. children on the PDI portion of the scales.

2.1.4 Childhood Development

2.1.4.1 New Zealand population—Kjellstrom et al. (1989)

Children in the New Zealand cohort were followed up at 6 years of age. Children were given a battery of 26 psychological tests, tests of scholastic aptitude, and behavioral tests. The following domains were assessed: general intelligence, language development, fine and gross motor coordination, academic attainment, and social adjustment. Maternal hair mercury concentration was associated with poorer scores on full-scale IQ tests (Wechsler Intelligence Scale for Children Revised), language development (Test of Language Development, spoken language quotient), and visual-spatial and gross-motor skills (McCarthy Scales of Children's Abilities). Multiple regression analyses were done on these endpoints: Test of Language Development, spoken language quotient (TOLD-SL); Wechsler Intelligence Scale for Children, Revised (WISC-R), performance IQ; WISC-R full-scale IQ; McCarthy Scales, perceptual performance; and McCarthy Scales, motor scales. Covariates in the regressions were these: maternal ethnic group, maternal age, maternal smoking and alcohol use during pregnancy, length of maternal residence in New Zealand, social class, primary language, siblings, sex, birthweight, fetal maturity, Apgar score, and duration of breast-feeding. Observations were weighted in the regression to deal with outliers. In the analyses there were statistically significant associations between maternal hair mercury and poorer scores on the following measures: full-scale IQ; language development (spoken language quotient), visual-spatial skills (perceptual-performance scale), and gross motor skills (motor scale). The poorer mean scores of the children in the high-mercury group were largely attributable to children of mothers with mercury concentrations above 10 ppm. In this group, mean average hair mercury was 13 to 15 ppm and mean peak was 25 ppm. Maternal hair mercury concentrations accounted for relatively small amounts of variance in the outcome measures and generally accounted for less than covariates such as social class and ethnic group.

In the original analyses of five test scores (Kjellstrom et al., 1986), hair mercury was used in regression analyses as a binary variable; that is, either >6 ppm or between 3 and 6 ppm. Their analyses found an association between high prenatal mercury exposure and decreased test performance. Later regression analyses by Crump et al. (1998), which used maternal hair mercury level as a continuous variable, did not find significant associations between mercury and children's test scores. However, this finding was highly influenced by a single child whose mother's mercury hair level (86 ppm) was more than four times that of any other. When this child was excluded, there were significant associations between hair mercury and TOLD-SL and MC-PP scores. When regression analyses were done on scores from all 26 scholastic and psychological tests, and the data on the influential point were omitted, scores on six

tests were significantly associated with mothers' hair mercury: Clay Reading Test-concepts, Clay Reading Test-letter test, McCarthy Scales-general cognitive index, McCarthy Scales-perceptual-performance scale, Test of Language Development-grammar completion, and Test of Language Development-grammar understanding.

2.1.4.2 SCDS pilot study—Myers et al. (1995c)

A portion of the pilot cohort of 789 children were given developmental assessments; these were children who were 66 months old within a 1-year testing window. Of the 247 eligible children, 217 were administered a test battery consisting of the McCarthy Scales of Children's Abilities, the Preschool Language Scale, and two subtests of the Woodcock-Johnson Tests of Achievement (letter-word identification and applied problems). The median maternal hair mercury concentration in that subsample of the pilot cohort was 7.1 ppm. Maternal hair mercury was associated with significantly lower general cognitive index (GCI) scores on the McCarthy scales. Scores declined approximately five points between the lowest and highest exposure categories. Similar associations were found on the perceptual-performance scale of the McCarthy scales and on the auditory comprehension scale of the Preschool Language Scale. Scores declined approximately 2.5 points across the range of maternal hair mercury concentrations. When outliers and influential points were removed from the regressions the statistical significance of the associations was lost for all except auditory comprehension (Preschool Language Scale Auditory Comprehension subscale). In the pilot phase of the SCDS, information was not collected on several key variables that frequently confound the association between neurotoxicant exposures and child development. Those variables are socioeconomic status, caregiver intelligence, and quality of the home environment.

2.1.4.3 SCDS Main Study—Davidson et al. (1998)

As part of the main SCDS, 711 children 66 months of age (of original cohort of 779) were evaluated using a battery of standardized neurodevelopmental tests. At this evaluation, mercury was measured in a 1 cm segment of the child's hair as an indicator of postnatal mercury exposure. The following were assessed: general cognitive ability (McCarthy Scales of Children Abilities), expressive and receptive language (Preschool Language Scale), reading achievement (letter-word recognition subtest of the Woodcock-Johnson Tests of Achievement), arithmetic (applied problems subtest of the Woodcock-Johnson Tests of Achievement), visual-spatial ability (Bender Gestalt Test), and social and adaptive behavior (Child Behavior Checklist). The scores of the six primary endpoints indicated no adverse effect of either prenatal or postnatal mercury exposure. The only significant associations were consistent with enhanced performance among children with increased exposure to methylmercury. Increased pre- and postnatal mercury concentrations were significantly associated with better scores on the total score of the Preschool Language Scale. For the applied problem test, increased postnatal Hg concentrations were associated with better scores. Among

boys, increased postnatal Hg concentrations were associated with fewer errors on the Bender Gestalt Test.

2.1.4.4 Faroe Population—Grandjean et al. (1997)

Testing was done at approximately 7 years of age on 917 of the surviving members of a 1986-1987 birth cohort of 1,022 singleton births. Maternal hair was sampled at parturition (geometric mean 4.3 ppm); children's hair mercury was measured at 12 months (geometric mean = 1.1 ppm) and 7 years of age (geometric mean = 3.0 ppm). Mercury was also measured in cord blood. The neuropsychological tests were these: computer-administered tests from the Neurobehavioral Evaluation System (NES) (finger tapping, hand-eye coordination, and continuous performance test); Tactual Performance Test; three subtests of the WISC-R (digit span, similarities, and block design); Bender Gestalt Test; California Verbal Learning Test-Children; the Boston Naming Test; and Nonverbal Analogue Profile of Mood States. Not all children could complete the entire battery; this was associated with increased mercury exposure for some tests such as the finger opposition test and mood test.

In multiple-regression analyses, increased cord-blood mercury concentration was significantly associated with worse scores on finger tapping, continuous performance test (in the first year of data collection), WISC-R digit span, Boston Naming Test, and California Verbal Learning Test-children. The investigators estimated that a tenfold increase in cord mercury concentration was associated with delays of 4 to 7 months in those neuropsychological domains. The maternal hair mercury concentration showed regression coefficients that were generally lower than those obtained with cord-blood mercury as the exposure indicator. For the finger-tapping test, maternal hair mercury was a better predictor of effect, especially for the both hands condition. The child's hair mercury measured at 12 months was a significant predictor for finger tapping with both hands and continuous performance test reaction time; by contrast hair mercury at the time of examination was significantly associated with continuous performance test reaction time, block designs, and Bender Visual Motor Gestalt errors.

When the Peters-Belson method for covariate adjustment was used, two additional endpoints (WISC-R block design, Bender Gestalt Test errors), were found to be associated with mercury exposure. Associations remained significant when the part of the cohort with maternal hair mercury concentrations greater than 10 ppm was excluded from the analyses. A term for the interaction between mercury and sex was not statistically significant, indicating that the effects were similar among boys and girls. In general, children's test scores were more strongly associated with cord-blood mercury concentration than with either maternal hair mercury concentration or mercury concentrations in samples of children's hair collected at 1 and 7 years of age.

Grandjean et al. (1998) also analyzed the Faroese data in a case-control fashion. Two groups were assembled: a case group of 112 children with maternal hair concentrations of 10 to 20 ppm at parturition, and a control group of 272 children with maternal hair mercury concentrations less than 3 ppm. Controls were matched to

cases on age, sex, year of examination, and caregiver intelligence. The median maternal hair Hg concentrations in the two groups were 1.8 and 12.5 µg/g, constituting a sevenfold difference. Median cord-blood mercury concentrations also differed substantially (59.0 µg/L in the case group versus 11.9 µg/L in the control group). On 6 of the 18 endpoints, the case group scored significantly lower than did the control group. The results of those analyses differ in certain respects from those of the main analyses. First, the set of endpoints on which the cases and controls differed is similar but not identical to the set of endpoints that was significantly associated with cord mercury concentration found in the main analyses. In the case-control analyses, a term for the interaction between mercury and sex was statistically significant for several scores: the Bender Gestalt Test error score, short-term reproduction on the California Verbal Learning Test-Children, all three finger-tapping conditions, continuous performance test reaction time, and average hand-eye coordination score. For all scores, adverse mercury effects were noted for boys but not girls.

2.1.4.5 Amazon Valley—Grandjean et al. (1999)

A study cohort was assembled numbering 351 children ages 7 to 12. The population, which was drawn from four riverine communities in Amazonian Brazil, had increased exposures to methylmercury because of their consumption of fish contaminated by upstream gold-mining activities. When data on all four villages were combined, children's hair mercury concentrations were significantly associated with their scores on finger tapping, Santa Ana dexterity test, WISC-III digit span, Stanford-Binet copying and recall, and Stanford-Binet bead memory. Adjustment for community generally reduced the magnitude of the associations, sometimes dramatically. Hair mercury concentrations and village residence were so highly confounded, however, that adjustment for village might be inappropriate.

2.1.4.6 French Guiana population—Cordier and Garel (1999)

Cordier and Garel (1999) studied a cohort from a gold-mining area in French Guiana. Median maternal hair concentration was 6.6 ppm with a range of 2.9 to 17.8 ppm. Children ages 5 to 12 years old (n = 206) were administered a battery of neuropsychological tests: finger tapping, three subtests from the Stanford-Binet (block design, copying designs, bead memory), and two subtests from the McCarthy scales (numerical memory, leg coordination). After adjustment for potential cofounders, increased maternal hair mercury concentrations were significantly associated with copying-design score; the effect was greater in boys. The data were reanalyzed to include only those observations from the region with highest mercury exposures (Upper Maroni). When observations were separated by gender, there was an association in boys between mercury exposure and poorer leg coordination and poorer block-design scores in girls.

2.1.4.7 Conclusions

There is ample evidence of low dose *in utero* mercury effects on neuropsychological indices in school-age children. In the New Zealand population, maternal hair mercury was associated with poorer scores on several measures: full-scale IQ, language development (spoken language quotient), visual-spatial skills (perceptual-performance scale), and gross-motor skills (motor scale). The poorer mean scores in the high-mercury group were largely attributable to the children of mothers with hair mercury above 10 ppm. One analysis by Crump et al. (1998) used maternal hair mercury as a continuous, rather than binary, variable; in this analysis there was no significant association with hair mercury. These analyses were heavily influenced by a single data point (a child with purported high developmental exposure who showed no abnormal scores). If data for this child are excluded, and parental education and age at testing are included as covariates, there are significant associates between mercury exposure and six scores.

In the SCDS pilot study, increasing maternal hair mercury was associated with the GCI and the perceptual performance scale of the McCarthy scales. Exclusion from analyses of several influential points reduced the significance of the mercury effect. As it was intended as a feasibility study, the cross-sectional, or pilot, SCDS did not collect information on socioeconomic status, caregiver intelligence, or quality of home environment. In the SCDS main study there was no observation of any adverse effect of prenatal or postnatal mercury exposure. The NRC report commented on the regression model for the GCI score:

> The R^2 (square of the multiple correlation coefficient) value (0.10) of the reduced regression model for the GCI score in the main SCDS study was identical to that in the pilot study. That also appeared to be true for scores on the Preschool Language Scale.... That finding is puzzling because the pilot-study models ... did not include several key covariates ... and because the regression coefficients for socioeconomic status and caregiver intelligence were statistically significant for total scores of the GCI and Preschool Language Scale in the main study cohort. Those differences suggest that maternal hair Hg concentration is very highly confounded with those key covariates in the Seychelles population, or they suggest that the associations between child neurodevelopment and the covariates differ substantially in the pilot and main study cohorts, or both.

In the Faroe population, mercury exposure measured in cord blood was associated with deficits on several measures: finger tapping, preferred hand; continuous performance test (first year of data collection, two scores); mean reaction time, WISC-R digit span; Boston Naming Test (with and without cues); and California Learning Test (short-term and long-term reproduction). Mercury effect was similar in males and females. Most test scores were more strongly associated with cord-blood mercury than with maternal hair mercury. In the case- control portion of the study, the case group scored significantly lower than the control group on 6 of 18 endpoints.

In two smaller populations there were observed effects of mercury exposure. Combining results from four communities in the Amazon basin showed a significant association of children's hair mercury with deficits on four measures. In a French

Guiana cohort (n = 206), it was shown that maternal hair mercury was associated with one measure (a Stanford-Binet subtest), particularly in boys.

2.1.5 Sensory, Neurophysiological, and Other Endpoints in Children

2.1.5.1 Faroe population—Grandjean et al. (1997)

In the Faroe Islands cohort, the evaluation of 7-year-old-children included assessments of visual acuity, near-contrast sensitivity, otoscopy and tympanometry, and some neurophysiological tests. Visual acuity, contrast sensitivity, auditory thresholds, and visual-evoked potentials were not significantly associated with prenatal methylmercury exposures. For brainstem auditory-evoked potential, peaks I, III, and V were slightly delayed at increased cord-blood mercury concentrations at both 20 and 40 Hz; interpeak latencies were not associated with mercury at either frequency.

2.1.5.2 Madeira population—Murata et al. (1999)

Many of the same neurophysiological tests that had been done in the Faroe Islands study were administered to 6- to 7-year-old children living in Madeira. This was a cross-sectional study of 149 subjects. For brainstem auditory-evoked potential, maternal hair mercury was significantly associated with I-III and I-V interpeak latencies at both 20 and 40 Hz, as well as with total latencies for peaks III and V at both frequencies. Those results are similar to the findings in the children tested in the first year of the Faroes cohort. For visual-evoked potentials on a pattern-reversal task, maternal hair mercury concentration was significantly associated with one of the three latencies, as well as with the N75-N145 and P100-N145 latencies.

2.1.5.3 Ecuador—Counter et al. (1998)

Auditory function in children and adults was investigated by Counter et al. (1998). The study sample consisted of 75 individuals (36 children and 39 adults) from a gold-mining region in Ecuador and 34 individuals (15 children and 19 adults) from nonmining areas as a control. Blood mercury concentrations were significantly higher in individuals (both adults and children) from the gold-mining area than in individuals from the control region (mean level of 17.5 µg/L versus 3.0 µg/L). Neurological examinations were carried out on all individuals. In children, blood mercury was significantly associated with hearing threshold at 3 kHz in the right ear only. No association was found for adults. A borderline association was found between blood mercury concentration and I-III interpeak transmission time on the left side in both children and adults. The authors concluded that overall auditory sensory-neural function and neural conduction time at the brainstem level were generally unaffected by elevated blood mercury levels in either children or adults.

2.1.5.4 Conclusions

There is increasing evidence of adverse endpoints other than cognitive development in mercury-exposed children. In the Faroes cohort, there were delays in some auditory-evoked potential peaks as a function of cord-blood mercury. Similar findings were reported for a smaller population from a fishing village in Madeira. A population of children in a gold-mining region of Ecuador showed an association between blood mercury and hearing threshold in the right ear at 3 kHz.

2.2 Choice of Study

Of the three large human developmental studies, two reported associations between low-dose *in utero* exposure to methylmercury and performance on standardized neurobehavioral tests. The Faroes investigators reported effects in the domains of attention, fine-motor function, confrontational naming, visual-spatial abilities, and verbal memory. Although similar results were reported for the New Zealand population (and in the Seychelles pilot study), there were no observations of adverse effects attributable to methylmercury in the main SCDS.

This section discusses issues relevant to the choice of critical study for calculation of a reference dose from among these three studies.

2.2.1 Critique of New Zealand Study

The study by Kjellstrom et al. (1986) included 57 fully matched groups of four 6-year-old children each as well as four incomplete sets, for a total of 237. As was the case for the Faroes study, these authors reported deficits in measures associated with methylmercury exposure. NRC noted that the New Zealand population's sources of methylmercury exposure and the study endpoints were similar to those examined in the Seychelles. While EPA was developing its RfD for the MSRC, the New Zealand data were available as a report that had not been subjected to standard peer-review procedures. In 1998, Crump and associates published a reanalysis of the New Zealand data that underwent peer review. This paper reported associations of prenatal methylmercury exposure with several endpoints (when one extreme outlier was excluded), including four endpoints that were not found to be related to methylmercury in the Seychelles study. NRC notes in its report that the New Zealand study has been criticized for errors in matching exposed children to controls and for testing exposed children and controls at different ages (Myers et al., 1998). Those errors occurred in the 4-year followup but were corrected in the 6-year followup. NRC notes that there is no reason to expect differential measurement error across the studies. An error of that type is likely to be nondifferential (i.e., unbiased), and it would reduce the likelihood of detecting associations between methylmercury exposure and neurobehavioral test scores.

The Kjellstrom et al. (1986) study collected data on several potential confounding factors and used a broad battery of standardized measures that were administered by trained examiners. It is likely that the exposure was relatively low-dose and not episodic, reflecting well-established food consumption patterns.

2.2.2 Control for Possible Confounding

Both the Faroes study and the SCDS evaluated most of the variables that have been linked to childhood cognitive development. Table 6-2 of the NRC report lists these and notes which study controlled for the particular variable. Although neither study controlled for all potential confounders, it was felt by the authors of the NRC report that the influences of those variables on cognitive outcome are probably too weak to account for any major inconsistencies between the two studies. The Confounders and Variables Panel of expert workshops sponsored by OSTP had earlier concluded that neither the SCDS nor the Faroese study was critically flawed, and that these studies were suitable for determination of the upper limit of a methylmercury NOAEL.

2.2.2.1 Place of Faroese residence—town versus country

At the 1998 OSTP workshop, the Faroes investigators noted that the maternal Ravens scores and the child verbal-test scores were generally higher among families residing in one of the three towns in the Faroes compared with those living in the countryside. This was thought to be due to social-class differences. It was suggested that because more fish and, in particular, whale meat, was consumed by rural residents, the associations of mercury exposure with child verbal-test scores could in fact reflect those social-class differences. However, analyses presented at the workshop showed that these associations remained significant, even after controlling for a dichotomous town-country control variable (Table 6-3 in the NRC report). NRC felt it would not be appropriate to control for town residence in all analyses. They made the following statement:

> Because fish and whale consumption constitute a large proportion of the rural diet, the disappearance of associations after controlling for residence could be due to the fact that residing in a rural area leads to increased Hg exposure which, in turn, causes an adverse outcome. It would not necessarily indicate that the lower social class associated with rural residence is the true cause of the Hg-associated deficit. The disappearance of an association between Hg and neurobehavioral effects under those circumstances would be very difficult to interpret, because the interpretation would depend upon what condition is considered the reason for the association between living in a rural area and poor outcome (i.e., lower social class or greater Hg exposure).

Another source of town versus country difference could be the distance traveled to the testing site, with resulting fatigue in the children from the countryside. However, analyses showed that the regression coefficients for prenatal mercury exposure remain significant even after controlling for child's residence.

2.2.2.2 Test administration

The neuropsychological test examiner was routinely controlled for in the Faroe Islands study (see NIEHS, 1998, Section 3.5), but was not controlled for in the SCDS. It was suggested at the OSTP workshop that if an examiner who is less adept at eliciting optimal performance from the subjects tested a large proportion of less-exposed children, the results could be affected. NRC noted:

> If those children performed more poorly than they otherwise would have on the test, an association between Hg concentration and test scores might be obscured by failure to control for the examiner. That result could also occur if an adept tester tested a large proportion of the more heavily exposed children, leading them to achieve higher scores than they would have if tested by other examiners.

2.2.2.3 Age at testing

The SCDS controlled for age at testing by converting the raw test scores to age-corrected standard scores with conversion tables based on U.S. norms (NIEHS, 1998). The Faroes investigators analyzed the raw scores by adjusting statistically for the child's age (measured in days since birth). NRC found the latter approach to be preferable. They noted, first, that the applicability of U.S. norms to these study populations is uncertain. In this context it should be noted that the Seychellois scores on the BSID were higher than U.S. averages at both 19 and 29 months. Second, NRC felt that the use of age-corrected standard scores can reduce the sensitivity of the test, because several adjacent raw scores are treated as equivalent in converting to standard scores. Last, they noted that age-corrected standard scores use 3-month intervals, which introduces a degree of arbitrariness in assigning a child to a particular group. The NRC report found the approach of controlling statistically for age by multiple regression to be appropriate, because the effect of age is likely to be linear across the relatively short age period (3 months in both studies); that is, over short time periods, development is most likely to take place at a constant rate.

2.2.2.4 Selection bias from exclusion of individuals with severe impairments

The OSTP workshop Confounders and Variables Panel (NIEHS, 1998) identified what they considered a serious potential issue with the SCDS. They noted that recruitment was limited to children with no severe debilitating conditions. This panel felt that such a restriction could lead to underestimation of effect when the shape of the dose-response curve is not known.

2.2.2.5 PCB exposure in the Faroese population

PCB exposure through maternal consumption of whale blubber was discussed at length at the OSTP workshop and in the report of the Confounders and Variables Panel. Using the data from the part of the cohort for which cord PCB was measured,

Grandjean et al. (1997) performed a series of analyses to ascertain if the PCB and mercury effects could be separated. Of the eight outcomes for which there was a significant association with cord-blood mercury, four were also associated with cord PCB. Those outcomes were generally related to verbal and memory performance, domains that have been associated with prenatal PCB exposure in other studies (Jacobson and Jacobson, 1996; Patandin et al., 1999). When PCBs and mercury were included together in the model, one outcome (continuous performance test reaction time) was independently related to mercury exposure (Grandjean et al., 1997, Table 5). For the other three outcomes, however, the associations with both PCB and mercury were not statistically significant. The Confounders and Variables Panel concluded that both PCB and mercury had adverse effects on the remaining three test measures, but that it was not possible to determine the relative contribution of each.

NRC concluded that there was no empirical evidence or theoretical mechanism to support the opinion that *in utero* Faroese exposure to PCBs exacerbated the reported methylmercury effect. They note that statistical tests for interaction between PCB and mercury show no interaction. NRC suggested that a likely explanation is that both of those contaminants adversely affect those outcomes, but their relative contributions cannot be determined given their co-occurrence in the Faroes population. They state it is unlikely that a difference in PCB exposure between the two populations explains the lack of developmental neurotoxic effects in the Seychelles.

PCB body burdens in the Seychellois are very low by comparison with North American and European populations. In 28 serum samples obtained from Seychelles study children, there were no detectable concentrations of any PCB congeners. In the Faroes study, prenatal PCB exposure was measured in 436 stored umbilical cord tissue samples. It was noted at the OSTP workshop that cord tissue PCB concentration has never been validated in relation to blood or milk concentration; because cord tissue is lean and PCBs are lipophilic, it may not be the most reliable indication of total PCB body burden.

In a second set of analyses, Budtz-Jørgensen et al. (1999) found that effect of prenatal PCB exposure was reduced when the data were sorted into tertiles by cord PCB concentrations. Regressions assessing mercury exposure and the five principal test outcomes were then run separately for each of the three groups. The regression coefficients for a mercury effect in the lowest PCB tertile were no weaker than those for the higher two PCB groups. This lends additional credence to a conclusion that the associations between mercury and test outcomes are not attributable to confounding by prenatal PCB exposure.

2.2.3 Population Differences in Susceptibility

Populations may be more or less susceptible to effects of a toxicant as a consequence of predisposing factors, such as nutritional status, exposure to other agents (see Section 2.2.1), or genetic susceptibility.

The SCDS cohort is predominantly African in descent; the Faroes cohort is Caucasian. The latter population has been somewhat isolated and thought to be descended from a small number of "founders." This could increase or decrease genetic susceptibility to effects of toxic insult. NRC noted that methylmercury neurodevelopmental effects were observed in a genetically heterogeneous and racially diverse sample studied in New Zealand, a population that was predominantly non-Caucasian.

Data on birthweight and gestation length in the Faroes and Seychelles show no indication of energy or macronutrient (protein and carbohydrate) deficiency. It is possible that members of either population could be deficient in micronutrients. It has been suggested that certain nutrients found in fish eaten by the Seychelles residents (e.g., omega-3 fatty acids and selenium) could attenuate adverse effects of methylmercury exposure. It should be noted that both the Faroese and New Zealand populations would be considered "high fish consumers" by comparison to U.S. norms, and both populations were observed to have measurable effects of mercury exposure. It is unlikely that general health status of the Faroese and Seychellois was a factor in enhancement or attenuation of mercury effects. Both populations receive excellent health care.

It has been noted in several scientific forums that the cohort in the main Seychelles study appears to have been robust for psychomotor development at early ages. The SCDS authors report a number of abnormal scores on the Denver Developmental Screening Test that is considered to be exceptionally low by U.S. norms. The population also was observed to have an unusually high mean PDI score and a very low rate of referral for mental retardation. The means and standard deviations of the cognitive measures administered at later ages were similar to U.S. norms. It is not clear what if any effect this developmental robustness has on susceptibility to any adverse effects of prenatal Hg exposure. Statistical power to find an adverse effect is discussed in Section 2.2.7.

2.2.4 Assessment of Prenatal Mercury Exposure

In the Faroes study, mercury in cord blood and maternal hair was measured; in the Seychelles, only maternal hair mercury was measured. The maternal hair samples obtained in the Faroes and Seychelles studies did not necessarily reflect the same period of pregnancy. The Seychelles samples were 9 cm lengths of hair reflecting average mercury exposure during pregnancy. The Faroes study analyzed mercury from hair samples of variable length, some 3 cm (reflecting late second and third trimester) and some 9 cm.

In the analyses of the Faroese data, cord-blood mercury concentration was significantly associated with a slightly larger number of endpoints than was maternal hair mercury. Given the estimated half-life of methylmercury and what is known of PBPK, it could be assumed that cord-blood mercury reflects the latter part of gestation. Hair mercury could reflect the entire pregnancy or could be segmentally analyzed to

provide snapshots of various times in gestation. Some of the effects reported in the Faroese cohort could be related to toxic responses in the latter stages of prenatal development. However, hair mercury concentrations in the Faroe Islands study were only a slightly weaker predictor of methylmercury effects than was cord blood. NRC concluded that it would be reasonable to expect that, if children were affected in the main Seychelles study, some indication of an association between child development and maternal hair mercury concentration would have been observed. It noted that the findings of developmental effects reported in New Zealand were based solely on maternal hair sample data averaged across the entire period of pregnancy. The difference in the observation of effects between the Faroes study and the SCDS is thus not an artifact of biomarkers of exposure.

2.2.5 Endpoints Assessed

In the opinion of most developmental scientists, the Faroes and Seychelles studies used very different neurobehavioral test batteries. According to the NRC report, the tests selected for use in the SCDS are considered apical or omnibus tests (e.g., the McCarthy Scales of Children's Abilities); these provide global scores that integrate performance over many separate neuropsychological domains. The investigators studying the Faroes population were working from a hypothesis that mercury would have multifocal domain-specific neuropsychological effects. Their test battery consisted of highly focused tests selected from those commonly used in clinical neuropsychology (e.g., California Verbal Learning Test-Children, and Boston Naming Test) and did not include an apical test of global function. Many of the subscales of the McCarthy Scales might be expected to provide measures comparable to some tests administered to the Faroese children. In their assessment of differences and points of comparability between the test batteries, NRC concluded the following: "although the Faroe Islands and SCDS test batteries include tests of language and memory, it is not appropriate to view the endpoints used in the studies to assess each domain to be equivalent either in terms of the specific skills assessed or the test sensitivity."

One test was administered to both populations: the Bender-Gestalt Test. The investigators used different scoring systems. The NRC report noted that in a paper by Trillingsgaard et al. (1985) scores derived using the more detailed Gottingen system were significantly associated with low-dose lead exposure, whereas scores on the Koppitz system were not. Thus the Gottingen system used in the Faroe Islands might be more sensitive.

A second important difference in the assessment batteries used in the Faroes study and SCDS is the age of the child at assessment; 7-year-olds were tested in the Faroe Islands in contrast to children 5.5 years of age in the SCDS. Assessments in the New Zealand cohort were done at 4 and 6 years of age. It is generally thought that developmental assessments are likely to be less sensitive in detecting subtle neurotoxic effects when they are administered during a period of rapid developmental change. The period covering ages 60 to 72 months (when the SCDS and New Zealand cohorts were evaluated) is such a time; individual differences in the rate of cognitive maturation

are likely to eclipse subtle differences in function attributable to a teratogenic exposure (Jacobson and Jacobson, 1991). The NRC panel also felt that in the SCDS, assessments of infants (particularly the 19- and 29-month BSID) were not given at optimal age points. Their report makes the following statement:

> Studies of prenatal exposure to alcohol and other substances that have administered the Bayley scales at multiple ages have repeatedly failed to detect effects at 18 months, probably because it too is a period of rapid cognitive maturation, involving the emergence of spoken language. Twenty-nine months is likely to be an insensitive testing point for the Bayley scales because it is at the end of the age range for which the version of this test used in the Seychelles was standardized, leading to a substantial risk of a "ceiling effect" (i.e., too many children receiving the highest possible scores on numerous items).

The overall conclusion of NRC, however, was that discrepancies between the Faroe Islands and the main Seychelles studies are probably not due to differences in the assessments. They point out that the New Zealand study observed associations between methylmercury exposure and scores on the McCarthy Scales of Children's Abilities (the primary outcome measure used in the SCDS) at about the same age of assessment as in the Seychelles study.

2.2.6 Episodic Versus Continuous Exposure

2.2.6.1 Significance of episodic versus continuous exposure

Exposure to methylmercury in the Seychelles is through daily consumption of fish—an average of 12 meals/week. Although the Faroese eat fish more frequently than does the average consumer in the United States (about three meals a week), a significant source of methylmercury exposure in this population is from eating pilot-whale meat. Pilot-whale meals are relatively infrequent (less than once per month on the average) (Grandjean et al., 1992) with additional intermittent snacks of dried whale (Grandjean et al., 1998). The whale-meat mercury concentration varies with the pod. An analysis of 466 whales showed an average concentration of 1.9 ppm, with a range of 0.59 to 3.30 ppm (Faroese Food Agency data quoted in NIEHS, 1998). In the New Zealand study, maternal consumption of a relatively high-mercury fish (shark) was presumed to be on a regular basis, although the actual frequency and pattern of exposure are unavailable.

The degree to which differences in exposure pattern between the studies accounts for differences in outcome is uncertain. It is presumed that the mercury body burden in the Faroe Islands study was the consequence of a "spike" exposure pattern, in contrast to a more continuous exposure pattern in the Seychelles study, which nonetheless resulted in a similar body burden in the two studies. The pattern of exposure can be a critical determinant of *in utero* toxicity. For example, the NRC report cites data in animals that showed that maternal ingestion of a given dose of alcohol over a short time caused greater neuronal impairment (Bonthius and West, 1990) and behavioral impairment (Goodlett et al., 1987) than that caused by gradual ingestion of the same total dose over several days. The frequency of exposure has a significant

influence on the variation in blood levels, even under steady-state conditions, and is dependent on blood half-life (Rice et al., 1989).

It is probable that both patterns of exposure are present in the population of the United States. Individuals in some ethnic groups engage in a subsistence-type fishing pattern, consuming fish as their major protein source. Most sport fishers, however, consume fish on an intermittent basis. It is not uncommon for piscivorous fish in inland waters to have mercury levels exceeding 1 to 2 ppm (MSRC, 1997), so that the body burden of mercury in this group of fish consumers would presumably be the result of episodic exposure to food sources with levels of mercury similar to those in the Faroe Islands. It therefore may be that the consumption pattern of the Faroe Islands population better represents the pattern of exposure in the majority of the U.S. population exposed to elevated levels of methylmercury than does the consumption pattern of the population of the Seychelles Islands.

2.2.7 Power of Studies

NRC commented on the power to detect subtle effects in the admittedly large human studies. They noted that it is possible that the differences in response between the Faroes study and the SCDS could be due to between-sample variability in the expression of neurotoxicity at low doses. NRC remarked that even large samples can have insufficient power to detect adverse effects if a relatively small number of subjects are exposed in the upper ranges of the exposure distributions, where those effects will presumably be found.

NRC said that the magnitude of the associations found in the methylmercury studies resembles that reported for other environmental contaminants, such as low-dose lead and PCBs. If the magnitude of an association is not large, it is not likely that it would be detected in every cohort studied. NRC noted by comparison that it is well established in the scientific community that a blood lead concentration in excess of 10 µg/dL places a child at increased risk of poor developmental outcomes. However, not all lead studies have found an association between exposure at this level and decreased performance, and substantial variability exists in the magnitudes of the reported effects (Bellinger, 1995). NRC notes for the SCDS, “the evidence consistent with such effects found in the pilot phase, coupled with the suggestion of unusual developmental robustness in the main study, suggest that the failure to detect apparent adverse effects in the main study could be due to the substantial sample-to-sample variation expected when trying to identify weak associations in an inherently ‘noisy’ system of complex, multidetermined neurobehavioral endpoints.”

In another comment on power, NRC says that power analyses based on total sample size can be misleading if adverse effects occur primarily among the most heavily exposed individuals, who typically constitute a small proportion of the sample. They note that of 700 children in the SCDS, only about 35 were exposed at levels concordant with maternal hair mercury of 15 ppm or higher. Because multiple-regression analysis examines associations that are averaged across the entire

distribution of exposure, associations that hold only for the most highly exposed children can be difficult to detect. "Thus, if adverse effects of prenatal MeHg exposure occur primarily in the upper range, the power to detect them will be limited, and it would not be surprising if associations found in one Seychelles cohort (the pilot study) were not detected in the next cohort (the main study)."

In this context it should be noted that Grandjean et al. (1997) published an analysis of their neuropsychological test data on 7-year-old children, wherein they excluded all scores from children born to mothers with 10 ppm or higher hair mercury. This decreased the number of observations by 15%. In the multiple-regression analyses, regression coefficients and p values were very similar to those obtained when data on the full cohort were used. This indicates that in this study population, adverse effects of mercury were detectable at exposures below 10 ppm maternal hair mercury.

2.2.8 Selection of Study

There is a large database on potential neurodevelopmental effects of methylmercury. In particular, three large, well-designed, prospective, longitudinal studies have been subjected to peer review and intensive analyses. Some results from these studies of large populations are in apparent conflict. The sections above reviewed some of the factors that have been suggested to account for the finding of adverse outcomes associated with *in utero* mercury exposure in the Faroes and New Zealand and the lack of this association in the SCDS. None of these factors represents a critical flaw in study design or execution. None of the factors adequately explains the differences in the study outcomes.

One strength of the New Zealand study is that an effect was shown in an ethnically heterogeneous sample; another advantage was that the study used developmental endpoints with predictive validity. NRC, however, had some reservations about using the New Zealand study as the basis for the methylmercury RfD. They note that it is a relatively small study with 237 subjects (by comparison to the population of up to 900 for the Faroes tests). NRC also notes that the New Zealand data have not had the exhaustive scientific scrutiny that have been applied to the SCDS and Faroes study. The advantages of the Faroes study include a larger sample size, the use of two different biomarkers of exposure, and extensive scrutiny in the epidemiological literature. The Faroes data have also undergone extensive reanalyses in response to questions raised by panelists in the NIEHS (1998) workshop and by this committee in the course of its deliberations. The SCDS shares many strengths of the Faroes study. However, EPA agrees with NRC that a positive study, one that shows statistically significant associations between prenatal mercury exposure and adverse outcomes, is the strongest public health basis for an RfD. The study selected by EPA for the basis of the methylmercury RfD is the report of developmental neurotoxicity in 7-year-old children in the Faroes.

2.3 Choice of Critical Effect (endpoint)

Several studies have reported significant associations between increased numbers of combined abnormal and questionable scores on standardized neurological examinations. NRC opined that the functional importance of these effects is uncertain. There is little evidence that relatively low-dose, long-term exposure has any significant effect on language or motor-skill developmental milestones. There is some evidence of an association between *in utero* mercury exposure and deficits on the DDST. The NRC put forth the opinion that this screening test is not as useful as others in developmental neurotoxicological testing.

There were significant associations reported for various neuropsychological endpoints in two studies. NRC presented BMDs and BMDLs for several endpoints in the positive Faroes and New Zealand studies as well as for the nonpositive Seychelles study (the next section discusses choices of model and choices made in BMDL calculation). Reproduced below is Table 7-2 from the NRC report (here as Table 2-1), which compares BMDs from the three studies in terms of maternal hair mercury. Included in this table are the New Zealand BMDs calculated after exclusion of the data from the highest exposed individual. NRC suggested that this hair mercury concentration of 86 ppm is not plausible. The text reads,

> a hair Hg concentration of 86 ppm is more than 4 times the next highest hair Hg concentration in the study. If the one-compartment pharmacokinetic model and EPA's standard default input assumption are used, it can be estimated that a 60-kg woman would have to eat an average of 0.5 pounds (227 g) of fish containing 2.2 ppm of Hg to reach a hair Hg concentration of 86 ppm. Consistent exposure at such a dose seems unlikely when the mean Hg concentration in fish from fish-and-chips shops, a principal source of exposure in New Zealand (Kjellström et al., 1986), is 0.72 ppm (Mitchell et al., 1982). On the basis of those considerations, the committee concluded that analyzing the New Zealand data without the data from that individual is appropriate.

Table 2-1. Benchmark Dose Calculations (ppm MeHg in maternal hair) from Various Studies and for Various Endpoints (NRC, 2000)

Study	Endpoint	BMD[a]	BMDL
Seychelles[b]	Bender Copying Errors	***[c]	25
	Child Behavior Checklist	21	17
	McCarthy General Cognitive	***	23
	Preschool Language Scale	***	23
	WJ Applied Problems	***	22
	WJ Letter/Word Recognition	***	22
Faroe Islands[d]	Finger Tapping	20	12
	CPT Reaction Time	17	10
	Bender Copying Errors	28	15
	Boston Naming Test	15	10
	CLVT: Delayed Recall	27	14
New Zealand[e]	TOLD Language Development	12	6
	WISC-R:PIQ	12	6
	WISC-R:FSIQ	13	6
	McCarthy Perceptual Performance	8	4
	McCarthy Motor Test	13	6

[a]BMDs are calculated from the K-power model under the assumption that 5% of the responses will be abnormal in unexposed subjects ($P_0 = 0.05$), assuming a 5% excess risk (BMR = 0.05).
[b]Data from Crump et al. (1998, 2000). "Extended" covariates.
[c] *** indicates value exceeds 100.
[d]Data from Budtz-Jørgensen et al. (1999).
[e]Data from Crump et al. (1998, 2000).

Abbreviations: WJ, Woodcock-Johnson Tests of Achievement; CPT, Continuous Performance Test; CVLT, California Verbal Learning Test; TOLD, Test of Language Development; WISC-R:PIQ, Wechsler Intelligence Scale for Children-Revised Performance IQ; WISC-R:FSIQ, Wechsler Intelligence Scale for Children-Revised Full-Scale IQ.

The range of BMDL values is relatively small (4 to 25 ppm maternal hair mercury). Inspection of this table shows that all the BMDs (and corresponding BMDLs) from the New Zealand study are lower than those from the other positive study in the Faroes. Often the most sensitive adverse endpoint is selected as the critical effect for calculation of a RfD. The most common surrogate for "most sensitive" is the lowest BMDL or bounded NOAEL (that is, NOAEL from a study wherein an effect was observed). The lowest BMDL is 4 ppm maternal hair mercury for the McCarthy Perceptual Performance Test calculated by Crump et al. (1998, 2000) on the New Zealand data (Kjellstrom et al., 1986). NRC had reservations about using the Kjellstrom (1986) data as the basis for the methylmercury RfD, with which EPA agreed (see Section 2.2.8). In this instance the choice is not of the lowest BMDL, but will be made from among the measures in the Faroese data.

Grandjean and colleagues reported significant associations between either maternal hair mercury or cord-blood mercury and decrements in several neuropsychological measures in
7-year-old Faroese children:

- Finger tapping—preferred hand (p= 0.05)
- Continuous performance test—first year of data collection
  – false negatives—(p = 0.02)
  – mean reaction time—(p = 0.001)
- WISC-R digit span (p = 0.05)
- Boston Naming Test
  – no cues (p = 0.0003)
  – with cues (p = 0.0001)
- California Verbal Learning Test
  – short-term reproduction (p = 0.02)
  – long-term reproduction (p = 0.05)

When an alternative approach to adjusting for covariates was used (Peters-Belson method) was used, two more measures showed significant associations:

- WISC-R block design (p = 0.05)
- Bender Gestalt Test errors (p = 0.05)

More endpoints were significantly associated with cord-blood mercury than with maternal hair mercury. Table 7-3 from the NRC report is reproduced below as Table 2-2; this presents calculations, in terms of cord-blood mercury concentrations, of BMDs and BMDLs for five Faroese endpoints.

The lowest of the above BMDLs is 46 µg/L mercury in cord blood for the continuous performance test reaction time scores. NRC recommended a different choice. They remarked that in a neuropsychological test battery, the reliability of the individual endpoints can be highly variable, so the most sensitive endpoint may not be the most appropriate choice. The Faroes investigators reported difficulties in administering the CPT; the data from the second half of the cohort were discarded for the analysis of this endpoint. The NRC panel thus suggested that a more appropriate choice would be to select the second most sensitive endpoint, the Boston Naming Test. Interestingly, this measure had the lowest BMDL in the analyses based on maternal hair mercury.

Table 2-2. Benchmark Dose Calculations (ppb methylmercury in cord blood) from the Faroe Islands Study for Various Endpoints

Endpoint	BMD[a]	BMDL
Finger Tapping	140	79
CPT Reaction Time	72	46
Bender Copying Errors	242	104
Boston Naming Test	85	58
CVLT: Delayed Recall	246	103

[a]BMDs are calculated from the K-power model under the assumption that 5% of the responses will be abnormal in unexposed subjects ($P_0 = 0.05$), assuming a 5% excess risk (BMR = 0.05).
CPT, Continuous Performance Test; CVLT, California Verbal Learning Test.
Source: NRC (2000); data from Budtz-Jørgensen et al. (1999).

3. CHOICE OF DOSE-RESPONSE APPROACH

3.1 Benchmark Versus NOAEL

In recent years, EPA has been moving to use of BMDs versus experimental NOAELs as the departure point for calculation of RfDs. The Agency is preparing guidance for application of this methodology. Guidance has been published in the Technical Support Document on Risk Assessment, Human Health Methodology for Ambient Water Quality Criteria.

NRC also made comments on the applicability or preference for BMD over NOAEL. They cite comments by several risk assessment scientists on statistical drawbacks to NOAELs. The NOAEL, for example, must correspond to one of the experimental doses; it can vary considerably across different experiments. In calculating an RfD, there is no statistical or other treatment of the data to adjust for the choice of dose groups by different experimenters. NRC notes that the identification of a no effect dose group is based on statistical comparisons between exposed and controls; thus, larger studies have higher power to detect small changes and tend to produce lower NOAELs. Furthermore, because NOAELs are identified as a consequence of pairwise comparisons, there is no widely accepted procedure for calculating a NOAEL in settings where exposure is measured on a relatively continuous scale.

In its guidance documents EPA lists some other advantages of BMD.

NRC recommended and EPA concurred with the use of a BMD approach to calculate the methylmercury RfD.

3.2 Choice of Exposure Metric

NRC discussed at length in their Chapter 4 the suitability as biomarkers of exposure of both hair and blood mercury. The measurement of mercury exposure in the study population serves two purposes when applied to risk assessment. The biomarker serves as the surrogate for the methylmercury dose to the target tissue, in this case fetal brain. As such, the biomarker is one of the coordinates of inputs to the dose-response models. From this perspective, the ideal biomarker is one that is closest pharmacokinetically to the target. Of the measurements available, cord blood represents the compartment closer to fetal brain than does hair, which is an excretion compartment.

The other use of biomarker in this risk assessment is as a surrogate for ingested dose, the unit in which an RfD is expressed. The ideal biomarker for this stage is closest pharmacokinetically or has the best correlation with ingested dose. Maternal hair or blood may be more suitable from this point of view.

Another point to consider in biomarker choice is temporality: Is the biomarker an adequate indicator of exposure during critical developmental windows? NRC noted that cord-blood mercury tends to reflect exposure in the later stages of pregnancy whereas hair mercury can be used to determine exposure at any point in pregnancy, given the appropriate sample. The NRC panel noted that for most assessment of hair mercury there will be significant uncertainty when attempting to relate a particular hair level to a time-specific dose to the fetal brain. In addition, there is no information on differential effects of methylmercury at different periods of gestation; it is in no way certain when critical developmental windows occur. Considering the information (or lack thereof) on time of exposure offered by each biomarker, there is no compelling reason to consider one more appropriate than the other.

NRC provided a table (see Table 6-1) that compares test performance associated with mercury concentration as a function of either cord-blood or maternal hair measurement. This comparison suggests that the cord-blood measure explains more of the variability in more of the outcomes than does maternal hair mercury.

In selecting the exposure metric, the above factors were considered. Cord blood is the biomarker most closely linked (at least conceptually) to the target organ. Cord blood is the marker for which there are the most associated adverse effects in the Faroes study. Neither cord-blood nor maternal hair mercury (as generally measured) provides a clear advantage in assessing exposure during putative critical developmental windows. Maternal hair mercury is conceptually closer to maternal ingested dose than is the cord-blood compartment. However, sensitivity analyses indicate that the maternal hair:maternal blood ratio is a key contributor to variability in calculations of ingested dose (Stern,1997; Clewell et al., 1999). On balance, the best choice for exposure metric for RfD calculation is cord-blood mercury.

3.3 Choice of BMD

In applying a BMD approach to data that are continuous in effect, there are several steps as defined by Gaylor and Slikker (1992). The first is to fit a regression model that characterizes the mean of the set of outcome measurements as a function of dose; the assumption of a normal distribution is made. Choice of model is described in Section 3.4. The second step is to define the cutoff for normal versus abnormal response. In the third step, the dose-specific probability of falling into the abnormal category is determined (P_0). One chooses a specific increase in the frequency of abnormal responses by comparison to background probability; this specific risk above background risk is the benchmark response, or BMR. The dose at which the BMR is reached is the BMD. The last step is to calculate the BMDL or 95% lower limit on the BMD.

In an extensive empirical comparison of NOAEL and BMD calculations, Allen et al. (1994) found that the NOAEL in a typically sized developmental toxicity study was, on average, six times larger than the BMDL, corresponding to a 5% risk. The NOAEL

was higher than even a 10% BMD, on average, by a factor of 3. Leisenring and Ryan (1992) came to somewhat similar conclusions based on analytical considerations.

For the analysis of the behavioral data, including the Faroe study, the NRC panel recommended that $P_0 = 0.05$: that is, that the cutoff for abnormal response be set at the lowest 5% (5^{th} percentile) of children. They further recommended that the BMR be set to 0.05, which would result in a doubling of the number of children with a response at the 5^{th} percentile of an unexposed population. Specification of P_0 for the Faroese data (or the other human methylmercury studies) is somewhat problematic because there are no subjects with true zero exposure. The mean response rate at zero is not actually based on observed data but is extrapolated from the fitted model.

The NRC panel felt that their choice of a P_0 of 0.05 and a BMR of 0.05 was justifiable in terms of being sufficiently protective of public health. The committee recognized, however, that the choice of P_0 and BMR is at the interface of science and policy and should be a science-informed policy judgment. EPA at this time has no established policy on acceptable risk level for the effects reported in the Faroese children. Our decision in the specific case of methylmercury is influenced by the public health conclusions that NRC articulated: the measured effects in the human studies are sentinels of adverse outcomes in children, related to their ability to learn and achieve success in educational settings. Thus, EPA accepts the NRC recommendation to set $P_0 = 0.05$ and BMR = 0.05 in this instance.

3.4 Choice of Model

A report prepared for EPA and subsequently published by Budtz-Jørgensen (1999) provided calculations of BMD and BMDL using square root and log transformations as well as calculations for K-power models. NRC used these results and similar calculations for the New Zealand and Seychelles studies to make some assessments of model suitability. They noted great variability in calculated BMDs and BMDLs as a function of model. This was so despite the inability of standard statistical assessments of model adequacy to distinguish between models based on the K-power model applied to untransformed data and linear models based on square-root or log dose. In unpublished analyses requested NRC Budtz-Jørgensen et al.; these were sensitivity analyses that repeated the regression models after omitting some of the highest observations. Their results suggested that the influence of the extreme observations did not explain the model-to-model variability.

NRC concluded that the most reliable and defensible results for the purpose of risk assessment are those based on the K-power model. They observed that in situations where there are no internal controls (i.e., no unexposed individuals) and where the dose response is relatively flat, the data will often be fit equally well by linear, square-root, and log models. The models can yield very different results for BMD calculations, however, because these calculations necessitate extrapolating to estimate the mean response at zero exposure level. Both the square-root and the log models take on a supralinear shape at low doses, leading to lower estimates of the BMD than

linear or K-power models. The mechanisms by which methylmercury exerts its neurotoxic effects in developing systems are speculative. No likely mode of action for methylmercury leads one to expect a supralinear dose-response at low dose. Thus, from a toxicological perspective, the K-power model has greater biological plausibility, because it allows for the dose-response to take on a sublinear form, if appropriate. The K-power model is typically fit under the constraint that K$1, so that supralinear models are ruled out.

NRC pointed out that the model sensitivity for BMD from the Faroes data appears in conflict with the concept, put forward by Crump and others, that by estimating risks at moderate levels, such as 5% or 10%, the BMD should be relatively robust to model specification. Budtz-Jørgensen et al. (2000) respond that this model dependence is a consequence of the lack of true controls (subjects with zero exposure). The majority of exposures in the Faroes resulted in hair mercury concentrations exceeding 5 ppm (or 24 ppb cord blood). The interquartile range for hair mercury was 3 to 8 ppm (13 to 40 ppb for cord blood) (Grandjean et al., 1992). Models fit to the Faroese data are in effect capturing the shape of the dose-response in this middle range of exposure. The NRC report (Figure 7-5) taken from Budtz-Jørgensen et al. (1999) shows dose-response curves fitted to hair Hg data for the linear, square-root, and log transformations. NRC notes that variations in estimated BMDs are not explained by differences in how well the models fit the bulk of the data, but rather by what the models predict for the mean response for unexposed individuals.

In reaching their conclusion on model choice, NRC concluded that biologically based arguments were needed. Their argument was as follows:

> One useful way to think of differences between the various models is that the linear model implicitly assumes an additive effect of Hg exposure, the log model assumes a multiplicative effect, and the square root lies somewhere in between. All three models fit essentially equally well to data that for the most part correspond to concentrations between 2 and 20 ppm in hair. However, the models differ fairly dramatically with regard to how they extrapolate to values below those levels. The linear model would predict that the change in mean outcome as MeHg concentration goes from 0 to 10 ppm in hair should be the same as the change observed in the mean outcome as concentration increases from 10 to 20 ppm. In contrast, the log model would predict that the change in mean outcome associated with any doubling of MeHg concentration should be the same as the change observed in the mean outcome as concentration increases from 10 to 20 ppm. Thus, the log model would predict that the same magnitude change in outcome would be expected as the concentration goes from 1 to 2 ppm or from 4 to 8 ppm as that observed for the concentration going from 10 to 20 ppm—that is, the extrapolation down to zero exposure will predict a very steep slope at low doses. Given the relative absence of exposures at very low levels, a decision should be made on biological grounds regarding which model makes the most sense for risk assessment. The committee believes that an additive (linear) or perhaps sublinear model is the most justifiable from a biological perspective, thus ruling out square-root and log-transformed models. For MeHg, the committee believes that a good argument can be made for the use of a K-power model with K constrained to be greater than or equal to 1.

3.5 Integrative Analysis

NRC presented an analysis that combined results from the SCDS, New Zealand, and Faroes studies. They estimated both a central tendency measure (21 ppm maternal hair mercury) and a lower limit based on a theoretical distribution of BMDs (7 ppm). The panel found the results to be supportive of their recommendations for BMD. They concluded, however, that use of this analysis as the basis for the RfD is premature.

3.6 Selection of the Point of Departure for the RfD

Based on all considerations in the preceding sections, the following is selected as the basis for the RfD. A benchmark approach using the results of the Boston Naming Test as expressed in ppb cord blood is chosen. The K-power model (K$1 to eliminate supralinearity) is the model choice, with $P_0 = 0.05$ and BMR = 0.05. Consistent with other uses of BMD, the 95% lower limit or BMDL is used as the point of departure for the RfD.

This results in a BMD of 85 ppb and a BMDL of 58 ppb. Corresponding values for hair Hg can be calculated by dividing the cord-blood concentration by a factor of 5 ppb of blood per ppm hair (Grandjean et al., 1992); the resulting BMDL is 12 ppm maternal hair mercury. This is slightly higher than the BMDL calculated directly from maternal hair mercury (10 ppm).

4. DOSE CONVERSION

The BMDL from the Faroes data on the Boston Naming Test was determined in units of ppb mercury in cord blood. In order to calculate an RfD, it is necessary to convert this to an ingested daily amount that would result in exposure to the developing fetus of 58 ppb mercury in blood. NRC (2000) offered advice on the use of these dose conversion procedures.

4.1 PBPK Models Versus One-Compartment Model

In estimating the 1995 RfD, EPA used a one-compartment model. Since publication of the MSRC, there have been evaluations of the use of this model and the parameter inputs as well as the discussion of PBPK models for methylmercury. For a description of the latter, refer to Chapter x.x of the Ambient Water Quality Criteria for Methylmercury. None of the existing models deal specifically with young children, nor are there data on methylmercury pharmacokinetics in children.

NRC briefly discussed the PBPK model published by Clewell et al. (1999). This model includes several fetal compartments that could be considered fetal submodels. NRC noted that this model is conceptually more accurate and flexible than the one-compartment model. The report also notes that the complexity of the model makes evaluation of it more problematic. Moreover, given the state of the data on methylmercury exposure, it would be necessary to use default values for some model inputs. These factors add to the overall uncertainty in the use of this or any of the other available PBPK models for methylmercury. EPA has chosen to use the one-compartment model for dose conversion for this RfD.

4.2 One-Compartment Model for Methylmercury

4.2.1 Description of Model

The model is described by the formula below:

$$d\ \mathrm{F}g/day\ ' \frac{C \times b \times V}{A \times f}$$

where

d	=	daily dietary intake (expressed as µg of methylmercury)
c	=	concentration in blood (expressed as µg/L)
b	=	elimination constant (expressed as $days^{-1}$)
V	=	volume of blood in the body (expressed as liters)
A	=	absorption factor (expressed as a unitless decimal fraction)

f = fraction of daily intake taken up by blood (unitless).

The formula can be solved for d as below:

$$d = \frac{c \times b \times V}{A \times f \times bw}$$

where

bw = body weight (expressed in kg).

In this one-compartment model, all maternal compartments are compressed to one: blood. It is assumed that the blood methylmercury concentration is at steady state. This assumption constitutes an area of uncertainty with the use of this model. One could either assume that the methylmercury concentrations of fetal blood and maternal blood are the same or adjust the cord-blood concentration to maternal levels using an empirically derived factor. There are some published indications that mercury in cord blood is 20% to 30% higher than in maternal blood (Dennis and Fehr, 1975: Pitkin et al., 1976; Kuhnert et al., 1981). Other publications show that there is no difference in concentration (Fujita and Takabatake, 1977; Sikorski et al., 1989). EPA has chosen to assume that maternal blood mercury is at the same level as fetal or cord blood and acknowledges that this is an additional area of uncertainty in the dose conversion.

4.2.2 Choice of Parameter Inputs—Distributions Versus Point Estimates

NRC presents an analysis of uncertainty and variability in the values to be used in the equation above. Although there are data from human studies that form the basis of the parameter estimates, it is clear that there is variability (and uncertainty) in these estimates. NRC notes that each of the model parameters is a random variable best described by a probability distribution. The ingested methylmercury concentration that leads to the benchmark cord-blood concentration is also a probability distribution determined by the combination of the distributions of the individual parameters. NRC cited two analyses of the variability and uncertainty in the ingested dose estimates based on the one-compartment model applied to maternal hair (Stern, 1997; Swartout and Rice, 2000) as well as similar analysis of a PBPK model (Clewell et al., 1999). Table 5-1 reproduces NRC's compilation of those analyses. NRC also supplied results of analyses that took maternal blood as the starting point, rather than maternal hair as was done in the published papers.

In 1995, EPA used central tendency estimates (or point estimates intended to reflect central tendency estimates) for all parameter inputs in the RfD dose conversion. Although it is a reasonable approach, it does not encompass the range of likely

parameter values or the range of estimated ingestion values. The RfD is not intended to protect only the mid-part of a population, but the whole population including sensitive subgroups. Thus, if one chooses to use central tendency or point estimates in the dose conversion, it is necessary to include an uncertainty factor in the final RfD calculation to ensure that pharmacokinetic variability is appropriately factored into the consideration of sensitive subgroups.

The choice of uncertainty factor can be informed by the analyses of variability presented by NRC. In general, all three analyses found similar ranges of variability due to pharmacokinetic factors. The ratios of estimated ingested doses at the 50th percentile/99th percentile ranged from 1.7 to 3.3. If one considers only the estimates using maternal blood as the starting point, then the range for all three studies is 1.7 to 3.0. NRC noted that variability was higher when maternal hair was the biomarker used, rather than blood mercury. In 1997, EPA identified the hair-to-blood ratio as a major contributor to the variability (and thus uncertainty) in estimating the ingested dose and in the RfD based on it. This provides an additional rationale for use of the cord-blood–based BMD.

In determining the methylmercury RfD, EPA chooses to use point estimates, rather than distributions, in the dose conversion and to account for uncertainty by application of a numerical uncertainty factor. NRC notes that use of parameter distributions and an ingested dose distribution (the "direct approach") does not eliminate uncertainty. In the direct approach, one would select an ingested dose corresponding to a BMD blood mercury concentration for the percentile of the population variability that is to be accounted for; that is, one would select the 95th or 99th (or some other suitable) percentile. The choice must be made among probability distributions predicted by analyses such as those done by Stern (1997) and Swartout and Rice (2000). NRC said that "the differences in the analyses are due to the use of different data sets for parameter estimates, and there is no clear basis for choosing one data set over another. Even when central-tendency estimates and uncertainty factors are used, the most appropriate value for each model parameter must be selected. Selection of different values for model parameters could underlie differences in the modeling results."

EPA chooses to make explicit choices for each dose-conversion parameter and to deal with both the uncertainty and variability implicit in those choices by the application of an uncertainty factor in the calculation of the RfD.

4.2.3 Choice of Parameter Inputs—Values for One-Compartment Model Terms

NRC recommended that in choices of point estimates EPA should consider the information and analyses in three publications: Stern (1997), Swartout and Rice (2000), and Clewell et al. (1999). All are recent contributions to the peer-reviewed literature. In addition, Swartout and Rice (2000) largely comprises analyses that received extensive scientific review as part of the MSRC. EPA found little in Clewell et al. (1999) that could be used directly to make parameter estimates, but rather used data and analyses from

the other two papers. The rationales for use of specific values for equation parameters follow.

Concentration in blood (c)

The concentration in blood is that corresponding to the BMDL, 58 ppb. As noted above, no numerical change is made to account for any potential differences between maternal blood mercury level and cord-blood concentration.

Fraction of mercury in diet that is absorbed (A)

After administration of radiolabeled methylmercuric nitrate in water to three healthy volunteers, uptake was reported to be >95% (Aberg et al., 1969). This value is supported by experiments in human volunteers conducted by Miettinen et al. (1971). These researchers incubated fish liver homogenate with radiolabeled methylmercury nitrate to produce methylmercury proteinate. The proteinate was then fed to fish for a week; the fish were killed, cooked, and fed to volunteers after confirmation of methylmercury concentration. The authors reported that the fraction of the administered dose not excreted in the feces within 3 to 4 days ranged from 91.2% to 97.0% with a mean of 94%. This fraction was assumed to be the amount absorbed. Stern (1997) noted that this method is most likely to result in an underestimate. It is generally felt that absorption of ingested methylmercury is high and not likely to vary a great deal. Use of an absorption factor of 0.95 as was done in the MSRC is reasonable.

Fraction of the absorbed dose that is found in the blood (f)

The MSRC notes that in 1995 EPA used data from Kershaw et al. (1980), Miettinen et al. (1971), and Sherlock et al. (1984) as the basis for the choice of a value of 0.05.

There are currently four published reports of the fraction of absorbed methylmercury dose distributed to blood volume in humans. Kershaw et al. (1980) reported an average fraction of 5.9% of absorbed dose in total blood volume, based on a study of five adult male subjects who ingested methylmercury-contaminated tuna. In a group of nine male and six female volunteers who had received ^{203}Hg-methylmercury in fish, approximately 10% of the total mercury body burden was present in 1 L of blood in the first few days after exposure; this dropped to approximately 5% over the first 100 days (Miettinen et al., 1971). In another study, an average value of 1.14% for the percentage of absorbed dose in kg of blood was derived from data on subjects who consumed a known amount of methylmercury in fish over a 3-month period (Sherlock et al., 1984). Average daily intake in the study ranged from 43 to 233 µg/day, and there was a dose-related effect on percentage of absorbed dose that ranged from 1.03% to 1.26% in 1 L of blood. Smith et al. (1994) administered radiolabeled methylmercury to seven subjects. The paper presented published modeled data rather than observations; the mean fraction of absorbed dose in blood was 7.7.% (SD, 0.88%).

Stern (1997) noted that while the Smith et al. (1994) and Kershaw et al. (1980) data could be fit by a log-normal distribution, the data sets are too small for a reasonable determination of the underlying distributions. Stern used the mean and standard deviation of those two data sets for average parameter values as inputs to the log-normal distribution; the average of the means is 0.067. Swartout and Rice (2000) used the observations published by Kershaw et al (1980), Miettinen et al. (1971), and Sherlock et al. (1984) as adjusted for 5 L of blood as inputs with a log-triangular distribution. The median value was 5.9% or 0.059, close to the values of 0.05 used in the MSRC and by other groups (e.g., Berglund et al., 1971, and WHO,1990). The median value of 0.059 was chosen by EPA for "f" in the dose conversion.

Elimination constant (b)

Currently, five studies report clearance half-times for methylmercury from blood or hair: Miettinen et al. (1971), Kershaw et al. (1980), Al-Shahristani et al. (1974), Sherlock et al. (1984), and Smith et al. (1994). The clearance half-lives for blood in these reports are quite variable, ranging from 32 to 189 days. In the Al-Shahristani et al. (1974) study, 10% of the sample population had mercury half-lives of 110 to 120 days. Average mercury half-lives from the five publications are 45 to 70 days. The MSRC used an average elimination constant of four of the studies (data from Smith et al. [1994] were not used). The corresponding elimination constant of 0.014 was also noted to be the average of individual values reported for 20 volunteers ingesting from 42 to 233 µg mercury/day in fish for 3 months (Sherlock et al., 1982).

Swartout and Rice (2000) applied a log-triangular distribution to the data from the five extant studies. They note that the distribution is highly skewed and that the median is 53 days; the corresponding elimination constant is 0.013.

Stern (1997) discussed the variability in the data sets. His analysis of variance indicated significant differences among the sets, which were eliminated when the Al-Sharistani data are removed. The author observed that the half-lives reported by Al-Sharistani are larger than those observed in the other studies. Stern offers the opinion that this may be due to the relatively large size of the Al-Sharistani data set by comparison to the others. Stern says that an alternative explanation is that the Al-Sharistani data reflect a genetic polymorphism in the metabolism occurring with higher frequency in the Iraqi population, which was the subject of this study. In his analyses, Stern (1997) treated the Al-Sharistani data both separately and in combination with the data from the other four studies. He reports a mean elimination constant of 0.011 for Al-Sharistani data alone; the combined data set mean elimination constant is 0.014.

The decision to select point estimates for dose conversion parameters was done with the acknowledgment that some of the variability around these parameters would be truncated. This is being compensated for in the use of a pharmacokinetic uncertainty factor. Nevertheless, it does not seem prudent to select a point estimate, which is meant to be reflective of population central tendency, from one data set only. The two central tendency estimates of Swartout and Rice (2000) and Stern (1997) are very close in value (0.013 versus 0.014); the differences are presumably due to the

application of different distribution types. The value of 0.014 is used for b in the dose conversion.

Volume of blood in the body (V)

In the MSRC, blood volume was estimated, as there were no data from the study population (the 81 pregnant women exposed in the poisoning episode in Iraq). It was noted then that blood volume is 7% of body weight, as determined by various experimental methods. MSRC assumed an increase of 20% to 30% (to about 8.5% to 9%) during pregnancy on the basis of the publication by Best (1961). Specific data for the body weight of Iraqi women were not found. Assuming an average body weight of 58 kg and a blood volume increase of 9% during pregnancy, a blood volume of 5.22 L was derived and was rounded to 5 L for the dose conversion.

Stern (1997) cited three studies (Brown et al., 1962; Retzlaff et al., 1969; Huff and Feller, 1956) wherein correlation of body weight and blood volume were demonstrated. All studies were of U.S. women, presumably not pregnant at the time of the study. The mean blood volumes for each study were 3.58 L, 3.76 L, and 3.49 L, respectively; the mean of the combined data set is 3.61 L. If one assumes a 30% increase in blood volume with pregnancy, this would be 4.67 L.

In their analysis, Swartout and Rice (2000) used data from a cohort of 20 pregnant Nigerian women (Harrison, 1966). Whole-blood volumes in the third trimester ranged from 4 to 6 L; the mean and median were both 5 L. Although 5 L is somewhat higher than the blood volume estimated from three studies of U.S. women, it is a reasonable value to use for V.

Body weight (bw)

The MSRC found no data on body weight for the study population and used a default value of 60 kg (rounded from 58) for an adult female. Swartout and Rice (2000) in their distributional analysis used the body weight data collected on the cohort of 20 pregnant Nigerian women (Harrison, 1966); this was the data set that they used for blood volume. Body weight during the third trimester of pregnancy ranged from 49.5 kg to 73.9 kg, with a geometric mean of 55 kg. Stern (1997) used the Third National Health and Nutritional Survey (NHANES III) data for women 18 to 40 years old (National Center for Health Statistics, 1995). The mean weight was 66.6 kg and the 50th percentile value was 62.8 kg. The EPA Methodology for Deriving Ambient Water Quality Criteria for the Protection of Human Health (U.S. EPA, 2000) also cites NHANES III data; in the Agency document, women of childbearing age were considered to be between the ages of 15 and 44 years old. The median body weight in this group was 63.2 kg and the mean was 67.3 kg. EPA also cites the earlier analyses of Ershow and Canter (1989); they do not state the age range but give a median of 64.4 kg and a mean of 65.8 kg. The recommendation in the EPA Methodology was to use a body weight value of 67 kg for a pregnant woman based on the relatively current data from NHANES III. This is the value used for bw in the dose conversion.

4.2.4 Dose Conversion Using the One-Compartment Model

For the RfC, the parameter values are as follows:

c = concentration in blood (expressed as 58 µg/L)
b = elimination constant (expressed as 0.014 $days^{-1}$)
V = volume of blood in the body (expressed as 5 L)
A = absorption factor (expressed as 0.9, unitless decimal fraction)
f = fraction of daily intake taken up by blood (0.059, unitless)
bw = body weight (expressed as 67 kg)

$$d = \frac{c \times b \times V}{A \times f \times bw}$$

$$d = \frac{58\ \mu g/L \times 0.014\ days^{-1} \times 5 L}{0.95 \times 0.059 \times 67\ kg}$$

$$d = 1.081\ \mu g/kg\text{-}day$$

rounded to 1.0 µg/kg/day.

5. CHOICE OF UNCERTAINTY FACTOR

5.1 Background

The RfD can be considered a threshold for a population at which it is unlikely that adverse effects will be observed. In estimating this level from either a NOAEL or a BMD, the risk assessor applies uncertainty factors; these are used to deal with both experimental and population variability and with lack of information that results in uncertainty in the risk estimate. For a discussion of uncertainty factors, refer to the Technical Support Document for Risk Assessment, Human Health Methodology for Ambient Water Quality Criteria.

In the MSRC, EPA published qualitative discussions and quantitative analyses of uncertainty and variability in the RfD based on the Iraqi data. Major sources of uncertainty identified were these: variability in susceptibility within the study cohort, variability in pharmacokinetic parameters for methylmercury (particularly biological half-life of methylmercury and the hair-to-blood ratio for mercury), response classification error, and lack of data on long term sequelae of *in utero* exposure. At that time a composite uncertainty factor of 10 was applied to account for these factors and the EPA policy choice to use an uncertainty factor in the absence of a two-generation reproductive bioassay.

NRC considered areas of uncertainty and variability relevant to the generation of an RfD based on data from the Faroes population and given the current state of the databases on both pharmacokinetics and effects of methylmercury. The panel concluded that not all sources of uncertainty or variability require addition of numerical uncertainty factors. NRC suggests that given the state of the human data on methylmercury, uncertainty factors be considered for two reasons:

- If the uncertainty could result in underestimation of the adverse effects of methylmercury exposure on human health.

- If there is reason to suspect that the U.S. population is more sensitive than the study populations to the adverse effects of methylmercury. NRC's recommendation was that an uncertainty factor of at least 10 be applied to a benchmark dose calculated from the Boston Naming Test results from the Faroe Islands study. EPA is in general agreement with NRC's conclusions and recommendations and considered them in the choice of the numerical uncertainty factor. Descriptions of areas of uncertainty and variability and choice of uncertainty factor are in the following sections.

5.2 Toxicodynamics

Individual response to methylmercury can vary as a function of many factors: age, gender, genetic makeup, health status, nutritional influences (including interaction among dietary components), and general individual toxicodynamic variability. Individual

sensitivity has been noted in the published human studies; NRC cited the example of members of the Iraqi population who seemed insensitive to high levels of mercury exposure. EPA believes there are insufficient data to conclude that the U.S. population is more or less sensitive than the reported human study populations. The U.S. population is extraordinarily diverse by any measures listed above, certainly by comparison to the Faroese population. The Faroese population is northern Caucasian, has been relatively isolated, and is thought to be descended from a small number of so-called founders who settled the islands many generations ago. In the heterogenous U.S. population, it is entirely likely that there are individuals both more and less sensitive to methylmercury toxicity than the cohort studied in the Faroes. As the RfD must be calculated to include sensitive subpopulations, variability in response to mercury is a consideration. EPA believes there are insufficient data to support a quantitative analysis of this area of variability and uncertainty for methylmercury.

5.3 Exposure Estimation as an Area of Uncertainty

Limitations in evaluation of exposure can be an additional source of uncertainty. As the RfD is based on a developmental outcome, there is particular concern for uncertainty in the linkage between time and intensity of exposure and critical periods of brain development. As noted before, cord-blood mercury generally reflects mercury exposure during late pregnancy and does not reflect temporal variability in exposure level. Use of any biomarker of methylmercury exposure can result in misclassification of exposure. Generally, exposure misclassification presents a bias to the null; that is, this source of error leads to decreased ability to detect a real effect. To the degree that there is exposure misclassification in the critical study, it would be expected to result in underestimation of the methylmercury effect.

5.4 Pharmacokinetic Variability

5.4.1 Cord:Maternal Blood Ratios

In its use of the one-compartment model for dose conversion, EPA chose to make no adjustment for potential differences between fetal and maternal blood mercury levels. Investigators have found that the placenta is not a barrier to the transfer of methylmercury from the mother to the developing fetus. Typically, there is a strong correlation between maternal blood mercury concentrations and fetal blood mercury concentrations as shown by cord blood. Although some investigators (notably Kuntz et al., 1982; Truska et al., 1989; and Sikorski et al., 1989) have found that there is approximately a 1:1 ratio of cord:maternal blood mercury, other researchers have reported cord blood to be higher than maternal blood in both mercury and methylmercury. Although methylmercury is preferentially bound to erythrocytes, the higher cord mercury concentration is not simply explained by differences in hematocrit (Kuhnert et al., 1981). Ratios of cord:maternal blood mercury greater than one are observed over exposures ranging from ~1 µg/L (e.g., Pitkin et al., 1976) to values >20 µg/L (e.g., Hansen et al., 1990). The extent to which mercury is concentrated in cord

blood differs among studies. In a large series of 497 women and their infants, Lauwerys et al. (1978) reported that cord blood was approximately 10% to 15% higher than maternal blood. However, cord blood has been reported to be higher than maternal blood by approximately 20% to 30% (Kuhnert et al., 1981; Pitkin et al., 1976); still other studies have shown substantially higher ratios (e.g., Soong et al., 1991). For example, Hansen et al. (1990), Dennis and Fehr (1975), and Bjerregaard and Hansen (2000) reported that the methylmercury concentration on the fetal side of the placenta is approximately twice as high as the maternal blood concentration. Within studies, individual cord blood may exceed maternal blood by as much as 300%. Cord blood has been reported to have a greater fraction of methylmercury than does maternal blood (Hansen et al., 1990; Ong et al., 1993). Some investigators report that total and methylmercury are higher in cord blood but that inorganic mercury is not elevated (Ong et al.,1993). Factors that determine these differences are not known.

There is little consistency in the published literature to support the calculation of a numerical adjustment factor to use in a dose conversion approach. Studies do not invariably indicate an increased cord-blood mercury by comparison to maternal levels. Those studies that do observe such an increase show large within-study variability. One study indicated that of x samples, y showed a cord:maternal ratio greater than 1, w were equal to 1, and v were less than 1. At this time no numerical adjustment is made, but it is clear that this constitutes an area of both pharmacokinetic variability and uncertainty.

5.4.2 Other Areas of Pharmacokinetic Variability

There is no specific evidence of genetic polymorphisms that affect methylmercury metabolism or excretion. Human studies have established, however, that there is great variability in some of the factors affecting the delivery of ingested methylmercury to target organs. The MSRC sensitivity analysis and the publication by Swartout and Rice (2000) noted that the greatest variability resided in the hair:blood ratio (not a factor in the current dose conversion), the fraction of absorbed methylmercury found in blood (f), and the half of methylmercury in blood (the reciprocal, b, in the current dose conversion).

NRC presented an analysis of methods of ingested dose reconstruction from biomarker measurements. NRC noted that cord-blood mercury is closely linked kinetically to the fetal brain compartment but less closely linked to ingested dose. As described in Section 4.0 of this document, EPA chose a one-compartment model and measures of cord-blood mercury for back calculation of the ingested dose of mercury. EPA also chose to use central tendency estimates for the parameters of the one-compartment model, rather than introduce an additional degree of uncertainty inherent in making choices of distribution shapes and the portion of the distribution that represents a sensitive population.

NRC presents analyses of uncertainty around dose conversion estimates, which are summarized in their Table 3.1 (reproduced below in Table 5-1).

Table 5-1. Comparison of Results from Three Analyses of the Interindividual Variability in the Ingested Dose of MeHg Corresponding to a Given Maternal hair or Blood Hg Concentration (reproduced from NRC, 2000)

Study	Maternal Medium	50th percentile[a] (µg/kg-d)	50th percentile/ 5th percentile[b]	50th percentile/1st percentile[c]
Stern (1997)	hair	0.03-0.05[d] (mean = 0.04)	1.8-2.4 (mean = 2.1)	2.3-3.3 (mean = 2.7)
	blood	0.01	1.5-2.2 (mean = 1.8)	1.7-3.0 (mean = 2.4)
Swartout and Rice (2000)	hair	0.08	2.2	Data not reported
	blood[e]	0.02	2.1	2.8
Clewell et al. (1999)	hair	0.08	1.5	1.8
	blood[f]	0.07	1.4	1.7

[a]Predicted 50th percentile of the ingested dose of methylmercury that corresponds to 1 ppm Hg in hair or 1 ppb in blood.
[b]Ratio of 50th percentile of ingested dose of methylmercury that corresponds to 1 ppm Hg in hair or 1 ppb in blood to the 5th percentile.
[c]Ratio of 50th percentile of ingested dose of methylmercury that corresponds to 1 ppm Hg in hair or 1 ppb in blood to the 1st percentile.
[d]Range reflects minimum and maximum values among eight alternative analyses.
[e]Data from J. Swartout, U.S. Environmental Protection Agency, personal commun.; June 9, 2000.
[f]Data from H.J. Clewell, ICF Consulting, personal commun.; April 19, 2000.

NRC discussed three independent analyses to characterize toxicokinetic variability in estimates of ingested dose corresponding to a BMD level in a particular biomarker, whether maternal hair or cord blood. These analyses were published by Stern (1997), Swartout and Rice (2000, after their work on EPA 1997), and Clewell et al. (1999). Each analysis used Monte Carlo simulation to combine probability distributions for each parameter of the model. For Stern (1997) and Swartout and Rice (2000), this was the one-compartment model shown in Section 4. Clewell et al. (1999) used a PBPK model with a fetal submodel. The analyses of the one- compartment model were done in a similar fashion; distributions for model parameters were determined from the published literature and shapes were set by the authors. Both analyses assumed correlations between some model parameters. Stern (1997) assumed that blood volume and body weight were correlated. Swartout and Rice (2000) made that assumption, as well as these correlations: hair-to-blood ratio and elimination rate constant, and fraction of absorbed dose in blood and body weight. The analysis based on the PBPK model also used parameter distribution values from the literature but included many more parameters than the one-compartment model (and more default distributions for model parameters).

The three published analyses all took maternal hair mercury as their starting point. NRC asked all three sets of authors to provide analyses of variability that used maternal blood as the starting point (as a surrogate for cord blood). These analyses were done by removing the hair:blood ratio from the model and running the Monte Carlo simulations.

Table 5-1 presents median estimates of ingested dose corresponding to 1 ppm maternal hair or 1 ppb maternal blood. Useful points of comparison are the ratios between the 50th percentile estimates and those at the end of the distribution (5th and 1st percentiles). Table 5-1 shows that using maternal blood as a starting point, the ratios of 50th percentile:1st percentile estimates ranges from 1.7 to 3.0. EPA's interpretation is that a factor of 3 will cover the toxicokinetic variability of 99% of the population. The choice of a factor of 3 also conforms to the standard EPA practice of a using a half-log to account for toxicokinetic variability.

5.5 Uncertainty in Choice of Critical Effect

Another critical area discussed by NRC is uncertainty around choice of a critical effect. NRC notes that developmental neurotoxicity is a sensitive indicator of methylmercury toxicity, but that there is some uncertainty as to the likelihood of other effects occurring at even lower levels of exposure. They cite indications of cardiovascular effects as well as neurotoxic effects uncovered later in life.

EPA agrees that there is a degree of uncertainty in our choice of critical effect; EPA believes this is not currently amenable to quantitative estimation but must be considered in the setting of the uncertainty factor. Summarized below are observations that support a concern that developmental neurotoxicity may not be the first effect of methylmercury exposure.

5.5.1 Cardiovascular Effects

There are some human data linking cardiovascular effects with exposure to elemental, inorganic, and organic forms of mercury. In addition, there are two recently published studies that show an association between low-level methylmercury exposure and cardiovascular effects. Sørensen et al. (1999) reported that in a study of 1,000 7-year-old Faroese children, diastolic and systolic blood pressures increased by 13.9 and 14.6 mm Hg, respectively, as the cord-blood mercury increased from 1 to 10 µg/L. They also reported a 47% decrease in heart rate variability (an indication of cardiac autonomic control) for the same increase in cord-blood mercury. Salonen et al. (1995) reported effects in adults from a study of 1,833 Finnish men. Over the 7-year observation period, men with hair mercury in the highest tertile (2 ppm or higher), had a 2.0 times greater risk of acute myocardial infarction than the rest of the study population.

As indicated by the Salonen (1995) study, the relatively subtle effects of methylmercury on cardiovascular indices can have public health implications. There is an analogous situation with lead exposure. Pirkle et al. (1985) reported on analyses of NHANES II data comparing the relationship between systolic and diastolic blood pressure to blood lead levels. They included in their model the 37% decrease in mean blood lead levels that was observed in white adult males between 1976 and 1980. Their calculation predicted a 4.7% decrease in the incidence of fatal and nonfatal

myocardial infarction over 10 years, a 6.7% decrease in the incidence of fatal and nonfatal strokes over 10 years, and a 5.5% decrease in the incidence of death from all causes over 11.5 years.

5.5.2 Persistent and Delayed Neurotoxicity

Another area of concern is the onset or exacerbation of neurological deficits in aging populations exposed *in utero* or as children. There are indications of this in the followup studies of the Minamata population wherein there is evidence that neurological dysfunction among people who have been exposed to methylmercury becomes exacerbated with aging. This heightened diminution of function is greater than that attributable to either age or methylmercury exposure alone. Specifically, Kinjo et al. (1993) surveyed 1,144 current patients with Minamata Disease (MD) aged 40 or over and an equal number of neighbor controls matched by age and sex. MD patients have symptoms of sensory disturbance at a high prevalence rate (e.g., hypoesthesia of mouth, ~20% to 29% of subjects; hypoesthesia of limbs, ~66% to 90% of subjects; dysesthesia of limbs, ~83% to 93%; weakness, ~75% to 84%), but these problems did not systematically increase with age. However, the MD patients did show as a function of age increased difficulties in speaking, tremor, stumbling, and difficulties with buttoning, clothing, or hearing. Although such changes also occurred among controls, evaluation of odds ratios showed that the MD patients had higher prevalence rates than the controls for 18 separate problems including those specifically listed above. Also evaluated were “acts of daily living” (ADL) that included the abilities to independently eat, bathe, wash, dress, and use the toilet. Among subjects under age 60 there were no significant differences in ADL abilities between MD patients and controls. However, among patients aged 60 or greater there were significantly lower ADL abilities among MD patients than among age-matched controls. A conclusion of the Kinjo et al. study is that the prevalence of deficits was relatively greater in cases compared with controls as a function of increasing age. In other words, exposure to methylmercury three decades earlier accelerated the aging process in aged individuals relative to younger ones.

There has also been evaluation of the health status of people living in methylmercury- polluted areas who were not designated as MD patients. Later followup by Fukuda et al. (1999) evaluated 1,304 adults who lived a methylmercury-polluted area near Minamata City in Kumamoto Prefecture in Japan (but were not designated MD patients) and 446 age-matched adults in a non-mercury–polluted area of Japan. All subjects were older than 40 years of age. A questionnaire survey evaluated 64 complaints that could be grouped as nonspecific, sensory, arthritic, and muscular. Complaints identified among male and female subjects that were significantly higher in methylmercury-contaminated areas included heart palpitation, dysesthesia, staggering when standing, resting and intention tremor in the hands, dizziness (especially when standing), low tone tinnitus, low pain sensation in hands and legs, and among women only, loss of touch sensations in hands and legs.

Animal studies lend support to the conclusion that methylmercury can have delayed effects that are uncovered with age. Spyker (1975) exposed mice during

gestation and lactation to methylmercury. Offspring noted to be normal at birth developed deficits in exploratory behavior and swimming ability at 1 month, neuromuscular and immune effects were noted as the animals reached 1 year of age, Rice (1989) exposed monkeys to 50 µg/kg/day methylmercury for the first 7 years of life. The animals were observed with motor incoordination only when they reached the age of 14; subsequent testing showed effects on somatosensory functioning (Rice and Gilbert, 1995). Rice (1998) also exposed monkeys *in utero* and for the first 4 years. Exposure to 10 to 50 µg/kg/day was observed to result in decreased auditory function compared with controls when the animals were tested at ages 11 and 19. The deficit at 19 years was relatively greater than at 11 years, providing evidence for an interaction of aging and methylmercury exposure on auditory impairment. All of these observations are consistent with a hypothesis that early life or *in utero* exposure to methylmercury can have adverse long-term sequelae that may not be detected in childhood.

5.5.3 Reproductive effects

EPA has a concern for potential reproductive effects of methylmercury. There are no studies of reproductive deficits in humans exposed to low-dose methylmercury. Bakir et al. (1973) did comment on the low number of pregnant women in the Iraqi population exposed to methylmercury in treated grain. They noted that among the 6,350 cases admitted to the hospital for toxicity, they would have expected 150 pregnancies; only 31 were reported. There are no two-generation reproductive assays for methylmercury. Shorter term studies in rodents and guinea pigs have reported effects including low sperm counts, testicular tubule atrophy, reduced litter size, decreased fetal survival, resorptions, and fetal malformations (Khera, 1973; Lee and Han, 1995; Hughes and Annau, 1976; Fuyuta et al., 1978, 1979; Hirano et al., 1986; Mitsumori et al., 1990; Inouye and Kajiwara, 1988). Burbacher et al. (1988) reported decreased conception rates, early abortions, and stillbirths in *Macaca fascicularis* monkeys treated with methylmercury hydroxide; the NOAEL for this study was 0.05 mg/kg/day. In a study of male *Macaca fascicularis* (Mohamed et al., 1987), a LOAEL for sperm abnormalities was 0.05 mg/kg/day.

The MSRC did an evaluation of the potential for methylmercury to be a germ-cell mutagen. Methylmercury is clastogenic but does not appear to cause point mutations. Methylmercury is widely distributed in the body, crossing both blood–brain and placental barriers in humans. Data indicate that methylmercury administered i.p. reaches germ cells and may produce adverse effects. When Suter (1975) mated female mice to treated males, he observed a slight reduction in both numbers of implantations and viable embryos; this was true for one mouse strain but not for another tested at the same time. When Syrian hamsters were treated intraperitoneally with methylmercury, aneuploidy but not chromosomal aberrations was seen in oocytes (Mailhes, 1983). Sex-linked recessive lethal mutations were increased in *Drosophila melanogaster* given dietary methylmercury (Ramel, 1972). Watanabe et al. (1982) noted some decrease in ovulation in hamsters treated s.c. with methylmercury, further indication that methylmercury is distributed to female gonadal tissue. Studies have reported increased incidence of chromosome aberrations (Skerfving et al., 1970, 1974) or sister chromatid

exchange (Wulf et al., 1986) in lymphocytes of humans ingesting mercury-contaminated fish or meat. Chromosome aberrations have been reported in cats treated *in vivo* and in cultured human lymphocytes in vitro. Evidence of DNA damage has been shown in a number of *in vitro* systems. The MSRC (U.S. EPA 1997) concluded that because there are data for mammalian germ-cell chromosome aberration and limited data from a heritable mutation study, methylmercury is placed in a group of high concern for potential human germ-cell mutagenicity. The only factor keeping methylmercury from the highest level of concern is lack of positive results in a heritable mutation assay.

In summary, there is increasing weight of evidence for effects other than neurodevelopmental that may be associated with low-dose methylmercury exposure.

5.6 Choice of Uncertainty Factor

The two major areas of uncertainty that must be addressed in the methylmercury RfD are interindividual toxicokinetic variability in ingested dose estimation and database insufficiency. For the former, EPA relied in part on the NRC analyses of variability in the pharmacokinetic factors underlying the conversion of a biomarker level of methylmercury to an ingested daily dose of methylmercury that corresponds to that level. Database insufficiency for methylmercury effects in humans is less amenable to quantitative analysis. Based on the discussions in the sections above, EPA identified several areas of database insufficiency.

In the calculation of this methylmercury RfD, a composite uncertainty factor of 10 is used. This is to account for the following factors:

- Pharmacokinetic variability and uncertainty in estimating an ingested mercury dose from cord blood. A factor of 3 is applied for this area.

- Database insufficiency
  - Lack of data on toxicodynamic variability. EPA most commonly applies a factor of 3 to deal with toxicodynamic variability and uncertainty.
  - Inability to quantify long-term sequelae.
  - Uncertainty as to selection of critical effect—insufficient data on cardiovascular effects and lack of a two-generation reproductive effects assay. EPA most commonly uses a factor of 3 to deal with the lack of a two-generation reproductive effects assay.

Given the overall robustness of the methylmercury database, but in consideration of the above areas of uncertainty, a composite factor of 10 is warranted.

6. CALCULATION OF THE RFD

For methylmercury, the RfD is calculated as follows:

$$RfD = \frac{BMD}{UF \times MF}$$

$$= \frac{1.0\ \mu g/kg\text{-}day}{10}$$

$$= 1 \times 10^{-4}\ mg/kg\text{-}day$$

or 0.1 µg/kg/day.

7. REFERENCES

Aberg, B., L. Ekman, R. Falk, U. Greitz, G. Persson, and J. Snihs. 1969. Metabolism of methyl mercury (Hg) compounds in man: excretion and distribution. Arch. Environ. Health 19:478-484.

Allen, B.C., R.J. Kavlock, C.A. Kimmel, and E.M. Faustman. 1994. Dose-response assessment for developmental toxicity. II. Comparison of generic benchmark dose estimates with no observed adverse effect levels. Fundam. Appl. Toxicol. 23(4):487-495.

Al-Shahristani, H., and K.M. Shihab. 1974. Variation of biological half-life of methyl mercury in man. Arch. Environ. Health 28:342-344.

Axtell, C.D., G.J. Myers, P.W. Davidson, A.L. Choi, E. Cernichiari, J. Sloane-Reeves, C. Cox, C. Shamlaye, and T.W. Clarkson. 1998. Semiparametric modeling of age at achieving developmental milestones after prenatal exposure to methylmercury in the Seychelles child development study. Environ. Health Perspect. 106(9):559-564.

Bakir, F., S. Damluji, L. Amin-Zaki, et al. 1973. Methylmercury poisoning in Iraq. Science 181:230-241.

Bellinger, D. 1995. Interpreting the literature on lead and child development: The neglected role of the "experimental system." Neurotoxicol. Teratol. 17(3):201-212.

Berglund, F., M. Berlin, G. Birke, R. Cederlof, U. von Euler, L. Friberg, B. Holmsteadt, E. Jonsson, et al. 1971. Methyl mercury in fish: a toxicologic-epidemiologic evaluation of risks. Report from an expert group. Nordisk Hygienisk Tidskrift, Stockholm Suppl. 4:19-364.

Bjerregaard P., and J.C. Hansen. 2000. Organochlordines and heavy metals in pregnant women from the Disko Bay area in Greenland. Sci. Total Environ. 17:245(1-3):195-202.

Bonthius, D.J., and J.R. West. 1990. Alcohol-induced neuronal loss in developing rats: Increased brain damage with binge exposure. Alcohol Clin. Exp. Res. 14(1):107-118.

Brown, E., J. Hopper, Jr., J.L. Hodges, Jr., B. Bradley, R. Wennesland, and H. Yamuchi. 1962. Red cell, plasma, and blood volume in healthy women measured by radiochromium cell-labeling and hematocrit. J. Clin. Invest. 41:2182-2190.

Budtz-Jørgensen, E., P. Grandjean, N. Keiding, et al. 2000. Benchmark dose calculations of methylmercury-associated neurobehavioral deficits. Toxicol. Lett. 112-113:193-199.

Budtz-Jørgensen, E., N. Keiding, and P. Grandjean. 1999. Benchmark modeling of the Faroese methylmercury data. Final Report to U.S. EPA.

Burbacher, T.M., M..K. Mohamed, and N.K. Mottett. 1988. Methyl mercury effects on reproduction and offspring size at birth. Reprod. Toxicol. 1:267-278.

Clarkson, T.W., L. Amin-Zaki, and S. Al-Tikriti. 1976. An outbreak of methyl mercury poisoning due to consumption of contaminated grain. Fed. Proc. 35:2395-2399.

Clewell, H.J. 2000. Personal communication, April 19, 2000. ICF Consulting.

Clewell, H.J., J.M. Gearhart, P.R. Gentry, T.R. Covington, C.B. Van Landingham, K.S. Crump, and A.M. Shipp. 1999. Evaluation of the uncertainty in an oral reference dose for methylmercury due to interindividual variability in pharmacokinetics. Risk Anal. 19:547-558.

Cordier, S., and M. Garel. 1999. Neurotoxic risks in children related to exposure to methylmercury in French Guiana. INSERT U170 and U149--Study financed by the Health Monitoring Institute (RNSP). National Institute of Health and Medical Research.

Counter, S.A., L.H. Buchanan, G. Laurell, and F. Ortega. 1998. Blood mercury and auditory neuro-sensory responses in children and adults in the Nambija gold mining area of Ecuador. Neurotoxicology 19(2):185-196.

Cox, C., D. Marsh, G. Myers, and T. Clarkson. 1995. Analysis of data on delayed development from the 1971-72 outbreak of methylmercury poisoning in Iraq: assessment of influential points. Neurotoxicology 16(4):727-730.

Crump, K., T. Kjellstrom, A. Shipp, A. Silvers, and A. Stewart. 1998. Influence of prenatal mercury exposure upon scholastic and psychological test performance: statistical analysis of a New Zealand cohort. Risk Anal. 18:701-713.

Crump, K., C. Landingham, C. Shamlaye, C. Cox, P. Davidson, G. Myers, and T. Clarkson. 2000. Benchmark concentrations for methylmercury obtained from the Seychelles child development study. Environ. Health Perspect. 108:257-263.

Crump, K., J. Viren, A. Silvers, H. Clewell, J. Gearhart, and A. Shipp. 1995. Reanalysis of dose-response data from the Iraqi methylmercury poisoning episode. Risk Anal. 15:523-532.

Dahl, R., R.F. White, P. Weihe, N. Sorensen, R. Letz, H.K. Hudnell, D.A. Otto, and P. Grandjean. 1996. Feasibility and validity of three computer-assisted neurobehavioral tests in 7-year old children. Neurotoxicol. Teratol. 19(4):413-419.

Davidson, P., G. Myers, C. Cox, C. Shamlaye, D. Marsh, M. Tanner, M. Berlin, J. Sloane-Reeves, E. Cernichiari, O. Choisy, A. Choi, and T. Clarkson. 1995. Longitudinal neurodevelopmental study of Seychellois children following in utero exposure to methylmercury from maternal fish ingestion: outcomes at 19 and 29 months. Neurotoxicology 16:677-688.

Davidson, P.W., G.J. Myers, C. Cox, C. Axtell, C. Shamlaye, J. Sloane-Reeves, E. Cernichiari, L. Needham, A. Choi, Y. Yang, M. Berlin, and T.W. Clarkson. 1998. Effects of prenatal and postnatal methylmercury exposure from fish consumption on neurodevelopment: outcomes at 66 months of age in the Seychelles child development study. JAMA 280:701-707.

Dennis, C.A., and F. Fehr. 1975. The relationship between mercury levels in maternal and cord blood. Sci. Total Environ. 3(3):275-277.

Ershow, A.G., and K.P. Canter. 1989. Total water and tapwater intake in the United States: population-based estimates of quantities and sources. Life Sciences Research Office, Federation of Amercian Societies for Experimental Biology, Bethesda, MD. (Prepared under NCI #263-MD-810264.)

Fujita, M., and E. Takabatake. 1977. Mercury levels in human maternal and neonatal blood, hair and milk. Bull. Environ. Contam. Toxicol. 18(2):205-209.

Fukuda, Y., K. Ushijima, T. Kitano, M. Sakamoto, and M. Futatsuka. 1999. An analysis of subjective complaints in a population living in a methylmercury-polluted area. Environ. Research 81:100-107.

Fuyuta, M., T. Fujimoto, and S. Hirata. 1978. Embryotoxic effects of methylmercuric chloride administered to mice and rats during organogenesis. Teratology 18(3):353-366.

Fuyuta, M., T. Fujimoto, and E. Kiyofuji. 1979. Teratogenic effects of a single oral administration of methylmercuric chloride in mice. Acta Anat (Basel) 104(3):356-362.

Gaylor, D.W., and W. Slikker. 1992. Risk assessment for neurotoxicants. In: Neurotoxicology. Tilson, H., and C. Mitchell, eds. New York: Raven Press, pp. 331-343.

Goodlett, C.R., S.J. Kelly, and J.R. West. 1987. Early postnatal alcohol exposure that produces high blood alcohol levels impairs development of spatial navigation learning. Psychobiology 15(1):64-74.

Grandjean, P., P. Weihe, P.J. Jorgensen, T. Clarkson, E. Cernichiari, and T. Videro. 1992. Impact of maternal seafood diet on fetal exposure to mercury, selenium, and lead. Arch. Environ. Health 47:185-195.

Grandjean, P., P. Weihe, and R. White. 1995. Milestone development in infants exposed to methylmercury from human milk. NeuroToxicology 16:27-34.

Grandjean, P., P. Weihe, R. White, F. Debes, S. Arak, K. Yokoyama, K. Murata, N. Sorensen, R. Dahl, and P. Jorgensen. 1997. Cognitive deficit in 7-year-old children with prenatal exposure to methylmercury. Neurotoxicol. Teratol. 20:1-12.

Grandjean, P., P. Weihe, R.F. White, N. Keiding, E., Budtz-Jørgensen, K. Murato, and L. Needham. 1998. Prenatal exposure to methylmercury in the Faroe Islands and neurobehavioral performance at age seven years. Response to workgroup questions for presentation on 18-20 November 1998. In: Scientific issues relevant to assessment of health effects from exposure to methylmercury. Appendix II-B.- Faroe Islands Studies. National Institute for Environmental Health Sciences. [Online]. Available: http://ntp-server.niehs.nih.gov/ Main_Pages/PUBS/MethMercWkshpRpt.html.

Grandjean, P., R.F. White, A. Nielsen, D. Cleary, and E. de Oliveira Santos. 1999. Methylmercury neurotoxicity in Amazonian children downstream from gold mining. Environ. Health Perspect. 107:587-591.

Harada, M. 1995. Minamata disease: methylmercury poisoning in Japan caused by environmental pollution. Crit. Rev. Toxicol. 25(1):1-24.

Harrison, K.A. 1966. Blood volume changes in normal pregnant Nigerian women. J. Obstet. Gynaec. Brit. Cwlth. 73:717-723.

Hirano, M., K. Mitsumori, K. Maita, and Y. Shirasu. 1986. Further carcinogenicity study on methylmercury chloride in ICR mice. Nipon Juigaku Zasshi (Jpn. J. Vet. Sci.) 48(1):127-135.

Huff, R.L., and D.D. Feller. 1956. Relation of circulating red cell volume to body density and obesity. J. Clin. Invest. 35:1-10.

Hughes, J.A., and Z. Annau. 1976. Postnatal behavioral effects in mice after prenatal exposure to methylmercury. Pharmacol. Biochem. Behav. 4(4):385-391.

Inouye, M., and Y. Kajiwara. 1988. Developmental disturbances of the fetal brain in guinea pigs caused by methylmercury. Arch. Toxicol. 62(1):15-21.

Jacobson, J.L., and S.W. Jacobson. 1996. Intellectual impairment in children exposed to polychlorinated biphenyls in utero. N. Engl. J. Med. 335(11):783-789.

Kershaw, T.G., T.W. Clarkson, and P.H. Dhahir. 1980. The relationship between blood levels and dose of methyl mercury in man. Arch. Environ. Health 35:28-36.

Khera, K.S. 1973. Reproductive capability of male rats and mice treated with methylmercury. Toxicol. Appl. Pharmacol. 24(2):167-177.

Kinjo, Y., H. Higashi, A. Nakano, M. Sakamoto, and R. Sakai. 1993. Profile of subjective complaints and activities of daily living among current patients with Minamata disease after 3 decades. Environ. Res. 63(2):241-251.

Kjellstrom, T., P. Kennedy, S. Wallis, and C. Mantell. 1986a. Physical and mental development of children with prenatal exposure to mercury from fish. Stage 1:

preliminary tests at age 4. Report 3080. Solna, Sweden: National Swedish Environmental Protection Board.

Kjellstrom, T., P. Kennedy, S. Wallis, et al. 1986b. Physical and mental development of children with prenatal exposure to mercury from fish. Stage 2: interviews and psychological tests at age 6. Solna, Sweden: National Swedish Environmental Protection Board, Report 3642.

Kjellstrom, T., P. Kennedy, S. Wallis, A. Stewart, L. Friberg, B. Lind, T. Wutherspoon, and C. Mantell. 1989. Physical and mental development of children with prenatal exposure to mercury from fish. Stage 2: interviews and psychological tests at age 6. Report 3642. Solna, Sweden: National Swedish Environmental Protection Board, p. 112.

Kuhnert, P.M., B.R. Kuhnert, and P. Erhard. 1981. Comparison of mercury levels in maternal blood, fetal cord blood, and placental tissues. Am. J. Obstet. Gynecol. 139(2):209-213.

Kuntz, W.D., R.M. Pitkin, A. Bostrom, and M.S. Hughes. 1982. Maternal and cord blood background mercury levels: a longitudinal surveillance. Am. J. Obstet. Gynecol. 143:440-443.

Lauwerys, R., J.P. Buchet, H. Roels, and G. Hubermont. 1978. Placental transfer of lead, mercury, cadmium, and carbon monoxide in women. I. Comparison of the frequency distributions of the biological indices in maternal and umbilical cord blood. Environ. Res. 15(2):278-289.

Lee, J.H., and D.H. Han. 1995. Maternal and fetal toxicity of methylmercuric chloride administered to pregnant Fischer 344 rats. J. Toxicol. Environ. Health 45(4):415-425.

Leisenring, W., and L. Ryan. 1992. Statistical properties of the NOAEL. Regul. Toxicol. Pharmacol. 15(2 Pt. 1):161-171.

Mailhes, J.B. 1983. Methyl mercury effects on Syrian hamster metaphase II oocyte chromosomes. Environ. Mutagen. 5:679-686.

Marsh, D., T. Clarkson, C. Cox, G. Myers, L. Amin-Zaki, and S. Al-Tikriti. 1987. Fetal methylmercury poisoning. Relationship between concentration in single strands of maternal hair and child effects. Arch. Neurol. 44:1017-1022.

Marsh, D.O, M.D. Turner, J.C. Smith, P. Allen, and N. Richdale. 1995. Fetal methylmercury study in a Peruvian fish-eating population. Neurotoxicology 16(4):717-726.

McKeown-Eyssen, G., J. Ruedy, and A. Neims. 1983. Methyl mercury exposure in northern Quebec. II. Neurologic findings in children. Am. J. Epidemiol. 118:470-479.

Miettinen, J.K., T. Rahola, T. Hattula, K. Rissanen, and M. Tillander. 1971. Elimination of ^{203}Hg-methylmercury in man. Ann. Clin. Res. 3:116-122.

Mitchell, J.W., T.E.U. Kjellström, and L. Reeves. 1982. Mercury in takeaway fish in New Zealand. N. Z. Med. J. 95(702):112-114.

Mitsumori, K., M. Hirano, H. Ueda, K. Maita, and Y. Shirasu. 1990. Chronic toxicity and carcinogenicity of methylmercury chloride in B6C3F1 mice. Fundam. Appl. Toxicol. 14:179-190.

Mohamed, M., T. Burbacher, and N. Mottet. 1987. Effects of methylmercury on testicular functions in Macaca fascicularis monkeys. Pharmacol. Toxicol. 60(1):29-36.

Murata, K., P. Weihe, S. Araki, E. Budtz-Jorgensen, and P. Grandjean. 1999. Evoked potentials in Faroese children prenatally exposed to methylmercury. Neurotoxicol. Teratol. 21:471-472.

Myers, G., D. Marsh, P. Davidson, C. Cox, C. Shamlaye, M. Tanner, A. Choi, E. Cernichiari, O. Choisy, and T. Clarkson. 1995a. Main neurodevelopmental study of Seychellois children following *in utero* exposure to methylmercury from a maternal fish diet: outcome at six months. Neurotoxicology 16:653-664.

Myers, G.J., P.W. Davidson, C. Cox, C.F. Shamlaye, M.A. Tanner, O. Choisy, J. Sloane-Reeves, D.O. Marsh, E. Cernichiari, A. Choi, M. Berlin, and T.W. Clarkson. 1995b. Neurodevelopmental outcomes of Seychellois children sixty-six months after *in utero* exposure to methylmercury from a maternal fish diet: pilot study. Neurotoxicology 16:639-652.

Myers, G.J., P.W. Davidson, and C.F. Shamlaye. 1998. A review of methylmercury and child development. Neurotoxicology 19(2):313-328.

Nordberg, G.F., and P. Strangert. 1976. Estimations of a dose-response curve for long-term exposure to methylmercuric compounds in human being taking into account availability of critical organ concentration and biological half-time: a preliminary communication. In: Effects and dose-response relationships of toxic metals. Nordberg, G.F., ed. Amsterdam: Elsevier, pp. 273-282.

NCHS (National Center for Health Statistics). 1995. [Section 4.2.3, body weight]

NIEHS (National Institute of Environmental Health Sciences). 1998. Scientific issues relevant to assessment of health effects from exposure to methylmercury. Workshop organized by Committee on Environmental and Natural Resources (CENR) Office of Science and Technology Policy (OSTP), The White House, November 18-20, 1998, Raleigh, NC.

NRC (National Research Council). 2000. Toxicological Effects of Methylmercury. Committee on the Toxicological Effects of Methylmercury, Board on Environmental

Studies and Toxicology, Commission on Life Sciences, National Research Council. Washington, DC: National Academy Press.

Ong, C.N., S.E. Chia, S.C. Foo, H.Y. Ong, M. Tsakok, and P. Liouw. 1993. Concentrations of heavy metals in maternal and umbilical cord blood. Biometals 6:61-66.

Patandin, S., C.I. Lanting, P.G. Mulder, E.R. Boersma, P.J. Sauer, and N. Weisglas-Kuperus. 1999. Effects of environmental exposure to polychlorinated biphenyls and dioxins on cognitive abilities in Dutch children at 42 months of age. J. Pediatr. 134(1):33-41.

Pirkle, J.L., J. Schwartz, J.R. Landis, and W.R. Harlan. 1985. The relationship between blood lead levels and blood pressure and its cardiovascular risk implications. Am. J. Epidemiol. 121:246-258.

Pitkin, R.M., J.A. Bahns, L.J. Filer, Jr., and W.A. Reynolds. 1976. Mercury in human maternal and cord blood, placenta, and milk. Proc. Soc. Exp. Biol. Med. 151(3):565-567.

Ramel, C. 1972. Genetic effects. In: Mercury in the environment. Friberg, L., and J. Vostal, eds. Cleveland: CRC Press, pp. 169-181.

Retzlaff, J.A., W.N. Tauxe, J.M. Khely, and C.F. Strobel. 1969. Erythrocyte volume, plasma volume, and lean body mass in adult men and women. Blood 33:649-664.

Rice, D.C. 1989a. Delayed neurotoxicity in monkeys exposed developmentally to methylmercury. Neurotoxicology 10:645-650.

Rice, D.C. 1989b. Brain and tissue levels of mercury after chronic methyl mercury exposure in the monkey. J. Toxicol. Environ. Health 27:189-198.

Rice, D.C. 1989c. Blood mercury concentrations following methyl mercury exposure in adult and infant monkeys. Environ. Res. 49:115-126.

Rice, D.C. 1998. Age-related increase in auditory impairment in monkeys exposed in utero plus postnatally to methylmercury. Toxicol. Sci. 44(2):191-196.

Rice, D.C., and S.G. Gilbert. 1995. Effects of developmental methylmercury exposure or lifetime lead exposure on vibration sensitivity function in monkeys. Toxicol. Appl. Pharmacol. 134(1):161-169.

Rice, D.C., D. Krewski, B.T. Collins, and R.F. Willes. 1989. Pharmacokinetics of methylmercury in the blood of monkeys (*Macaca fascicularis*). Fundam. Appl. Toxicol. 12:23-33.

Salonen, J.T., K. Seppanen, K. Nyyssonen, H. Korpela, J. Kauhanen, M. Kantola, J. Tuomilehto, H. Esterbauer, F. Tatzber, and R. Salonen. 1995. Intake of mercury from fish, lipid peroxidation, and the risk of myocardial infarction and coronary, cardiovascular, and any death in Eastern Finnish men. Circulation 91(3):645-655.

Schwartz, J.G., T.E. Snider, and M.M. Montiel. 1992. Toxicity of a family from vacuumed mercury. Am. J. Emerg. Med. 10(3):258-261.

Sherlock, J., J. Hislop, D. Newton, G. Topping, and K. Whittle. 1984. Elevation of mercury in human blood from controlled chronic ingestion of methylmercury in fish. Hum. Toxicol. 3:117-131.

Sherlock, J.C., D.G. Lindsay, J. Hislop, W.H. Evans, and T.R. Collier. 1982. Duplication diet study on mercury intake by fish consumers in the United Kingdom. Arch. Environ. Health 37(5): 271-278.

Sikorski, R., T. Paszkowski, P. Slawinski, J. Szkoda, J. Zmudzki, and S. Skawinski. 1989. The intrapartum content of toxic metals in maternal blood and umbilical cord blood. Ginekol. Pol. 60(3):151-155.

Skerfving, S. 1974. Methylmercury exposure, mercury levels in blood and hair, and health status in Swedes consuming contaminated fish. Toxicology 2:3-23.

Skerfving, S., K. Hansson, and J. Lindsten. 1970. Chromosome breakage in humans exposed to methyl mercury through fish consumption. Arch. Environ. Health 21(2):133-139.

Smith, J.C., P.V. Allen, M.D. Turner, B. Most, H.L. Fisher, and L.L. Hall. 1994. The kinetics of intravenously administered methylmercury in man. Toxicol. Appl. Pharmacol. 128:251-256.

Soong, Y-K, R. Tseng, C. Liu, and P-W Lin. 1991. Lead, cadmium, arsenic, and mercury levels in maternal and fetal cord blood. J. Formosan Med. Assn. 90:59-65.

Sorensen, N., K. Murata, E. Budtz-Jorgensen, P. Weihe, and P. Grandjean. 1999. Prenatal methylmercury exposure as a cardiovascular risk factor at seven years of age. Epidemiology 10:370-375.

Spyker, J.M. 1975. Assessing the impact of low level chemicals on development: behavioral and latent effects. Fed. Proc. 34(9):1835-1844.

Stern, A.H. 1997. Estimation of the interindividual variability in the one-compartment pharmacokinetic model for methylmercury: implications for the derivation of a reference dose. Regul. Toxicol. Pharmacol. 25:277-288.

Steurwald, U., P. Weibe, P. Jorgensen, K. Bjerve, J. Brock, B. Heinzow, E. Budtz-Jorgensen, and P. Grandjean. 2000. Maternal seafood diet, methylmercury exposure, and neonatal neurologic function. J. Pediatr. 136(5):599-605.

Suter, K.E. 1975. Studies on the dominant lethal and fertility effects of the heavy metal compounds methyl mercuric hydroxide, mercuric chloride, and cadmium chloride in male and female mice. Mutat. Res. 30:365-374.

Suzuki, T., J. Yonemoto, H. Satoh, A. Naganuma, N. Imura, and T. Kigama. Normal organic and inorganic mercury levels in the human feto-placental system. J. Appl. Toxicol. 4(5):249-252.

Swartout, J. 2000. Personal communication, June 9, 2000, U.S. Environmental Protection Agency.

Swartout, J., and G. Rice. 2000. Uncertainty analysis of the estimated ingestion rates used to derive the methylmercury reference dose. Drug Clin. Toxicol. 23(1):293-306.

Trillingsgaard, A., O.N. Hansen, and I. Beese. 1985. The Bender-Gestalt Test as a neurobehavioral measure of preclinical visual-motor integration deficits in children with low-level lead exposure. In: WHO Environmental Health, Document 3. Neurobehavioral methods in occupational and environmental health, Second International Symposium, Copenhagen, Denmark, Aug. 6-9, 1985. Copenhagen, Denmark: World Health Organization, pp. 189-193.

Truska, P., I. Rosival, G. Balazova, J. Hinst, A. Rippel, O. Palusova, and J. Grunt. 1989. Placental concentrations of cadmium, lead, and mercury in mothers and their newborns. J. Hygiene Epidemiol. Microbiol. Immunol. 33(2):141-147.

Tsuchiya, H., K. Mitani, K. Kodama, and T. Nakata. 1984. Placental transfer of heavy metals in normal pregnant Japanese women. Arch. Environ. Health 39(1):11-17.

U.S. EPA (United States Environmental Protection Agency). 1980. Ambient water quality criteria document for mercury. Prepared by the Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office, Cincinnati, OH for the Office of Water Regulation and Standards, Washington, DC. EPA/440/5-80-058. NTIS PB 81-117699.

U.S. EPA. 1997. Mercury study report to Congress. Vol. IV: an assessment of exposure to mercury in the United States. U.S. EPA, Office of Air Quality Planning and Standards and Office of Research and Development. EPA/452/R-97-006.

Watanabe, T., T. Shimada, and A. Endo. 1982. Effects of mercury compounds on ovulation and meiotic and mitotic chromosomes in female golden hamsters. Teratology 25(3):381-384.

WHO (World Health Organization). 1976. Environmental Health Criteria: Mercury. Geneva, Switzerland: World Health Organization, p. 121.

WHO. 1990. Environmental health criteria 101. Methylmercury. Geneva, Switzerland: World Health Organization.

Wulf, H.C., N. Kromann, N. Kousgaard, J.C. Hansen, E. Niebuhr, and K. Alboge. 1986. Sister chromatid exchange (SCE) in Greenlandic Eskimos. Dose-response relationship between SCE and seal diet, smoking, and blood cadmium and mercury concentrations. Sci. Total. Environ. 48(1-2):81-94.

Yang, J., Z. Jiang, Y. Wan, I.A. Qureshi, X.D. Wu. 1997. Maternal-fetal transfer of metallic mercury via the placenta and milk. Ann. Clin. Lab. Sci. 27(2):135-141.