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Water Quality Criterion for the Protection of Human Health: Methylmercury

Final

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NOTICE

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Potential areas for conflict of interest were investigated via direct inquiry with the peer reviewers and review of their current affiliations. No conflicts of interest were identified.

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EXECUTIVE SUMMARY

About This Document

This document is the basis for a human health Ambient Water Quality Criterion (AWQC) for methylmercury. This AWQC replaces the AWQC for total mercury in published in 1980 and partially updated in 1997. Under Section 304(a) of the Clean Water Act, EPA must periodically revise criteria for water quality to accurately reflect the latest scientific knowledge on the kind and extent of all identifiable effects of pollutants on human health.

This document uses new methods and information described in the Methodology for Deriving Ambient Water Quality Criteria for the Protection of Human Health (2000) (2000 Human Health Methodology) (U.S. EPA, 2000a,b). These new methods include updated approaches to determine toxicity dose-response relationships for both carcinogenic and noncarcinogenic effects, updated information for determining exposure factors, and new procedures to determine bioaccumulation factors.

The Mercury Study Report to Congress (MSRC) (U.S. EPA, 1997), an eight-volume report prepared by the U.S. Environmental Protection Agency (EPA) and submitted to Congress in 1997, serves as a primary information source on methylmercury. However, as the state of the science for methylmercury is continuously and rapidly evolving, the information from the MSRC has been supplemented by inclusion of published information since 1997.

Exposure to Methylmercury

The major pathway for human exposure to methylmercury is consumption of contaminated fish. Dietary methylmercury is almost completely absorbed into the blood and is distributed to all tissues including the brain; it also readily passes through the placenta to the fetus and fetal brain.

Major Health Effects of Methylmercury

Methylmercury is a highly toxic substance with a number of adverse health effects associated with its exposure in humans and animals. Epidemics of mercury poisoning following high-dose exposures to methylmercury in Japan and Iraq demonstrated that neurotoxicity is the health effect of greatest concern. These epidemics led to observation of methylmercury effects on the fetal nervous system. High-dose

human exposure results in mental retardation, cerebral palsy, deafness, blindness, and dysarthria in utero and in sensory and motor impairment in adults. Although developmental neurotoxicity is currently considered the most sensitive health endpoint, data on cardiovascular and immunological effects are beginning to be reported and provide more evidence for toxicity from low-dose methylmercury exposure.

Three large prospective epidemiology studies in the Seychelles Islands, New Zealand, and the Faroe Islands were designed to evaluate childhood development and neurotoxicity in relation to fetal exposures to methylmercury in fish-consuming populations. Prenatal methylmercury exposures in these three populations were within the range of some U.S. population exposures. No adverse effects were reported from the Seychelles Islands study, but children in the Faroe Islands exhibited subtle developmental dose-related deficits at 7 years of age. These effects include abnormalities in memory, attention, and language. In the New Zealand prospective study, children at 4 and 6 years of age exhibited deficiencies in a number of neuropsychological tests.

In addition to the three large epidemiological studies, studies on both adults and children were conducted in the Amazon; Ecuador; French Guiana; Madeira; Mancora, Peru; northern Quebec; and Germany. Effects of methylmercury on the nervous system were reported in all but the Peruvian population.

Other Health Effects of Methylmercury

Methylmercury causes chromosomal effects but does not induce point mutations. The MSRC 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. There is no two-generation study of reproductive effects, but shorter term studies in rodents, guinea pigs and monkeys have reported observations consistent with reproductive deficits. There are no data to indicate that methylmercury is carcinogenic in humans, and it induces tumors in animals only at highly toxic doses. Application of the proposed revisions to the Guidelines for Cancer Risk Assessment (EPA 1999)leads to a judgment that methylmercury is not likely to be carcinogenic for humans under conditions of exposure generally encountered in the environment.

Quantitative Risk Estimate for Methylmercury

The quantitative health risk assessment for a noncarcinogen relies on a reference dose (RfD). This is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious health effects during a lifetime. To derive an RfD, one first establishes a no adverse effect level (NOAEL) for a particular endpoint. This can be done by inspection of the available data or by using a mathematical modeling procedure to estimate the NOAEL; the latter approach was used for methylmercury. Next the NOAEL is divided by a numerical uncertainty factor to account for areas of variability and uncertainty in the risk estimate.

There has been considerable discussion within the scientific community regarding the level of exposure to methylmercury that is likely to be without an appreciable risk of deleterious health effects during a lifetime. In 1999, the Congress directed EPA to contract with the National Research Council (NRC) of the National Academy of Sciences to evaluate the body of data on the health effects of methylmercury. NRC was to concentrate on new data since the 1997 MSRC, and to provide recommendations regarding issues relevant to the derivation of an appropriate RfD for methylmercury. NRC published their report, *Toxicological Effects of Methylmercury*, in 2000. EPA generally concurred with the NRC findings and recommendations. The NRC document was used as a resource in determining the EPA RfD for methylmercury documented here.

Choice of Study

The adverse effect of methylmercury observed at lowest dose is neurotoxicity, particularly in developing organisms. The brain is considered the most sensitive target organ for which there are data suitable for derivation of an RfD. There is an extensive array of peer-reviewed, well-analyzed data from human studies of low-dose exposure to methylmercury. NRC and EPA considered three epidemiologic longitudinal developmental studies suitable for quantitative risk assessment: the Seychelles Child Development Study (SCDS); the ongoing studies of children in the Faroe Islands; and the study of children in New Zealand. All cohorts consisted of children exposed in utero through maternal consumption of mercury-contaminated fish or marine mammals. In all studies there were biomarkers of maternal exposure (hair), and in the Faroes study cord blood was also used as an additional measure of fetal exposure. The SCDS yielded no evidence of impairment related to methylmercury exposure, but the two other studies have found dose-related adverse effects on a number of

neuropsychological endpoints. EPA chose to base the RfD on data from the Faroes study. The SCDS has no findings of effects associated with methylmercury exposure, and thus is not the best choice for a public health protective risk estimate. While the New Zealand study does show mercury-related effects it relatively small by comparison to the other two. Advantages of the Faroes study include these:

- Large sample size ($n > 900$ for some measures)
- Good statistical power as calculated by conventional means
- Use of two different biomarkers of exposure
- Comprehensive and focused neuropsychological assessment
- Assessment at an age and state of development when effects on complex neuropsychological functions are most likely to be detectable
- Statistically significant observations which remain after adjusting for potential PCB effects
- Extensive scrutiny in the epidemiological literature

The Faroe Islands study was used for derivation of the RfD.

Estimation of the No Adverse Effect Level

A benchmark dose analysis was chosen as the most appropriate method of quantifying the dose-effect relationship. The level chosen was a Benchmark Dose Lower Limit (BMDL); this was the lower 95% limit on a 5% effect level obtained by applying a K power model ($K \geq 1$) to dose-response data based on mercury in cord blood. The BMDL was chosen as the functional equivalent of a no-adverse-effect level for calculation of the RfD.

Choice of Endpoint

Several endpoints are sensitive measures of methylmercury effects in the Faroese children. EPA considered the recommendations of the NRC and EPA's external scientific peer review panel in coming to a decision as to the appropriate endpoint. The NRC recommended the use of a BMDL of 58 ppb mercury in cord blood from the Boston Naming Test (BNT). The external peer panel felt that the BNT scores showed an effect of concomitant PCB exposure in some analyses. They preferred a PCB-adjusted BMDL of 71 ppb mercury in cord blood for the BNT. A difficulty with this choice is that this BMDL is based on scores from only about one-half of the total cohort. The peer panel further suggested using a composite index across several measures in the Faroes data set. EPA prepared a comparison of the

endpoints recommended by NRC and peer reviewers; this also included the BMDLs from the NRC integrative analysis and geometric means of four scores from the Faroes. These BMDLs and corresponding estimates of ingested methylmercury are within a very small range. Rather than choosing a single measure for the RfD critical endpoint, EPA considers that this RfD is based on several scores. These test scores are all indications of neuropsychological processes related to the ability of a child to learn and process information.

Calculation of Ingested Methylmercury Dose

In the risk assessment discussion EPA uses the NRC-recommended BMDL of 58 ppb mercury in cord blood as an example in the dose conversion and RfD calculation. The BMDL in terms of mercury in cord blood was converted to an estimate of ingested methylmercury. This was done by use of a one-compartment model similar to that used in the MSRC. Single-parameter estimates were used rather than a distributional approach. It was assumed that the cord blood methylmercury level was equal to maternal blood level. The ingested dose of methylmercury that corresponds to a cord blood level of 58 ppb is 1.081 μg/kg bw/day.

Uncertainty Factor

Several sources of variability and uncertainty were considered in the application of a composite uncertainty factor of 10. This included a factor of 3 for pharmacokinetic variability and uncertainty; one area of pharmacokinetic uncertainty was introduced with the assumption of equivalent cord blood and maternal blood mercury levels. An additional factor of 3 addressed pharmacokinetic variability and uncertainty. Other areas of concern include inability to quantify possible long-term sequelae for neurotoxic effects, questions as to the possibility of observing adverse impacts (such as cardiovascular effects) below the BMDL, and lack of a two-generation reproductive effects assay.

Methylmercury Reference Dose

The RfD derived in this assessment is 0.1 μg/kg bw/day or $1x10^{-4}$ mg/kg bw/day. The RfD for methylmercury was not calculated to be a developmental RfD only. It is intended to serve as a level of exposure without expectation of adverse effects when that exposure is encountered on a daily basis for a lifetime. In the studies so far published on subtle neuropsychological effects in children, there has been no definitive separation of prenatal and postnatal exposure that would permit dose-response modeling.

That is, there are currently no data that would support the derivation of a child (vs. general population) RfD.

Relative Source Contribution

The assessment of methylmercury exposure from common media sources (e.g., diet, air) and relative source contribution (RSC) estimates follows the 2000 Human Health Methodology. The RSC is used to adjust the RfD to ensure that the water quality criterion is protective, given other anticipated sources of exposure. The exposure assessment characterizes the sources of methylmercury exposure in environmental media, providing estimates of intake from the relevant sources for children, women of childbearing age, and adults in the general population. Based on available data, human exposures to methylmercury from all media sources except freshwater/estuarine and marine fish are negligible, both in comparison with exposures from fish and compared with the RfD. Estimated exposure from ambient water, drinking water, nonfish dietary foods, air, and soil are all, on average, at least several orders of magnitude less than those from freshwater/estuarine fish intakes. Therefore, these exposures were not factored into the RSC. However, ingestion of marine fish is a significant contributor to total methylmercury exposure. For the methylmercury criterion, the RSC is the estimated exposure from marine fish intake. This is subtracted from the RfD when calculating the water quality criterion. One hundred percent of the mercury in marine fish was assumed to be present as methylmercury. The estimated average exposure to methylmercury from marine fish is 2.7 x 10-5 mg/kg-day. This exposure represents almost 30% of the RfD.

Methylmercury Bioaccumulation

Methylmercury is a chemical that bioaccumulates and biomagnifies in aquatic food webs. The fates of mercury and methylmercury in the environment are complex processes affected by numerous biotic and abiotic factors that are subjects of ongoing research. Methylation of mercury is a key step in the entrance of mercury into food chains. The biotransformation of inorganic mercury forms to methylated organic forms in water bodies can occur in the sediment and the water column. Inorganic mercury can be absorbed by aquatic organisms but is generally taken up at a slower rate and with lower efficiency than is methylmercury. Methylmercury continues to accumulate in fish as they age. Predatory organisms at the top of aquatic and terrestrial food webs generally have higher methylmercury concentrations because methylmercury is typically not completely eliminated by organisms and is

transferred up the food chain. Nearly 100% of the mercury that bioaccumulates in upper-trophic-level fish (predator) tissue is methylmercury.

Numerous factors can influence the bioaccumulation of mercury in aquatic biota. These include, but are not limited to, the acidity (pH) of the water, length of the aquatic food chain, temperature, and dissolved organic material. Physical and chemical characteristics of a watershed, such as soil type and erosion or proportion of area that is wetlands, can affect the amount of mercury that is transported from soils to water bodies. Interrelationships among these factors are poorly understood and are likely to be site-specific. No single factor (including pH) has been correlated with extent of mercury bioaccumulation in all cases examined. Two lakes that are similar biologically, physically, and chemically can have different methylmercury concentrations in water, fish, and other aquatic organisms.

The Methylmercury Criterion is a Fish Tissue Residue Criterion

EPA concluded that it is more appropriate at this time to derive a fish tissue (including shellfish) residue water quality criterion for methylmercury rather than a water column-based water quality criterion. This decision considered issues of mercury fate in the environment, the NRC report on the toxicological effects of mercury, and in particular the methylmercury peer review comments. EPA believes a fish tissue residue water quality criterion is appropriate for many reasons. Such a criterion integrates spatial and temporal complexity that occurs in aquatic systems and that affects methylmercury bioaccumulation. A fish tissue residue water quality criterion is more closely tied to the CWA goal of protecting the public health because it is based directly on the dominant human exposure route for methylmercury. The concentration of methylmercury is also generally easier to quantify in fish tissue than in water and is less variable over the time periods in which water quality standards are typically implemented in water quality-based. Thus, the data used in permitting activities can be based on a more consistent and measurable endpoint. A fish tissue residue criterion is also consistent with how fish advisories are issued. Fish advisories for mercury are based on the amount of methylmercury in fish tissue that is considered acceptable, although they are usually issued for a certain fish or shellfish species in terms of a meal size. A fish tissue residue water quality criterion should enhance harmonization between these two approaches for protecting the public health.

The methylmercury water quality criterion is, thus, a concentration in fish tissue. It was calculated using the criterion equation in the 2000 Human Health Methodology rearranged to solve for a protective concentration in fish tissue rather than in water.

$$TRC = \frac{BW \times (RfD - RSC)}{\sum_{i=2}^{4} FI_i}$$

Where:

TRC = Fish tissue residue criterion (mg methylmercury/kg fish) for freshwater and estuarine fish

RfD = Reference dose (based on noncancer human health effects) of 0.0001 mg methylmercury/kg body weight-day

RSC = Relative source contribution (subtracted from the RfD to account for marine fish consumption) estimated to be 2.7×10^{-5} mg methylmercury/kg body weight-day

BW = Human body weight default value of 70 kg (for adults)

FI = Fish intake at trophic level (TL) i (i = 2, 3, 4); total default intake is 0.0175 kg fish/day for general adult population. Trophic level breakouts for the general population are: TL2 = 0.0038 kg fish/day; TL3 = 0.0080 kg fish/day; and TL4 = 0.0057 kg fish/day.

The resulting Tissue Residue Criterion is 0.3 mg methylmercury/kg fish. This is the concentration in fish tissue that should not be exceeded based on a total fish and shellfish consumption-weighted rate of 0.0175 kg fish/day. EPA strongly encourages States and authorized Tribes to develop a water quality criterion for methylmercury using local or regional data rather than the default values if they believe that such a water quality criterion would be more appropriate for their target population.

1.0 INTRODUCTION

1.1 PURPOSE OF THIS DOCUMENT

This document provides guidance to States and Tribes authorized to establish water quality standards under the Clean Water Act (CWA) to protect human health, pursuant to Section 304(a) of the CWA. Under the CWA, States and authorized Tribes are to establish water quality criteria to protect designated uses. While this document constitutes the U.S. Environmental Protection Agency's (EPA's) scientific recommendations regarding concentrations of methylmercury in fish and shellfish that protect human health, this document does not substitute for the CWA or EPA's regulations, nor is it a regulation itself. Thus, it cannot impose legally binding requirements on EPA, States, Tribes, or the regulated community, and may not apply to a particular situation based upon the circumstances. State and Tribal decision makers retain the discretion to adopt approaches on a case-by-case basis that differ from this guidance when appropriate. EPA may change this guidance in the future.

This document establishes a water quality criterion for methylmercury. The U.S. Environmental Protection Agency (EPA) originally published an Ambient Water Quality Criterion (AWQC) for total mercury in 1980. That AWQC was partially updated in 1997 to incorporate a change in the reference dose (RfD). As required under Section 304(a) of the Clean Water Act, EPA must periodically revise criteria for water quality to accurately reflect the latest scientific knowledge on the kind and extent of all identifiable effects on human health from the presence of pollutants in any body of water. The criterion uses new methods and information described in the *Methodology for Deriving Ambient Water Quality Criteria for the Protection of Human Health* (2000) (2000 Human Health Methodology) and in the Methodology's accompanying *Federal Register Notice* (U.S. EPA, 2000a,b). These new methods include updated approaches to determine toxicity dose-response relationships for both carcinogenic and noncarcinogenic effects, updated information for determining exposure factors, and new procedures to determine bioaccumulation factors.

Development of a methylmercury criterion involves some unique considerations compared with many of EPA's past efforts in the water quality criteria program. Traditionally, EPA has established recommended 304(a) criteria to protect human health as ambient concentrations in water. For those pollutants that bioaccumulate, such as methylmercury, exposure through the food pathway is estimated by using a bioaccumulation factor (BAF). However, following review of available data and

recommendations made by external peer reviewers (U.S. EPA, 2000c), EPA determined that it is more appropriate to base the methylmercury criterion on a fish tissue residue concentration than on an ambient water concentration. This determination was partly based on the current scientific understanding of the fate of mercury and methylmercury in aquatic ecosystems. Another factor was the limited information on sources of mercury and the conversion to methylmercury (and its bioavailability). Additional considerations were the difficulty in measuring methylmercury in the water column and relating it to concentrations in aquatic organisms. EPA believes that the latest data and science on methylmercury exposure, effects, and environmental fate support the derivation of a fish tissue residue criterion.

1.2 PRIMARY DATA SOURCE

Much of the information in this document has been taken from the *Mercury Study Report to Congress* (MSRC) (U.S. EPA, 1997b-h). This comprehensive, eight-volume study was prepared by EPA and submitted to Congress in 1997 to fulfill the requirements of section 112(n)(1)(B) of the Clean Air Act, as amended in 1990. The MSRC provides an assessment of the magnitude of U.S. mercury emissions by source, the health and environmental implications of those emissions, and the availability and cost of control technologies. As the state of the science for methylmercury continues to evolve, information from the MSRC has been supplemented by data and analyses published since 1997. The health effects information used in the derivation of the reference dose (RfD) for the fish tissue residue concentration is based on the recommendations of the National Academy of Sciences National Research Council report, *Toxicological Effects of Methylmercury* (NRC, 2000). For additional discussion on the NRC recommendations, see Section 4 of this criteria document. The comments of the methylmercury RfD scientific peer review panel also guided the risk assessment.

1.3 CHEMICAL AND PHYSICAL PROPERTIES

The water quality criterion is being derived for methylmercury (CAS No. 22967-92-6). Synonyms for methylmercury include MeHg, methylmercury ion, methylmercury ion (1+), methylmercury (1+), methyl mercury, and methylmercury(I) cation (Prager, 1997). A commonly occurring form of methylmercury is methylmercuric chloride ($CH_3Hg^+Cl^-$), a stable salt form that exists as a white crystal. This compound is often used in laboratory dosing experiments investigating the toxicological properties of methylmercury. Because methylmercury exists as a free ion only in minute quantities (Prager, 1997), the chemical and physical data provided below are for the chloride salt.

The table below presents available chemical and physical data for methylmercuric chloride (ATSDR, 1999; Kaufman, 1969).

Chemical formula	CH_3HgCl
Chemical structure	$CH_3—Hg^+ Cl^-$
Molecular weight	251.10 (g/mol)
Physical state (25°C)	White crystal
Boiling point (at 25 mm Hg)	No data
Melting point	170°C
Density (25°C)	4.06 g/mL
Vapor pressure (25°C)	0.0085 mm Hg
Water solubility (21°C)	<100 mg/L
Log octanol/Water partition coeff.	No data
Odor threshold (air)	No data
Conversion factors (air)	1 ppm = 10.27 mg/m^3; 1 mg/m^3 = 0.0974 ppm

2.0 TOXICOKINETICS

This section presents information on the absorption, distribution, metabolism, and excretion of methylmercury in humans and animals. This information is summarized from Volume V, Chapter 2 of the *Mercury Study Report to Congress* (MSRC) (U.S. EPA, 1997e).

2.1 ABSORPTION

2.1.1 Oral Absorption

Methylmercury is efficiently absorbed from the gastrointestinal tract following ingestion. Approximately 94%-95% of methylmercury in fish ingested by volunteers was absorbed from the gastrointestinal tract (Aberg et al., 1969; Miettinen, 1973). Aberg et al. (1969) found uptake of greater than 95% of radiolabeled methylmercuric nitrate administered in water to human volunteers.

Data from studies on rats, cats, and monkeys support these absorption estimates (ATSDR, 1999). Studies on rats indicate rapid and complete absorption of inhaled methylmercury vapor into the bloodstream (Fang, 1980). Female cynomolgus monkeys administered 0.5 mg mercury per kilogram of methylmercuric chloride by oral gavage experienced complete absorption within 6 hours (Rice, 1989).

2.1.2 Absorption via Other Routes

Limited information is available on absorption via inhalation and dermal routes. There is one reported human dermal exposure when a 48-year-old chemistry professor inadvertently spilled drops (0.4-0.5 mL) of dimethylmercury from her pipette into her latex gloves. Penetration of dimethylmercury through the gloves occurred instantaneously. Mercury hair level was elevated to almost 1,100 ppm, with a half life of 74.6 days. Five months after exposure, the woman experienced severe neurotoxicity and died 9 months later (Blayney et al., 1997; Nierenberg et al., 1998).

Skog and Wahlberg (1964) evaluated the dermal absorption of the methylmercuric cation in guinea pigs. The test material was applied as the dicyandiamide salt. Absorption was estimated by disappearance of the applied compound and by appearance of mercury in kidney, liver, urine, and blood. Approximately 3% to 5% of the applied dose was absorbed during a 5-hour period.

Indirect evidence in animals indicates that inhaled methylmercury vapor is absorbed readily through the lungs. Fang (1980) showed a correlation between tissue mercury levels and both exposure level and exposure duration in rats exposed to radioactively labeled methylmercury vapor. The percent absorbed was not quantified.

2.2 DISTRIBUTION

After absorption from the gastrointestinal tract, methylmercury is readily absorbed into the blood and distributes to all tissues, including the brain and fetus. The fraction of the absorbed dose that is found in the blood has been estimated in three studies. Kershaw et al. (1980) reported an average fraction of 0.059 of the 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). Sherlock et al. (1984) derived an average value of 1.14% for the percentage of absorbed dose in 1 kg of blood from data on subjects who consumed a known amount of methylmercury in fish over a 3-month period. Average daily intake in the study ranged from 43 to 233 µg/day. There was a dose-related effect on percentage of absorbed dose that ranged from 1.03% to 1.26% in 1 L of blood. Each of these values was multiplied by 5 to yield the total amount in the blood compartment, as there are approximately 5 L of blood in an adult human body.

Methylmercury in the blood is found predominantly in the red cells (Kershaw et al., 1980; Thomas et al., 1986). It is distributed throughout the body following absorption from the gastrointestinal tract into the blood (Clarkson, 1972; Hansen, 1988; Hansen et al., 1989; Nielsen and Andersen, 1992; Soria et al., 1992; Suzuki et al., 1984). Although the distribution of methylmercury in the body is generally uniform, at least one animal study indicates that high levels can be found in the kidney. Rice (1989b) administered 0.025 or 0.05 mg mercury/kg-day as methylmercuric chloride in apple juice to cynomolgus monkeys for approximately 2 years. Kidney tissue concentrations of mercury ranged from 10 to 28 ppm in the cortex and 1 to 10 ppm in the medulla when assessed more than 200 days after cessation of treatment. In contrast, mercury concentration was less than 2 ppm in the other tissues evaluated.

Methylmercury easily penetrates the placental barrier in humans and animals (Hansen, 1988; Hansen et al., 1989; Nielsen and Andersen, 1992; Soria et al., 1992; Suzuki et al., 1984). Several studies

have demonstrated mercury in newborn cord blood. The relationship to maternal blood is variable (Grandjean et al., 1999). Information on this relationship is discussed in Section 4.5.4.1.

The distribution of methylmercury in animals may vary by age and sex (Thomas et al., 1982,, 1986, 1988). Female rats exposed to methylmercury had higher peak levels of mercury in the kidney (primarily as methylmercury) than males; inorganic mercury levels did not differ significantly between the sexes (Thomas et al., 1986). Accumulation of mercury was found to be higher in the bodies of neonatal rats (Thomas et al., 1988) than in adult rats (Thomas et al., 1982). Ten days after administration of methylmercury, 94% of the dose was still detected in neonates while approximately 60% was retained in adults (Thomas et al., 1988). The longer retention of mercury in neonates may result from multiple factors, including the high levels of mercury accumulated in the pelt of neonates owing to lack of clearance (Thomas et al., 1988) and the lack of a fully developed biliary transport system in neonates (Ballatori and Clarkson, 1982).

2.3 METABOLISM

The time required for methylmercury metabolism to inorganic mercury may account for the latent or silent period observed in epidemiological studies from methylmercury poisoning incidents in Japan and Iraq. During the latent period (both during and after the cessation of exposure) the patient feels no untoward effects. It is possible that a number of biochemical changes may take place in parallel during this period, and some may not be causatively related to the clinical outcome. Ganther (1978) hypothesized that the carbon-mercury bond in methylmercury undergoes homolytic cleavage to release methyl free radicals. The free radicals are expected to initiate a chain of events involving peroxidation of lipid constituents of the neuronal cells. The onset of symptoms is delayed for the period of time that cellular systems are able to prevent or repair effects of lipid peroxidation. When the cellular defense mechanisms are overwhelmed, rapid and progressive degeneration of the tissue results. In the Iraqi poisoning incident, the latent period before toxic signs were noted varied from a matter of weeks to months. In contrast, the latency observed in the Japanese poisoning incident was as long as a year or more. The difference in duration may in part be due to the presence of selenium in the fish ingested by the Japanese population.

Rat liver microsomes can metabolize methylmercury into inorganic mercury via the NADPH-cytochrome P-450 reductase, also known to control hydroxyl radical production in liver microsomes (Suda and Takahashi). To a lesser degree, an oral dose of methylmercuric chloride may also be

converted into inorganic mercury via the intestinal flora (Nakamura et al, 1977; Rowland et al., 1980). The intestinal wall is poor in absorbing the inorganic mercury, thus almost all of it is excreted. Studies in mice appear to indicate that toxicity from exposure to dimethylmercury results from the biotransformation of dimethylmercury to methylmercury (Ostland, 1969). Following acute exposure to methylmercury, most of the mercury in the brain is in the organic form; however, with chronic exposures, a greater amount is in the inorganic form, suggesting that the rate of demethylation increases with long-term exposure (Aschner and Aschner, 1990). Rice (1989a, 1989) demonstrated that tissue half-life of methylmercury in the brain may be significantly longer than the blood half-life.

In rats, methylmercury in the body is relatively stable and is only slowly demethylated to form mercuric ion (Norseth and Clarkson, 1970). The demethylation appears to occur in tissue macrophages (Suda and Takahashi, 1986), intestinal microflora (Nakamura et al., 1977; Rowland et al., 1980), and fetal liver (Suzuki et al., 1984).

2.4 EXCRETION

In humans, approximately 90% of the absorbed dose of methylmercury is excreted in the feces (U.S. EPA, 1997e). Excretion via the urine is relatively minor but slowly increases with time; at 100 days after dosing, urinary excretion of mercury accounted for 20% of the daily amount excreted. The urinary excretion of mercury may reflect the deposition of demethylated mercury in the kidneys and its subsequent excretion. In humans the major routes of excretion are via the bile and feces.

Feces are also the predominant route of methylmercury elimination in adult animals (Farris et al., 1993; Hollins et al., 1975; Thomas et al., 1987). Biliary excretion of methylmercury and its demethylation in gastrointestinal flora have been reported in rats (Farris et al., 1993). After a single oral dose of methylmercury, the major elimination route was the feces (65% of the administered dose as inorganic mercury and 15% of the administered dose as methylmercury) and the minor route was urine (1% of the administered dose as inorganic mercury and 4% of the administered dose as methylmercury) (Farris et al., 1993). Following administration of methylmercuric nitrate, 33% of the administered dose was excreted in 49 days; 0.18% to 0.27% excretion in the urine in 10 days and 3.3% urinary excretion in 49 days. This continued for up to 71 days postingestion (Miettinen, 1973). Forty to 50 days postingestion, <0.12% of the administered dose of mercury was found per gram of hair. The half-life for methylmercury appeared to be 70-74 days. In humans the whole body half-life of methylmercury was estimated to be between 70 and 80 days (Aberg et al., 1969; Miettinen, 1973; Bernard and Purdue, 1984).

Mercury is excreted into the hair of methylmercury-exposed humans and animals. Incorporation of mercury into hair is irreversible, and hair analysis is thus a useful tool for monitoring exposure to methylmercury. Segmental analysis of hair may be used to provide a historical record of exposure patterns.

Methylmercury is excreted in breast milk (Bakir et al., 1973; Sundberg and Oskarsson, 1992). The ratio of mercury in breast milk to mercury in whole blood was approximately 1:20 in women exposed to methylmercury via contaminated grain in Iraq between 1971 and 1972 (Bakir et al., 1973). Evidence from the Iraqi poisoning incident also showed that lactation decreased blood mercury clearance half-times from 75 days in males and nonlactating females to 42 days in lactating females; the faster clearance due to lactation was confirmed in mice (Greenwood et al., 1978). In mice, of the total mercury in the breast milk, approximately 60% was estimated to be methylmercury. Skerfving (1988) has found that 16% of mercury in human breast milk is methylmercury. Studies in animals indicate that the mercury content of breast milk is proportional to the mercury content of plasma (Sundberg and Oskarsson, 1992; Skerfving, 1988).

In rat and monkey neonates, excretion of methylmercury is severely limited (Lok, 1983; Thomas et al., 1982). In rats dosed prior to 17 days of age, essentially no mercury was excreted (Thomas et al., 1982). By the time of weaning, the rate of excretion had increased to adult levels. The failure of neonates to excrete methylmercury may be associated with the inability of suckling infants to secrete bile (Ballatori and Clarkson, 1982) and the decreased ability of intestinal microflora to demethylate methylmercury during suckling (Rowland et al., 1977).

Currently, five studies report clearance half-lives for methylmercury. Three studies suggest a half-life of approximately 70 to 80 days (Aberg et al., 1969; Bernard and Purdue, 1984; Miettinen, 1973). Smith et al. (1994) reported a half-life of 44 days in a study of seven adult males treated intravenously with methylmercury. In this study, methylmercury and inorganic mercury concentrations in blood and excreta were determined separately based on differential extractability into benzene. The predominant species in the blood was methylmercury; there was no detectable methylmercury in the urine. Al-Shahristani and Shihab (1974) calculated a "biological half-life" of methylmercury in a study of 48 male and female subjects who had ingested seed grain contaminated by organic mercurials. The half-life, determined from distribution of mercury along head hair, ranged from 35 to 189 days with a mean of 72 days.

The relatively long half-life of methylmercury in the body results partly from reabsorption of methylmercury secreted into the bile (hepatobiliary cycling) (Norseth and Clarkson, 1971). In this cycle, methylmercury forms a complex with glutathione in the hepatocyte and the complex is secreted into the bile via a glutathione carrier protein (Clarkson, 1993). The methylmercury-glutathione complex in the bile may be reabsorbed from the gallbladder and intestines into the blood. This cycle is terminated when intestinal microorganisms demethylate methylmercury to form mercuric ion (Rowland et al., 1980). Mercuric mercury is poorly absorbed from the intestines and the fraction that is not reabsorbed is excreted in the feces. As noted above, approximately 90% of the absorbed dose of methylmercury is ultimately excreted in the feces as mercuric mercury.

2.5 BIOLOGICAL MONITORING

Distribution of methylmercury to hair and blood provides a means for biological monitoring of methylmercury exposure. This section provides an overview of the use of hair and blood for assessing exposure and outlines the available methods for quantitation.

2.5.1 Blood

Methylmercury distributes freely throughout the body, and thus blood is a good medium for estimating short-term exposure. Blood levels may not necessarily reflect methylmercury intake over longer periods, as an individual's intake may fluctuate (Sherlock et al., 1982; Sherlock and Quinn, 1988).

The characteristic partitioning of mercury in the blood permits identification of the form of mercury to which an individual has been exposed. Measurements of blood hematocrit and mercury concentrations in both whole blood and plasma can be used to calculate the red blood cell to plasma mercury ratio. In the case of methylmercury, examination of this ratio enables estimation of interference from exposure to high levels of elemental or inorganic mercury (Clarkson et al., 1988).

2.5.2 Hair

Scalp hair is a useful indicator for estimating methylmercury exposure (Phelps et al., 1980). Mercury is incorporated into scalp hair at the hair follicle in proportion to its content in blood. The hair-to-blood ratio in humans has been estimated as approximately 250:1 expressed as μg mercury/g hair to mg mercury/l blood. Uncertainty in measurements, interindividual variation in body burden, differences

in hair growth rates, and variations in fresh and saltwater fish intake have led to estimates ranging from 190:1 to 370:1 and higher (Birke et al., 1972; Skerfving, 1974; Phelps et al., 1980; Turner et al., 1980; Sherlock et al., 1984). Once incorporated into the hair, the mercury is stable, and can give a longitudinal history of blood methylmercury levels (Phelps et al., 1980; WHO, 1990). The identity of the predominate chemical species (inorganic or methylmercury) depends on exposure patterns and the extent of methylmercury demethylation.

Chemical analyses to determine mercury content of hair assay total mercury rather than chemical species of mercury. As a result, the fraction of hair mercury that is methylmercury is an estimate based on knowledge of environmental and occupational exposure patterns (U.S. EPA, 1997f). Analysis of hair mercury levels may be confounded by several factors, including adsorption of mercury vapor onto the hair strands, natural hair color, hair treatment, and growth rate (Francis et al., 1982; Suzuki, 1988).

Analysis of mercury in maternal hair has been utilized to estimate the fetal burden. This approach has been validated by Cernichiari et al. (1995), who collected blood samples and autopsy brains from terminally ill neonates in a population exposed to methylmercury via fish consumption. Maternal blood and hair samples were also obtained. The concentrations of total mercury in six major brain regions of the neonates were highly correlated with the concentration of mercury in a 1-cm segment of maternal hair next to the scalp (correlation coefficients 0.6 to 0.8, $p<0.01$). These correlations were confirmed by a series of comparisons utilizing maternal hair, maternal blood, neonate blood, and neonate brain tissue.

2.5.3 Methods of Analyzing Mercury Concentrations in Biological Samples

The most common methods used to determine mercury levels in biological media include atomic absorption spectrometry, neutron activation analysis, X-ray fluorescence, and gas chromatography. Another method is anodic stripping voltammetry (Liu et al., 1990). Gas chromotography-electron capture is the only method capable of differentiating methylmercury from other species, whereas cold vapor atomic absorption spectrometry will detect mercury at parts per billion in both urine (Magos and Cernik, 1969) and blood samples (Magos and Clarkson, 1972). Mercury content in hair has been measured by cold vapor atomic absorption spectrometry, atomic fluorescence spectrometry, X-ray fluorescence, and neutron activation analysis (Zhuang et al., 1989).

Another method for analyzing biological samples containing methylmercury is with the use of *Pseudomonas putida* strain FB1. The method is considered very reliable and specific for methylmercury

quantification because chemical inference is negligible. The *Pseudomonas putida* bacteria is capable of converting methylmercury to methane gas and elemental mercury (Baldi and Filippelli, 1991), thus allowing the detection of 15 ng of methylmercury in 1 g of biological tissue with a coefficient of variation of 1.9%.

New methods, such as inductively coupled plasma-mass spectrometry (Kalamegham and Ash, 1992) for analyzing mercury in biological samples are being developed, but are considered very costly and unaffordable by many laboratories. For additional detail on other methods, please refer to the Toxicological Profile for Mercury (Update) (ATSDR, 1999) and in the World Health Organization (WHO) report Methylmercury (IPCS, 1990).

2.6 PHARMACOKINETIC MODELS

A number of extrapolations are generally required in risk assessments, including high-dose to low-dose extrapolations, route-to-route extrapolations, cross-species extrapolations, and extrapolations for varying exposure durations. Physiologically based pharmacokinetic (PBPK) modeling can increase the accuracy of these extrapolations if one has data to use in the model parameters. (Clewell and Andersen, 1985, 1989; Clewell, 1995a; Andersen et al., 1995).

For methylmercury, PBPK modeling in the risk assessment process is used to estimate the relationship between the measure of exposure used in epidemiological studies (mercury in hair and blood) and the daily ingested dose used to determine a reference dose. Several human PBPK models have been developed (Luecke et al., 1994, 1997; Smith et al., 1994; Gearhart et al., 1995; Clewell et al., 1999) to address this issue. Two animal models (Farris et al., 1993; Gray, 1995) were also developed to describe the disposition and metabolism of methylmercury and its major metabolite, mercuric mercury, in rats. A brief description of the pharmacokinetic models developed for methylmercury is presented here.

A PBPK model was developed by Farris et al. (1993) to simulate the disposition of methylmercury and its primary metabolite, inorganic or mercuric mercury, in the adult rat. Farris et al. (1993) also conducted metabolism and distribution studies in rats to collect the data needed to understand the processes that influence the pharmacokinetics of both methylmercury and mercuric mercury. This model incorporated time-dependent compartment volume changes, compartment volume-dependent clearance rates, and the recycling of mercury as a result of hair ingestion during grooming. The Farris model served as the foundation for several subsequent models developed for methylmercury.

On the basis of the modeling results reported by Farris et al. (1993), Smith et al. (1994) developed a simple human PBPK model. Smith et al. (1994) assumed that methylmercury behaved as a single pool while the behavior of its metabolite (inorganic mercury) varied in different tissues. Smith et al. (1994) also conducted experimental studies in human volunteers to monitor levels of methylmercury and inorganic mercury in the blood, urine, and feces following a single intravenous injection of a tracer dose of methylmercury. The modeling results indicated that inorganic mercury accumulated in the body and was the predominant form of mercury present at longer times following administration. The biological half-life of methylmercury in the body was estimated to be 44 days, with an estimated 1.6% of the body burden excreted each day.

Gray (1995) developed a PBPK model for methylmercury in the rat that could be used to evaluate the developmental toxicity observed following *in utero* exposure to methylmercury. The model consists of a maternal model with a fetal submodel. This model can be used to obtain fetal and maternal organ methylmercury concentration-time profiles for any maternal dosing regimen, including the dosing patterns used in rat developmental neurobehavioral studies.

Luecke et al. (1994) developed a generic PBPK model for human pregnancy that was applied (Luecke et al., 1997) to both rat and human kinetic data for methylmercury. This model consists of four submodels and incorporates the changes observed in both the mother and the fetus during the time course of pregnancy. Both rat and human data have been simulated using the model following various routes of exposure to methylmercury.

Stern (1997) identified data on the distribution of parameters in the one-compartment model from the published literature. Available data specific to women between the ages of 18 and 40 were used; data between men and women were also used to determine statistical differences, if any. Blood volume and body weight were assumed to be correlated. A similar approach was used by Swartout and Rice (2000). In that analysis, however, some of the parameters are described by different distributional shapes or by distributions from different data sources than those used by Stern (1997).

Swartout and Rice (2000) performed an uncertainty analysis of the estimated ingestion rates used to derive the methylmercury reference dose. The uncertainty arising from the calculation of ingestion dose levels in mg/kg per day corresponding to measured concentrations of mercury in hair is estimated through a Monte Carlo analysis of the EPA dose conversion model. The Monte Carlo model was modified to include a methylmercury elimination concentration that was converted to an equivalent half-

life, and a term was added to account for measurement error of hair-mercury concentrations. The authors assumed correlations between several pairs of parameters: the hair-to-blood ratio and the elimination-rate constant, body weight and blood volume, and the fraction of the absorbed dose in the blood and body weight. Applying the results of this analysis and assuming the input correlations to the benchmark dose of 11 ppm mercury in hair used in the derivation of the methylmercury RfD results in a lower 95% confidence limit of 4.07×10^{-4} mg/kg-day. The dose conversion factor simulation is 8.0×10^{-5} with a 90% confidence interval of 3.7×10^{-5} to 1.6×10^{-4}. The corresponding dose conversion value used in the derivation of the methylmercury reference dose is 9.8×10^{-5}. The 90% confidence interval spans a three fold to five fold range of ingestion doses for any given concentration of mercury in hair. The hair-to-blood mercury concentration ratio contributed to the variance of the output.

Gearhart et al. (1995) developed a multicompartment adult and fetal model to analyze epidemiological data for a methylmercury risk assessment. This model was recently reparameterized by Clewell et al. (1999) for use in a Monte Carlo variability and sensitivity analysis. The model structure, a modification of the model developed by Farris et al. (1993), consists of a maternal model with a fetal submodel. Changes in both maternal and fetal tissues during gestation are described. The model has the capability to estimate maternal hair and blood concentrations following ingestion of methylmercury, as well as the resulting fetal cord blood concentrations. This model was used to address the relationship between mercury in maternal hair and daily ingested dose, which has been identified as a major issue in conducting a risk assessment for methylmercury. The results of Monte Carlo analysis using the model provided an estimate of the variability in ingestion rates associated with a measured hair concentration. The predicted variability (ratio of median to 5th percentile equals 1.5) is comparable to similar analyses performed using a simple compartmental model (U.S. EPA, 1997e; Stern, 1997). The results of a sensitivity analysis of the model suggest that the most important determinants of pharmacokinetic variability for methylmercury are the hair:blood partition, body weight, and hair growth rate.

3.0 TOXICOLOGICAL BASIS FOR CRITERIA

This section of the *Water Quality Criteria for the Protection of Human Health* document for methylmercury relies heavily on information provided in the *Mercury Study Report to Congress* (MSRC) (U.S. EPA, 1997e) for summaries of studies published before 1997. Data published after 1997 are summarized in this chapter. The *Water Quality Criteria for the Protection of Human Health* document for methylmercury is not intended to be an exhaustive survey of the voluminous health effects literature available; rather, it includes detailed information on studies that form the basis for EPA's hazard identification and dose-response assessment. The database on neurodevelopmental effects of methylmercury is quite extensive. Developmental neurotoxicity is currently considered the most sensitive health endpoint. Data on cardiovascular and immunological effects are beginning to be published and may provide a more sensitive endpoint for low-dose methylmercury effects. This chapter will focus on developmental neurotoxic, cardiovascular, and immunological toxic effects of methylmercury exposure. The reader is referred to the MSRC for information on other toxic effects of methylmercury.

3.1 INTRODUCTION

Methylmercury is a highly toxic substance with a number of adverse health effects associated with its exposure in humans and animals. Human exposure following high-dose poisonings in Japan and Iraq resulted in effects that included mental retardation, cerebral palsy, deafness, blindness, and dysarthria in individuals who were exposed *in utero* and sensory and motor impairment in exposed adults. Chronic, low-dose prenatal methylmercury exposure from maternal consumption of fish has been associated with more subtle endpoints of neurotoxicity in children. Results from animal studies also show effects on cognitive, motor, and sensory functions. The following section focuses on studies reporting neurotoxicity as an endpoint for methylmercury exposure.

3.2 NEUROTOXICITY

3.2.1 Human Studies

3.2.1.1 Minamata and Niigata, Japan

Minamata Bay, Japan

The first documented widespread human methylmercury poisoning occurred in Minamata, Japan, between 1953 and 1960. Over time the source of the poisoning was traced to consumption of contaminated fish and seafood from Minamata Bay. An industrial plant was found to have discharged waste containing mercury directly into the waters of the bay. The initial cases of what was later called Minamata disease were two young women with what appeared to be encephalitis. Public awareness of the situation grew after the sudden deaths of cats in the surrounding area. Cats were brought into Minamata in February 1957 to study the possible health impact of environmental exposure to methylmercury. Within 32 to 65 days after arrival, all developed similar symptoms (e.g., excessive salivation, violent rotational movements, inability to walk in a straight line, and collapsing death or voluntarily jumping into the sea to drown) (Harada, 1995). This episode revealed the potential neurotoxic effects on humans exposed to methylmercury.

Adult Minamata Disease

Officially, approximately 2,200 persons have Minamata disease. Many other cases of the disease have either not been reported or were misdiagnosed. Many had eaten contaminated fish and shellfish for quite some time before the symptoms appeared (Iwata et al., 1975). In human patients, the early stage of Minamata disease brought gross disturbance of the central nervous system, which affected approximately 88 people living in the area around Minamata Bay. Of those 88 people, 12 died within 100 days, while the others had permanent disability. Among those with permanent disability, symptoms included appallic symptoms and idiotic disorders, with nervous symptoms resulting from widespread disturbance of brain cortices. In those with advanced illnesses from moderate poisoning, symptoms included tremor, disturbance of sensation, severe generalized ataxia, dysarthria, concentric constriction of the visual fields, and difficultly in hearing (Takeuchi et al., 1975).

The most common clinical signs observed in adults were paresthesia, ataxia, sensory disturbances, tremors, impairment of hearing, and difficulty in walking. Examination of the brains of severely affected patients who died revealed marked atrophy of the brain (55% normal volume and weight), with lesions in the cerebral cortex and cerebellar cortex, and changes in the nerve fibers, cystic cavities, and spongy foci (Harada, 1995). Microscopically, entire regions of the brain were devoid of neurons, granular cells in the cerebellum, Golgi cells, and Purkinje cells. In addition to effects on the brain, methylmercury is known to have direct effects on the visual field. Korogi et al. (1997) presented results from a study on the comparison of magnetic resonance imaging findings of the striate cortex with visual field deficits in patients with Minamata disease. Results from this study indicated that the central 10° and 15° of vision represent 20% and 30% of the surface area of the striate cortex, respectively. The central portion of the visual fields occupied the posterior area as well as a greater proportion of the striate cortex. The visual field deficits in patients with Minamata disease correlated well with the magnetic resonance findings of the striate cortex. In severe cases of Minamata disease, the visual fields are identical with bilateral homonymous hemianopsia, with sparing of central vision (Korogi et al., 1997).

Delayed Onset-Type Minamata Disease

Mercury content in the hair and blood samples of Minamata patients was not analyzed until 1959. This was due in large part to the latency of the disease; the Minamata incident had apparently continued for such a protracted period that symptoms were delayed in appearing. In some cases, symptoms appeared more than 5 years after methylmercury intake ceased. Symptoms of delayed Minamata also were complicated by other diseases or aging. In the case of maternal exposure, symptoms usually did not appear until 5 to 8 years after the birth of the child. At this time, hair samples from mothers ranged from 1.82 to 191 ppm, while that of their offspring (congenital patients) ranged from 5.25 to 110 ppm (Harada, 1995).

Congenital Minamata Disease

Awareness of the developing fetus as a sensitive subpopulation came to light when a number of children were born with congenital cerebral palsy. These patients experienced symptoms such as mental retardation, primitive reflex, cerebellar ataxia, disturbances in physical development and nutrition, dysarthria, deformity of the limbs, hyperkinesia, hypersalivation, paroxysmal symptoms, strabismus, and pyramidal symptoms. Pathological findings of congenital Minamata disease patients include general atrophy and hypoplasia of the brain cortex and abnormality of the cytoarchitecture, remaining matrix

cells, hypoplasia of the corpus callosum, intramedullary preservation of the nerve cells, and dysmyelination of the pyramidal tract. In the cerebellum, hypoplasia of the granular cell layer and other layers as well as degeneration of granular cells were observed (Harada, 1995).

In a small fishing village called Yudo, 7 cases of cerebral palsy and 10 cases of infantile Minamata disease were found in a total of 50 households. Between 1955 and 1958, there were 188 births in the small fishing villages of Yudo, Tsukinowa, and Modo, with a 9.0% incidence of cerebral palsy, while the overall national incidence ranged from 0.2% to 2.3% (Harada, 1995).

Extensive investigations of congenital Minamata disease were undertaken and 20 cases that occurred over a 4-year period were documented. The exact number of congenital Minamata disease patients is not known, as some undiagnosed patients were already deceased. At present, 64 cases have been confirmed as congenital Minamata disease. In all instances congenital cases showed a higher incidence of symptoms than did the cases where exposure occurred as an adult. The congenital patients are unable to perform ordinary functions of living (Harada, 1995).

From 1950 to 1969, a total of 151 umbilical cords were collected from residents of the Minamata area. Included in this pool were 25 patients with congenital Minamata disease. Levels of methylmercury in the umbilical cords ranged from 0.35 ppm in 1952 to 0.96 ppm in 1955. The methylmercury levels in the cords from patients with congenital Minamata disease showed higher values than the cords of patients who had Minamata disease (0.72 ppm), mental retardation (0.74 ppm), other diseases (0.22 ppm), and no symptoms (0.28 ppm) (Harada et al., 1999).

Kinjo et al. (1993)

A case-control study examined the relationship between health complaints of patients with Minamata disease and exposure to methylmercury. A total of 1,144 Minamata disease patients older than 40 years of age were surveyed. A control group was also established; this group included nonexposed people living in neighboring towns, matched by age and sex. A questionnaire was used to obtain information on subjective complaints and activities of daily living (ADL). Results from analysis of the data indicated that Minamata disease patients had significantly higher rates of all complaints than did controls. Subjective complaints of Minamata disease patients, overall, were more prevalent than in controls. The results remained unchanged with age when the subjective complaints were categorized into two groups: those where frequency increased with age and those related to sensory disturbance. The

authors noted that the reason for the high prevalence rate of sensory disturbance among current Minamata disease patients is unclear. The data from the ADL questionnaire, when analyzed, were used to estimate functional capability in the elderly. Results indicate that ADL was significantly lower for Minamata disease patients aged 60 and over in comparison with controls. The authors conclude that ADL disability in Minamata disease patients is accelerated by aging. Overall, the prevalence of deficits was relatively greater in cases compared with controls as a function of increasing age.

Harada et al. (1998)

In 1995, Harada et al. (1998) measured mercury concentration in hair samples from 191 fishermen and family members living in mercury-polluted areas in the Minamata region of Japan. The study participants fished for a living and had previously consumed methylmercury-contaminated fish and shellfish caught in this region. Estimates of fish consumption were not provided. The study population comprised 83 men and 108 women who ranged in age from 32 to 82 years. Data on subjective symptoms and lifestyle factors were collected by questionnaire. In addition, each participant was administered relevant neurological tests (test details not provided) by a group of neurologists. Mercury concentrations in hair were less than 10 ppm in 185 out of 191 subjects. The mean concentrations were 5.0 ± 3.4 ppm and 2.1 ± 1.1 ppm for men and women, respectively. All six subjects with hair concentrations greater than 10 ppm were men. The mean concentration for men in the study was only slightly higher than the mean value of 4.6 ppm for normal nonexposed Japanese men. There appeared to be an upward trend in hair mercury concentration associated with increased frequency of fish consumption. Although the hair mercury concentrations approached what was considered normal (≤10 ppm in hair samples), the study participants exhibited a high incidence of a variety of neurological conditions. More than 85% of subjects reported subjective symptoms including numbness, forgetfulness, pain in the extremities, focal cramps, headache, and motor disturbances. Clinical findings included sensory disturbance, ataxia, speech impediment, hearing impairment, constriction of visual fields, and tremor. "Stocking and glove" sensory disturbance (a hallmark of Minamata disease) occurred in 69% of the participants. A dose-response relationship between clinical symptoms and hair concentration was not evident, indicating that hair level data were of limited use for diagnosis of chronic Minamata disease.

Fukuda et al. (1999)

A study was completed in Kumamoto, Japan, near Minamata City, to evaluate the relationship between the number of neurological complaints from symptoms and methylmercury exposure. A total of

1,304 exposed adults living in a methylmercury-polluted area and 446 nonexposed age-matched adults, living in an area not known to be polluted with methylmercury, participated in an interview and questionnaire survey. The data from 64 participants of the survey were analyzed by comparison of prevalence, factor analysis, and cluster analysis. Results indicated that the exposed population had more neurological complaints in comparison with those not exposed. The factor analysis proposed four factors: arthritic, muscular, sensory, and nonspecific complaints. All four were higher in the exposed population in comparison with the nonexposed. The authors suggest that the increased neurological and nonspecific complaints may be due to past exposure to methylmercury.

Futatsuka et al. (2000)

A case-control study was conducted to estimate the role of various risk factors, including methylmercury exposure, for diseases such as liver disease, renal disease, and diabetes mellitus. The study population included 1,500 subjects over 40 years of age living in the town of Tsunagi since 1984. The town of Tsunagi was methylmercury polluted, with 36.9 diagnosed Minamata disease patients for every 1,000 population. Urine, blood, physical, and ultrasonographic examinations were administered to determine evidence of liver disease, renal disease, and diabetes mellitus. Personal interviews were conducted to collect information on risk factors and specific details on the complaints. Results from this study indicated that prevalence of disease, liver disease, renal disease, and diabetes mellitus was not higher in the methylmercury-polluted area compared with other areas in Japan. However, subjects in the polluted area had more complaints than those in the nonpolluted area. The authors concluded that past exposure to methylmercury may have influenced these results.

Niigata, Japan

From 1963 to 1965, patients with Minamata disease-like symptoms were reported in the basin of the Agano River in Niigata. Methylmercury, a residual product from acetoaldehyde synthesis, was released from a manure factory located 70 km up the river. Untreated wastewater from the factory drained into the Agano River, contaminating the fish and shellfish population. By 1973, 325 patients with Minamata disease were identified. This poisoning was later named "Niigata Minamata disease." Similar to the incident in Minamata, the symptoms progressed even after cessation of exposure. Numbness in the extremities and in the perioral area was the most frequently reported (Iwata et al., 1975). In the Niigata incident, the maternal hair mercury concentration immediately after giving birth to a congenital patient was 293 ppm. The maternal symptoms associated with this level of exposure were

mild, with sensory disturbances and other Minamata disease-related symptoms. The level of mercury exposure required to initiate the onset of Minamata disease was established at 50 ppm maternal mercury hair level. Because of the previous experience in Minamata with methylmercury poisoning, women with hair mercury levels above 50 ppm were advised not to become pregnant. As a consequence, there was only one case of congenital Minamata disease in the Niigata incident (Harada, 1995).

3.2.1.2 Iraq Outbreak

In fall 1971, 90,000 metric tons of methylmercury-treated seed grain were imported through the southern seaport of Basra, Iraq, and distributed freely throughout the countryside. Because the grain was delivered at planting time, residents of the area baked the grain into bread. There are no records on the size of the population who consumed grain treated with methylmercury fungicide. Nor are there reliable estimates of the number of people who ate methylmercury-treated grain and developed signs and symptoms but did not seek medical attention. It was not until late December 1971 that the first case of methylmercury poisoning was recorded. Within 2 months, 6,530 hospital admissions and 459 hospital deaths were recorded from methylmercury ingestion. Included in this exposed population were pregnant women (Bakir et al., 1973). Children exposed *in utero* manifested severe sensory impairments such as blindness and deafness, general paralysis, hyperactive reflexes, cerebral palsy, and impaired mental development (Amin-Zaki et al., 1974).

A study was conducted by Marsh et al. (1987) to investigate the relationship between methylmercury exposure, as measured by maternal hair concentrations during pregnancy, and associated adverse effects in offspring. A total of 81 mother-infant pairs participated; maternal hair mercury levels served as the index for prenatal exposure and were measured by x-ray fluorescent spectrometric analysis to range from 1 to 674 ppm. Clinical evaluations were conducted along with interviews with the mother about labor, delivery, any abnormalities at birth, size of the baby, early childhood development, and age at which infants achieved developmental milestones. These milestones included sitting without support, standing and walking unaided, and speaking two or three meaningful words. Developmental retardation was indicated by the child's inability to walk a few steps unsupported by 18 months of age or to speak two or three meaningful words by 24 months of age. Additional questions included any observations of involuntary movements, seizures, impaired vision or hearing, lack of coordination, and the mother's general impression of the child's physical and mental development. The interview was limited by the mothers' recall of the age of their children; moreover, this culture did not use Western calendars to record family events. The physical examination of the child included observation; head circumference

and body length measurements; cranial nerve signs; speech; limb tone, strength; deep tendon reflexes; plantar responses; coordination; dexterity; primitive reflexes; sensation; posture; and ability to sit, stand, walk, and run. Neurological examinations scored 0 to indicate normal functions and 3 to indicate definite abnormality. Unclear readings were denoted with points for borderline findings, whereas scores of 0-3 reflect no definite abnormality. The highest score in the most severely affected child was 11.

The impact of methylmercury on neurological function of infants exposed *in utero* during the Iraqi poisoning incident is described in a series of reports by Amin-Zaki et al. (1974, 1976, 1979, 1981), Marsh et al. (1980, 1981, 1987), and Seafood Safety (1991). The major symptoms observed in this epidemic closely resembled those recorded in Minamata, Japan. The predominant symptom noted in adults was paresthesia, and it usually occurred after a latent period of 16 to 38 days following initiation of exposure. Additional dose-dependent symptoms observed in the more severely affected individuals included ataxia, blurred vision, and constriction of the visual field leading to blindness in severe cases, slurred speech and hearing difficulties. Fatalities from methylmercury exposure usually resulted from failure of the central nervous system (Bakir et al., 1973). Of the 28 children with the highest exposures, 7 had seizures, whereas none of the 53 children with the lowest exposures experienced seizures. Maternal hair mercury levels for those seven children ranged between 78 and 674 ppm.

Results indicate that boys appeared to be more severely affected than girls. Statistically significant differences were apparent for regressions for boys and girls, where boys had the steeper slope to indicate increased severity in late walking and talking than girls.

Cox et al. (1989) performed an analysis of the Iraqi data to identify the threshold for adverse neurodevelopmental effects if one existed. A variety of statistical models such as logit, hockey-stick, and nonparametric kernel-smoothing methods were used in the attempt. Analyses were limited by the lack of data on the background prevalence of poor outcomes among Iraqi children. The authors estimated a population threshold of approximately 10 ppm for the outcomes investigated. The uncertainty associated with such an estimate, however, is highly dependent upon the assumed background prevalence of poor outcomes (e.g., motor retardation, neurological abnormality) (Cox et al., 1989). In another attempt at reanalyzing the data, Crump et al. (1995) reported that the estimate of the population threshold was highly dependent on the choice of the model and highly sensitive to the definition of abnormality. For example, delayed walking was heavily influenced by four cases of delayed walking among children with corresponding maternal hair mercury levels below 150 ppm. Crump et al. (1995) concluded that the statistical upper limit of the threshold could be as high as 255 ppm. Furthermore, their maximum

likelihood estimate of the threshold using a different parametric model was said by the authors to be virtually zero.

Cox et al. (1995) analyzed the Iraqi data on late walking in children exposed to methylmercury *in utero*. The results indicated that dose-response analyses based on late walking endpoints were unreliable because of four influential observations in the group of responders with hair mercury levels below 150 ppm. Based on visual interpretation of the plot of the data, the four observations are isolated from the remainder of the responders and would be expected to have considerable influence on the threshold estimate. No quantitative sensitivity analysis was performed to further investigate the effect of removing one or more of these data points. The authors point out that if the four data points were to represent background, the threshold for late walking would be greater than 100 ppm. This is, however, considered unlikely given that no responses were observed in the 37 individuals with lower levels of exposure.

3.2.1.3 Peru

A prospective study (Marsh et al., 1995) was conducted in Mancora, Peru, between 1981 and 1984 but not published until 1995. Mancora was selected as the study site based on a number of criteria, but mainly for its dependence on marine fish as a large source of dietary protein. A diet high in seafood was presumed to be associated with methylmercury exposure. Study participants consisted of 369 pregnant women and 194 of their children. Maternal hair samples were collected from the final group of 131 mother-infant pairs to analyze for methylmercury content. The geometric mean hair level was 7.05 ppm, with a range of 0.9 to 28.5 ppm. The peak maternal hair methylmercury levels during pregnancy ranged from 1.2 to 30 ppm, with a geometric mean of 8.3 ppm. Neurological examinations were administered to children. Frequencies were reported for tone decreased; tone increased; limb weakness; reflexes decreased; Babinski's sign, which is an indicator of a pyramidal-tract abnormality; primitive reflexes; and ataxia. This study identified no significant relationship between maternal hair methylmercury levels and measures of infant development or neurological signs. The authors suggested that marine fish may contain elements, such as selenium, that reduce the toxicity of methylmercury, thereby masking any neurological effects associated with methylmercury exposure.

3.2.1.4 Northern Quebec, Canada

A cross-sectional study of 234 Cree Indian children between the ages of 12 and 30 months on July 1, 1978, was conducted by McKeown-Eyssen et al. (1983). These children resided in four northern

Quebec communities known to have the highest levels of methylmercury exposures within Quebec. Maternal hair mercury level was the index to reflect prenatal exposure. Methylmercury levels of the hair were measured in alternate 1-cm segments, beginning with the scalp-end segment. The average maternal hair methylmercury concentration was 6 ppm, with only 6% of the samples exceeding 20 ppm. Physical and neurologic examinations were administered to the children, with the additional measures of special senses, cranial nerve function, sensory function, muscle tone, stretch reflexes, coordination, persistence of Babinski's response, and a summary of signs for the absence or presence of neurologic abnormality. At 4 years of age, four measures of the Denver Developmental Scale (gross and fine motor development, language development, and personal and social skills) were administered to assess the child's development. Associations between exposure and neurological outcome were analyzed by multiple regression analyses adjusted for alcohol and caffeine intake, tobacco use, age of mother, and multiparity.

No significant association between methylmercury exposure and neurological deficits was identified in girls. Abnormality of tendon reflexes was evidenced in 11.4% of the boys and 12.2% of the girls, but was only significantly associated with maternal hair mercury in boys. The prevalence of abnormality of muscle tone or reflexes was found to increase seven times with each increase of 10 ppm of the prenatal exposure index. However, the authors caution the interpretation of the results on boys because the abnormality of muscle tone or reflexes tended to consist of isolated abnormalities of mild severity that are of doubtful clinical importance. In addition, there was no dose-response relationship.

3.2.1.5 Seychelles Islands

The Seychelles Child Development Study (SCDS) was initiated in 1981 to examine the effects of low-dose fetal exposure to methylmercury from maternal consumption of fish. The SCDS was planned and conducted in two separate stages. The preliminary cross-sectional stage of the study sought to provide additional detail and guidance on how to design the main study. The main study, started in 1989, was a double-blind, prospective, longitudinally designed study that followed a cohort of infant-mother pairs from 6 months to 66 months postgestation.

Demographics

The Seychelles Islands is a Westernized archipelago in the middle of the Indian Ocean, more than 1,500 kilometers from the eastern coast of mainland Africa. The Seychellois population is of African and European origin with some minority groups from India and China. English, French, and Creole are

the three official national languages, with Creole being the most popular language at home. A majority (~85%) of the population consume a high amount of marine fish on a daily basis. In general, the Seychellois population is considered quite healthy, with easy access to good health care and education (Marsh et al., 1995).

Cross-Sectional Pilot Study (Myers et al., 1995b,c)

From 1987 to 1988, a cohort of 789 mother-infant pairs was selected after exclusion criteria were exercised. The fetal exposure index used was maternal hair total mercury. The levels ranged from 0.59 to 36.4 ppm, while the median level in this study was 6.6 ppm total mercury. The Denver Developmental Screening Test-Revised (DDST-R) was administered and a medical and neurological examination was performed for each child between 5 and 109 weeks of age. Covariates were selected for statistical analysis because of their potential to bias the assessment of the association between maternal mercury and developmental outcomes. These covariates included gender, birth weight, Apgar score, age at testing, and medical history. Mother's age, use of alcohol and tobacco, and medical history also were used. When DDST-R scores of questionable and abnormal results were grouped, mercury effects were seen and were more pronounced in boys and declined as age of testing increased. In general, males had higher response rates on the DDST-R than females, independent of mercury level. No association, however, was observed between mercury exposure and overall neurological examination results. The authors cautioned the interpretation of the results because the developmental association with fetal mercury exposure disappeared when DDST-R scores of "questionable" were treated in the standard manner as passes.

A subset (217 children) of the children from the pilot study cohort (Myers et al., 1995a) was tested at 66 months of age with the same battery of tests as planned for the main study at similar age. Maternal hair mercury levels during pregnancy ranged from 1.0 to 36.4 ppm, while the median level was 7.1 ppm. Nine endpoints were evaluated in this second evaluation: the McCarthy Scales of Children's Abilities that yield the general cognitive index (GCI), perceptual performance, memory, and motor ability; the Preschool Language Scale that yields total language score and subscores for verbal ability and auditory comprehension; and the letter-word identification and applied problems subscales of the Woodcock-Johnson Tests of Achievement. The association between maternal hair mercury concentration and outcome was assessed by multiple regression analysis. Prenatal mercury exposure correlated with outcomes at 66 months on the McCarthy GCI and perceptual performance subscale and with total language and auditory comprehension scores. After removing outliers and influential points, however,

mercury effects were no longer significant except for the Preschool Language Scale auditory comprehension subscale.

Prospective Longitudinal Main Study

A double-blinded, prospective longitudinal study was initiated with a new cohort of 740 mother-infant pairs that were selected between 1989 and 1990. These participants resided on the island of Mahe, which is one of the largest islands in the archipelago of the Seychelles where 90% of all Seychellois citizens live. Maternal hair mercury level was used as the marker of fetal mercury exposure. The levels ranged from 0.5 ppm to 26.7 ppm, with a median of 5.9 ppm. The cohort was followed from ages 6.5 months to 66 months, with evaluations occurring uniformly at four critical periods (6.5, 19, 29, and 66 months of age) (Myers et al., 1995). Tests of 7-year-old children have also been done, but results are not yet published. Age-appropriate tests were administered at the time points indicated in Table 3-1.

6-Month Evaluation (Myers et al., 1995c)

At 6 months of age, all children were administered a standardized test of visual recognition memory (Fagan Infantest); a standardized screening test to measure personal-social, fine motor adaptive, language, and gross motor development (DDST-R); and a general medical and neurological examination. Covariates of this main study included those evaluated in the pilot study, with the addition of birth order, gestational age of the child, primary caregiver intelligence, maternal and paternal educational levels, history of breastfeeding, language spoken at home, and family income. Medical conditions related to poor neurodevelopmental outcomes were also included as covariates in the statistical analysis. The study results indicate no association at 6 months of age with DDST-R, neurological examination, and Fagan Infantest. However, males had lower scores on both tests than females.

19- and 29-Month Evaluations (Davidson et al., 1995)

At 19 months of age, children were evaluated with the Bayley Scales of Infant Development (BSID), while the primary caregiver was administered the Raven Standard Progressive Matrices. The cohort was evaluated again at 29 months. Infant intelligence was measured by BSID Mental and Psychomotor Scales. To measure adaptive behaviors, a modified version of the BSID Infant Behavior Record was completed at 29 months. Between the ages of 42 and 56 months, children were administered

Table 3-1. Developmental domains evaluated and tests applied in the Seychelles Islands Child Development Main Study

Developmental Domain	Age of Child (months)			
	6.5	19	29	66
Marsh et al. (1995)				
Global-cognitive	DDST-R	BSID MDI	BSID MDI	MSCA GCI
Visual-perceptive	—	Kohen-Raz	Kohen-Raz	Bender-Gestalt MSCA Perceptual
Speech-language	DDST-R	—	—	MSCA Verbal PLS Total Language Aud. Comprehension Verbal Ability
Memory	Fagan Infantest	—	—	MSCA Memory
Visual attention	Fagan Infantest	—	—	—
Neuromotor exam	Neurological DDST-R	BSID PDI	BSID PDI	Bender-Gestalt MSCA Motor
Behavioral	DDST-R	—	BSID IBR	CBCL
Learning-achievement	—	—	—	Woodcock-Johnson
Auditory response	—	—	—	Audiometry Tympanometry
Davidson et al. (1998)				
Global-cognitive	—	—	—	MSCA GCI
Visual-perceptive	—	—	—	Bender-Gestalt
Speech-language	—	—	—	PLS Total Score
Behavioral	—	—	—	CBCL
Learning-achievement	—	—	—	Woodcock-Johnson Letter and Word Recognition, Applied Problems

Symbols and Abbreviations: — = No test administered; BSID = Bailey Scales of Infant Development; IBR = Infant Behavior Record; MDI = Mental Developmental Index; PDI = Psychomotor Developmental Index; CBCL = Child Behavior Checklist; DDST-R = Denver Developmental Screening Test - Revised; GCI = General Cognitive Index; MSCA = McCarthy Scales of Children's Abilities; PLS = Preschool Language Scale.
Source: Marsh et al. (1995); Davidson et al. (1998).

the Pre-School Caldwell-Bradley Home Observation for Measurement of the Environment (HOME). Hair samples were collected from all children at both 19 and 29 months of age for analysis of total mercury concentration to determine postnatal exposure. The median maternal hair mercury concentration during pregnancy for the 738 mother-infant pairs in the cohort at 19 months was 5.8 ppm. Twenty-two percent of the children at 19 months had child hair mercury levels ≥ 10 ppm (Myers et al., 1997). The same covariates and modeling strategy were used as in the primary analysis. No effects of mercury were detected on the BSID scores at either age. Results of this study indicate that one functional behavior—the examiner's subjective rating of the child's test session activity level—was related to maternal hair mercury levels in the mothers of male children: activity level decreased as maternal hair mercury level increased. Independent of mercury exposure; activity level was rated higher in males. Authors of this study conclude that these two results suggest that prenatal exposure to mercury may lower activity level in males. This result should be interpreted with caution as it is not yet clear whether the lower activity in males is a direct result of increased mercury exposure.

19-Month Evaluation of Walking and Talking (Myers et al., 1997)

The 19-month cohort was selected for evaluations of two developmental milestones. Data for age of first walking (n = 720) and talking (n = 680) were obtained from the primary caregiver of each child. Age at walking was defined as the age when the child was able to walk without support, while age at talking was defined as the age the child first said words other than "mama" and "dada." The mean age for walking was 10.7 months for girls and 10.6 months for boys, while for talking it was 10.5 months for girls and 11.0 months for boys. Multiple regression analysis was used to assess the relationships between each developmental milestone, maternal hair mercury levels, and covariates. Covariates evaluated are the same as those included in the study reported by Davidson et al. (1995) described in the previous paragraph. In this study, there was a marginally significant relationship between prenatal mercury exposure from eating fish and the age at which males started to walk, but this depended on four statistical outliers. No association between prenatal mercury exposure and either the age at which females started to walk or either gender started to talk was found.

Semiparametric Modeling of the 19-Month Data (Axtell et al., 1998)

In addition to the multiple regression analysis used in the prospective longitudinal main study of the SCDS, a semiparametric generalized additive model was used to identify nonlinearities in the relationship between prenatal methylmercury exposure and developmental milestone achievements. The

specific milestones evaluated in the main SCDS cohort at 19 months of age (n = 738 children) were age that children walked and said words. Walking was defined as the number of steps without support and talking was any word except "mama" or "dada." Maternal hair total mercury was used as an index of fetal exposure. No significant nonlinear relationships with mercury were identified in any of the models for age at talking; this implies that the original linear regression models were appropriate for this analysis. A General Additive Model analysis indicated that the relationship between maternal hair mercury level and age at walking may not be linear. Walking appeared at a later age as exposure increased in the range from 0 to 7 ppm. Walking appeared slightly earlier with increasing mercury levels above 7 ppm. However, there was no evidence from any models that higher levels of mercury exposure resulted in further delays in walking. There is no biological or developmental hypothesis to explain the increase in age of walking at lower levels and not at higher levels.

66-Month Evaluation (Davidson et al., 1998)

An evaluation was conducted on 711 mother-child pairs at 66 months of age. At this age, six neurobehavioral tests were administered: McCarthy Scales of Children's Abilities, the Preschool Language Scale, the Woodcock-Johnson Applied Problems and Letter and Word Recognition Tests of Achievement, the Bender Gestalt Test, and the Child Behavior Checklist (CBCL). Maternal hair mercury and child hair mercury were measured. Mercury exposure was assessed by total mercury in segments of maternal hair representing growth during pregnancy. The mean maternal hair total mercury level was 6.8 ppm while the mean child hair total mercury level at age 66 months was 6.5 ppm. The covariates evaluated include all those included in the previous study period, in addition to hearing status of the child and Hollingshead socioeconomic status of the family. Two multiple linear regression analyses were performed for each of the six primary measures. Secondary analyses tested the hypothesis that associations between developmental outcomes and total mercury exposure might be nonlinear. Four of the six measures (all except for Bender Gestalt and Woodcock-Johnson Applied Problems Tests of Achievement) showed better scores in the highest methylmercury groups compared with lower groups for both prenatal and postnatal exposure. For both prenatal and postnatal methylmercury exposure, no adverse developmental effects were reported for toddlers. Postnatal exposure at 66 months, however, was associated with a small but statistically significant increase on several developmental outcomes even though there is no reason to suppose that such effects are associated with exposure to methylmercury. There are studies, however, that indicate the methylmercury levels in the infant were surrogate for the length of breastfeeding, which is reported to have a positive association with developmental outcomes (Grandjean et al. 1992).

New Analysis—CBCL Main Cohort 66 Months (Myers et al., 2000)

No effect of mercury was identified on the Child Behavior Check List (CBCL) at 66 months of age in the main cohort of the Seychelles study as determined by the total T score (Davidson et al., 1998). The CBCL is a report inventory scored by the caregiver that assesses eight domains: withdrawn, somatic complaints, anxious/depressed, social problems, thought problems, attention problems, delinquent behavior, and aggressive behavior. An analysis of these subscales was performed on the 711 children assessed on this test (Myers et al., 2000). No effect of mercury was identified on individual subscales.

New Analysis—Main Cohort 66 Months (Axtell et al., 2000; Palumbo et al., 2000)

The investigators performed additional analyses of the 66-month data to evaluate the possibility of nonlinear relationships associated with mercury exposure (Axtell et al., 2000). Endpoints included the six primary variables analyzed previously: McCarthy GCI, Preschool Language Scale (PLS), Wodcock-Johnson Applied Problems, Woodcock-Johnson Letter/Word Recognition, Bender copying errors, and CBCL total T score. Generalized additive models, which make no assumptions about the relationship between exposure and test score, were used. Maternal hair levels during pregnancy were used as a measure of prenatal exposure and child's hair mercury at 66 months was used for postnatal exposure. Nonlinearities were identified between prenatal exposure and PLS and CBCL, and between postnatal exposure and McCarthy GCI. For the PLS the trend involved a decrement of 0.8 points (poorer performance) from 0-10 ppm and an increase of 1.3 points above 10 ppm. For the CBCL there was an increase (representing a poorer score) between 0 and 15 ppm and a decrease above 10 ppm. The GCI increased (improved) by 1.8 points through 10 ppm mercury in the child's hair and declined by 3.1 above 10 ppm. Although these results are difficult to interpret, they provide limited evidence of an adverse effect of mercury exposure below 10 ppm maternal hair on two measures, and a somewhat greater association of adverse effects with child's hair mercury above 10 ppm on the GCI. As pointed out by the authors, there are fewer data points above 10 ppm (this is especially true for child's hair mercury), and therefore trends above this level are estimated less precisely.

The investigators in the Seychelles study further examined by multiple linear regression the results of the McCarthy GCI administered at 66 months (Palumbo et al., 2000). They analyzed the standard MSCA subscales and also constructed subscales to approximate the domains of cognitive functioning assessed in the Faroe Islands study: attention, executive function, expressive language, receptive language, nonverbal memory, visuospatial ability, visuomotor ability, and gross motor ability. They

found a positive association between child's hair mercury at 66 months and the standard memory subscale, with no other associations identified. As with all previous analyses of these variables, the raw scores were converted to "normative" scores. As pointed out by an OSTP panel (NIEHS 1998, Section 3.5 of the Confounders and Variables Section), the applicability of U.S. norms to this population is unclear, and the use of standardized scores may decrease sensitivity by collapsing different raw scores to one standard score.

Pilot Cohort Analysis at 108 Months (Davidson et al., 2000)

Further evaluation was performed on a portion of the Seychelles pilot cohort at 108 months of age (Davidson et al., 2000). Eighty-seven children were tested on five subtests of the WISC-III (Information, Block Design, Vocabulary, Digit Span, and Coding), California Verbal Learning Test (CVLT), Boston Naming Test (BNT), Beery-Buktenica Development Test of Visual Motor Integration (VMI) (copying geometric figures), Finger Tapping, grooved pegboard, Trailmaking (tracing the correct route through a form with a pencil), and the design memory subtest of the Wide Range Assessment of Memory and Learning (WRAML) (drawing each of four geometric designs from memory). Performance on BNT, VMI, and grooved pegboard showed a positive association (better performance) related to mercury exposure in males, with no effects identified in females. There were trends toward poorer performance related to mercury exposure for grooved pegboard in females ($p = 0.07$) as well as marginal p values on the full model that were not further analyzed (Finger Tapping, digit span). The investigators did not report power calculations, but with such a small number of subjects the power was probably quite low, so these largely negative results need to be interpreted with caution.

Benchmark Analysis (Crump et al., 2000)

A benchmark analysis (Crump et al., 2000) was conducted on data from the SCDS, with the goal of providing an alternative basis for deriving an appropriate human exposure level for methylmercury. The data modeled included responses from the neurological test batteries conducted at 6.5, 19, 29, and 66 months of age. In addition, data for developmental milestones (age first walked and age first talked) were analyzed. Maternal hair mercury concentrations measured in this study ranged from 0.5 to 26.7 ppm and averaged 6.8 ppm.

Most of the measured endpoints in the SCDS were recorded as continuous responses, and the k-power model, the Weibull model, and the logistics models for continuous data were applied. Test scores

below a predetermined value, $P_0 = 0.05$, were considered abnormal. For this analysis, the BMR was defined as 10% (BMR = 0.1). (For a description of modeling terms see Section 4.3).

In cases where responses were recorded as quantal responses (abnormal/normal), the data were modeled using the Weibull dose-response model for quantal data. Quantal responses reported in children in the Seychelles study included deep tendon reflexes, limb tone, overall neurological responses, and psychomotor index. In addition, each continuous response was converted to a quantal response by considering a response abnormal if it was more than 2 standard deviations away (in the adverse direction) from the mean response of the entire cohort, and then analyzed using the Weibull model. In these analyses, the BMD was defined in the same way as in the analyses of the continuous response.

The analyses of continuous response were conducted without covariates. Analyses with P_0 specified were conducted using both an expanded set and a reduced set of covariates for the children: sex, birth weight, birth order, whether or not the child was breastfed, medical history, maternal age, maternal smoking and alcohol use during pregnancy, maternal medical history, language spoken in home, score from home visit, Raven group (caregiver's intelligence quotient), maternal and paternal education level, family income, gestational age, Hollingshead socioeconomic scale, auditory scores, and the child's mercury level. Covariates were not included in the analyses of quantal responses or in the analyses of continuous responses in which x_0 was specified.

Parameter estimates were obtained using the maximum likelihood method, and statistical confidence bounds were computed by the profile likelihood method. The BMDL was defined conventionally as the 95% statistical lower confidence bound on the BMD. Results indicated that the most reliable analyses were represented by 144 calculated lower statistical bounds on the BMD (BMDL, or the lower statistical bound on maternal mercury hair level corresponding to an increase of 0.1 in the probability of an adverse response) derived from the modeling of continuous responses.

The results of BMD modeling are shown in Table 3-2. The average value of the BMDL in these 144 analyses was 25 ppm mercury in maternal hair, with a range of 19 to 30 ppm. With the exception of the linear model, which produced larger BMDLs, the dose-response models applied to continuous end points all produced comparable BMDLs.

Table 3-2. BMDL values (expressed as ppm mercury in maternal hair) for neurological responses and developmental milestones from the Seychelles Child Development Study

Endpoint	Model						
	Weibull				*K*-Power		
	P_0[a]		χ_0[b]	Quantal	P_0[a]		χ_0[b]
	None	Exp.[c]	None	None	None	Exp.	None
6.5 Months							
Deep tendon reflexes	—	—	—	22.8	—	—	—
Limb tone	—	—	—	20.9	—	—	—
Overall neurological	—	—	—	15.8	—	—	—
Fagan visual recognition memory	26.0	26.0	27.4	19.7	26.0	26.0	26.9
Fagan attention	25.7	25.9	27.0	23.7	25.5	25.6	26.4
19 Months							
Mental development index	23.7	23.4	26.0	22.6	24.3	24.1	25.6
Psychomotor index	—	—	—	22.3	—	—	—
29 Months							
Mental development index	24.1	24.4	25.7	21.9	24.0	24.2	24.8
Psychomotor index	—	—	—	22.5	—	—	—
66 Months							
Bender gestalt errors	26.9	26.7	28.5	22.7	26.7	26.7	27.5
Child behavior checklist total	27.2	27.2	29.0	19.4	20.0	26.9	27.8
McCarthy general cognitive index	24.4	24.2	26.5	22.7	24.7	24.6	25.9
Preschool language total score	25.2	25.1	26.8	22.7	24.7	24.7	25.5
Woodcock-Johnson							
Applied problems	23.1	23.5	25.3	22.7	23.9	24.3	25.5
Letter-word recognition	23.7	23.7	25.3	22.7	23.8	23.9	24.7
Developmental milestones							
Age first walked unassisted	24.9	24.0	25.9	22.7	24.4	23.2	26.8
Age first talked	24.6	23.5	25.9	20.3	25.0	24.1	25.9

[a] Abnormal defined as a response >2 standard deviations in adverse direction from mean response of entire cohort.
[b] Abnormal defined so that 5% of responses are abnormal ($p_0 = 0.05$).
[c] Exp. denotes use of an expanded range of covariates.

Source: Crump et al., 2000.

3.2.1.6 New Zealand

A study was conducted in the northern New Zealand islands to study the effects of prenatal methylmercury exposure on children exposed *in utero* from maternal fish consumption. Between 1982 and 1983, 11,000 mother-infant pairs were requested to submit hair samples and fill out a detailed diet questionnaire. Of those 11,000 pairs approximately 1,000 of these mothers had consumed fish more than three times per week for the 9 months of pregnancy. Seventy-three had hair mercury levels above 6 ppm, with the highest level being 86 mg/kg. This study was conducted in two stages.

Preliminary Tests at Age 4 (Kjellstrom et al., 1986)

From the 73 mothers with high mercury exposure (> 6 ppm) during pregnancy, a total of 31 matched pairs were selected to participate in a study on the effects of prenatal methylmercury exposure on children exposed *in utero* from maternal consumption of fish. A reference child matched for mother's ethnic group, age, and child's birthplace and birth date was located for each child selected from the high-fish-consumption group. Mercury exposure during gestation was determined from maternal hair analysis. The average hair concentrations for high-exposure mothers and the reference group were 8.8 ppm and 1.9 ppm, respectively. At 4 years of age, the children were tested using the DDST. Standardized vision tests and sensory tests were also performed to measure development of these components of the nervous system. The prevalence for developmental delay in children was 50% for progeny of high-mercury mothers and 17% for progeny of mothers of the control group. These results were statistically significant. Analysis of the DDST results by sector showed that developmental delays were most commonly noted in the fine motor and language sectors, but the differences between the experimental and control groups were not significant. The authors concluded that children born to mothers with mean hair mercury levels above 6 ppm have twice the risk of delayed development, as tested by the DDST, in comparison with the control group.

Psychological Tests at Age 6-7 (Kjellstrom et al., 1989)

In 1985 when the children were 6 to7 years of age, a follow-up study was conducted. In this study, 61 of the 74 high-exposure children were compared with three control groups with lower prenatal mercury exposure. Average maternal hair mercury concentrations in the control groups were 3 to 6 ppm and 0 to 3 ppm, respectively. The high-exposure group, with maternal hair mercury levels ranging from 6 to 86 ppm, was matched with controls for maternal ethnic group, age, smoking habits, residence, and sex

of the child. Each child was tested with a battery of 26 scholastic, psychological, and behavioral tests, which included Test of Language Development (TOLD), the Wechsler Intelligence Scale for Children (WISC), and McCarthy Scale of Children's Abilities as described in Table 3-3. Confounding factors such as language used at home, maternal and paternal occupation, maternal alcohol consumption, and number of children in the household were controlled using linear multiple regression analysis.

Table 3-3. Developmental domains evaluated and tests applied in studies of New Zealand children with prenatal exposure to mercury from fish

Developmental Domain	Age of Child (years)	
	4	6
General cognitive	—	MSCA general WISC-R Performance IQ, Total IQ
Visual-perceptual	Sheridan-Gardiner Letter Matching test Miniature Toy Test	MSCA perceptual
Speech-language	DDST	TOLD Spoken Language Quotient MSCA Verbal WISC-R Verbal Peabody Picture Vocabulary Test (1981)
Memory	—	MSCA memory
Motor	DDST	MSCA motoric
Learning-achievement	—	Clay Diagnostic Survey Concepts, Letter Test, and Word Test MSCA quantitative Burt Word Recognition Test Key Math Diagnostic Arithmetic Test
Personal-social	DDST	Everts Behaviour Rating Scale

Symbols and Abbreviations: — = No test administered; DDST = Denver Developmental Screening Test; MSCA = McCarthy Scales of Children's Abilities; TOLD = Test of Language Development; WISC-R = Wechsler Intelligence Scale for Children - Revised.
Source: Kjellström et al., 1986; 1989.

An average hair mercury level of 13 to 15 ppm during pregnancy was consistently associated with decreased test performance. Results of the psychological test variables were influenced by ethnic background and social class. After controlling for confounding factors and eliminating outliers, the association between prenatal methylmercury exposure and decreased performance in psychological tests remained unchanged. The children who had the poorest performance in the WISC IQ test at age 6 also had a high prevalence of abnormal or questionable DDST scores at age 4, indicating that the effects evidenced in this follow-up study confirm those found in the preliminary study at age 4. The authors

conclude that effects of methylmercury leading to developmental delays may later lead to deficits in psychological tests.

Benchmark Modeling of the 1985 Data (Crump et al., 1998)

Crump et al. (1998) performed a reanalysis and BMD modeling of the Kjellstrom et al. study results. Crump et al. used actual hair mercury levels as opposed to an indicator variable for mercury level in hair; additional confounding factors, such as parent's education and age at which the child was tested were also controlled for. They also and evaluated all 26 scholastic and psychological tests (illustrated in Table 3-4) administered to the 237 6 to 7-year old children. No significant associations between mercury exposure and children's test scores were identified. This finding, however, was highly influenced by one child whose mother's hair mercury level was 86 ppm, fourfold higher than observed for any other mother. When this outlier was omitted, scores on six tests were found to be significantly associated with maternal hair mercury concentrations: Clay reading test-concepts, Clay reading test-letter test, McCarthy-general cognitive test, McCarthy-perception, TOLD-grammar completion, and TOLD-grammar understanding. BMDs calculated from five tests (TOLD-spoken language quotient, WISC-performance IQ, WISC-full scale IQ, McCarthy perceptual, and McCarthy-motoric) ranged from 32 to 73 ppm and BMDL of 17 to 24 ppm, respectively. When the child with the highest maternal hair mercury was excluded, the BMDs ranged from 13 to 21 ppm with BMDLs spanning 7.4 to 10 ppm (Table 3-4).

Table 3-4. BMD and BMDL values (expressed as maternal hair mercury concentration, ppm) for neurobehavioral endpoints in New Zealand children evaluated at 6 to 7 years of age

Test	All New Zealand children		Child with highest maternal mercury concentration omitted	
	BMD[a]	BMDL[b]	BMD	BMDL
TOLD – spoken language	45	20	15	9.5
WISC – performance IQ	73	24	15	10
WISC–full-scale IQ	51	21	15	10
McCarthy–perception	32	17	13	7.4
McCarthy–motoric	55	21	21	9.8

[a] A background prevalence (P_0) of abnormal response of 5% and a benchmark response of 10% were used for these calculations.
[b] 95% lower confidence bound on BMD.
Abbreviations: TOLD = Test of Language Development; WISC = Wechsler Intelligence Scale for Children.
Source: Crump et al. (1998).

3.2.1.7 Faroe Islands

A large human prospective longitudinal study was conducted in the Faroe Islands to determine if increased methylmercury exposure is related to decreased neurobehavioral function. Before the prospective study, a pilot study was conducted to assess the magnitude of fetal mercury exposure in the Faroes. At 12 months of age, a follow-up evaluation was conducted and then a prospective study was initiated with children born at consecutive deliveries within a 22-month period at nearby hospitals.

Demographics

The Faroes is a group of 18 islands located in the North Atlantic between Scotland and Iceland. The Faroese population is homogenous with respect to cultural and socioeconomic factors. The culture is mainly Scandinavian, with a traditional stable family unit that has easy access to good health care, education, and social systems. Dietary deficiencies are virtually nonexistent, alcohol intake is low, rate of preterm delivery of low-birth-weight infants is also low, and rate of breastfeeding is high for at least 12 months (Budtz-Jorgensen et al., 2000). Seafood constitutes a major part of the average diet in fishing communities in the North Atlantic like the Faroe Islands (Grandjean et al., 1995). The major source of methylmercury exposure is pilot whale, which according to ancient tradition was hunted and distributed within the community (Grandjean et al., 1997). Other components of the Faroese diet include lamb, potatoes, dairy products, and foods imported from other countries (Steurwald et al., 2000).

Pilot Study (Grandjean et al., 1992)

A pilot study was conducted by Grandjean et al. (1992) to assess the magnitude of fetal mercury exposure in the small fishing village of Lorvik, Faroe Islands. Blood samples were collected from a group of 53 women of fertile age, between 20 and 50, identified through a municipal register. Between 1986 and 1987, 1,023 umbilical cord blood samples were also collected at consecutive deliveries at three local hospitals. Women had a median blood mercury level of 12.1 μg/L, with values that ranged from 2.6 to 50.1 μg/L. The median mercury concentration in cord blood for all 250 samples exceeded 40 μg/L, while 20 samples had levels higher than 100 μg/L. Hair samples had mercury content that exceeded 10 ppm, and five samples exceeded 25 ppm. In 34 hair samples the measured mercury levels exceeded 15 ppm. Mercury concentrations tended to be 20% to 65% higher in cord blood than in the venous blood of mothers. Highly increased mercury concentrations in maternal hair and umbilical cord blood were related to maternal consumption of pilot whale.

12-Month Evaluation (Grandjean et al., 1995b)

At 12 months of age, 583 children were selected for further evaluation. These children were followed for 1 year after birth. Three age-appropriate developmental milestones were evaluated: sitting, creeping, and standing. The age at which the child achieved a developmental milestone was not associated with indices of prenatal mercury exposure, either from cord blood (average of 174 μg/L) or maternal hair (approximately 15% of mothers had concentrations above 50 nmol/g). Infants who reached the milestone criteria early had significantly higher mercury concentrations in their hair at 12 months than those who did not. The child's hair mercury concentration was found to be highly correlated to the period of breastfeeding. Breast milk may transfer contaminants such as methylmercury, but it is also known to confer certain advantages such as maternal antibodies. The authors concluded that if methylmercury exposure from human milk had any adverse effect on milestone development in these 12 month-old infants, the effect was compensated for by advantages offered through breastfeeding.

Computer-Assisted Neurobehavioral Tests in 7-Year-Olds (Dahl et al., 1996)

In this study, 917 children were evaluated at 7 years of age. The study focused on computer-assisted neurobehavioral tests and whether or not they could serve as meaningful parameters of neurotoxicity; three Neurobehaviroal Evaluation System (NES) tests were administered with slight modifications. The NES tests were selected to assess motor speed (Finger Tapping [FT]), sustained attention (Continuous Performance Test [CPT]), and motor coordination (Hand-Eye Coordination [HEC] Test). The CPT was modified to use animal silhouettes as a stimuli instead of letters to accommodate those children who had not yet started school and were unfamiliar with the alphabet.

Finger Tapping was relatively easy for most children, but the HEC test was considered too difficult. Of the 914 children who completed the full HEC, 755 had fewer than 25% nonresponses. Decreased visual acuity, strabismus, use of eyeglasses, and contrast sensitivity were markedly associated with decreased performance, especially on the CPT. Boys and older children performed better than girls and younger children, but this was due to increased familiarity with computers and use of a joystick. The authors concluded that maternal hair mercury and cord blood mercury were clearly associated with NES results, especially in the FT and CPT tests.

Main Prospective Longitudinal Study of 7-Year-Olds (Grandjean et al., 1997)

The cohort consisted of 917 children at 7 years of age who survived from the original cohort established in the pilot study. Indices of prenatal exposure included cord blood and maternal hair, and the index for postnatal exposure was children's hair mercury. The geometric mean cord blood mercury concentration was 22.8 μg/L, and the concentration found in children's hair averaged 11.68 ppm. Detailed neurobehavioral and physical examinations and neuropsychological and neurophysiological testings were performed. The neuropsychological tests (Table 3-5) included NES FT Test, NES HEC Test, Tactual Performance Test, NES CPT, Wechsler Intelligence Scale for Children - Revised (WISC-R), WISC-R Similarities, WISC-R Block Designs, Bender Gestalt Test, California Verbal Learning Test-Children [CVLT]), Boston Naming Test (BNT), and Nonverbal Analogue Profile of Mood States. These tests were chosen for their sensitivities in detecting neuropathological abnormalities. The neurophysiological tests were chosen to exclude those with electrical stimulation or long measurement times. These tests include pattern reversal visual-evoked potentials with binocular full-field stimulation, brain stem auditory-evoked potentials (BAEP), and postural sway.

Fewer than 60% of the children completed three of the most difficult tests. The WISC-R Similarities Test, NES HEC Test, and Nonverbal Analoguous Profile of Mood States were found to be too difficult for many of the children to reveal the subtle neurotoxic effects associated with methylmercury. The geometric mean cord blood mercury concentration for the 85 children who failed or refused to take the mood test was 29.5 μg/L, compared with 22.3 μg/L in children who voluntarily completed it. Reciprocal motor coordination and simultaneous finger movement showed no relation to mercury exposure. In the finger opposition test, however, 465 children with geometric mean blood concentrations of 21.8 ug/L mercury performed optimally, whereas those with blood concentrations of 23.9 μg/L had questionable or deficient performances.

Mercury-related abnormalities were not identified in either the neurophysiological or clinical examination. However, in the neuropsychological testing, statistically significant mercury-related dysfunction was observed. This was most pronounced in the areas of language, attention, and memory, and to a lesser extent visuospatial and motor functions. After adjustment of covariates and exclusion of children with maternal hair mercury above 10 ppm, the association remained. This indicates effects of methylmercury at doses lower than that which result in 10 ppm maternal hair mercury. In the neurophysiological test, girls showed significantly shorter latencies of evoked potentials than boys in the electrophysiological tests. For the BAEP latencies, peak I at 40 Hz and 20 Hz was slightly delayed at

Table 3-5. Developmental domains evaluated and tests applied in studies of Faroese children at age 7 years

Developmental Domain	Test
Grandjean et al. (1997) - Main Prospective Study	
General cognitive	WISC-R Similarities
Visuospatial	WISC-R Block Designs Bender Motor Visual Gestalt Test
Attention	NES2 Continuous Performance WISC-R Digit Spans Forward
Speech-language	Boston Naming Test
Memory	California Verbal Learning Test
Motor	NES2 Finger Tapping NES2 Hand-Eye Coordination NES2 Tactual Performance
Personal-social	Nonverbal Analogue Profile of Mood States
Grandjean et al. (1998) - Nested Case Control Study	
General cognitive	WISC-R Similarities
Visuospatial	WISC-R Block Designs Bender Visual Motor Gestalt Test
Attention	NES2 Continuous Performance WISC-R Digit Spans Forward
Speech-language	Boston Naming
Memory	California Verbal Learning Test
Motor	NES2 Finger Tapping NES2 Hand-Eye Coordination
Personal-social	—

Symbols and Abbreviations: — = No test administered; NES2 = Neurobehavioral Evaluation System; WISC-R = Wechsler Intelligence Scale for Children - Revised.

Source: Grandjean et al., 1997, 1998.

increased prenatal mercury exposures and the delays for peaks III and V were statistically significant, but the interpeak latencies showed no associations with mercury. Body sway showed a slight negative association with mercury exposure in all four conditions: eyes open, no foam; eyes closed, no foam; eyes open with foam; and eyes closed with foam.

Four tests were selected for further analysis. Tests were chosen to reflect each of the following brain functions: motor function (Finger Tapping with preferred hand), attention (CPT reaction time), visuospatial performance (error score on the Bender Visual Motor Gestalt Test), language (Boston Naming Test after cues), and memory (long-delay recall on the California Verbal Learning Test). After

adjustment for covariates using the Peters-Belson method, children with scores in the lowest quartile were identified and distributed into quartile groups of mercury exposure (< 15, 15-30, 30-50, and > 50 μg/L). These results indicate that there is a statistically significant trend for the attention, language, and memory test with increasing prenatal mercury exposure (Grandjean et al., 1997).

Pilot whale blubber is also consumed by the Faroese population, and this could result in increased exposure to PCBs, a potential confounding factor. A subset (n = 436) of the cord tissue samples was evaluated for PCBs; inclusion of PCB exposure as a covariate in the regression analysis affected only the regression for the BNT. The authors conclude that results of the expanded data analysis do not suggest that the mercury effect can be explained by concomitant PCB exposure, or that PCB exposure enhances the mercury-associated effects.

Reevaluation of the Evoked Potentials in the Prospective Study (Murata et al., 1999a)

Significant associations with delays in evoked potential latencies and mercury exposure (Murata et al., 1999a) initiated the reanalyses of the data from the prospective longitudinal study. This analysis is limited to only children born during the first half of the cohort generation in 1993. Data from the second year were excluded because of shorter BAEP latencies and delayed latency on the visual-evoked potentials. Three sets of mercury exposure data were utilized in regression analyses: (1) mercury in cord blood (geometric mean of 23.0 μg/L, range of 3.3-351 μg/L), (2) mercury in maternal hair at parturition (geometric mean of 4.49 ppm, range of 0.9-39.1 ppm), and (3) mercury in the child's hair (geometric mean of 3.42 ppm, range of 0.04-26.4 ppm). The mercury concentration in maternal hair was a significant predictor for peak III latency and the I-III interval, where the child's own hair mercury concentration at the time of examination was not associated with these response variables. The cord blood concentration was, however, a significant predictor, supporting the notion that the latency delays are related to increased prenatal methylmercury exposure.

Nested Case-Control Study (Grandjean et al., 1998)

Following the evaluation of 7-year-olds in the prospective longitudinal study, the data were evaluated as a nested case-control study. From the original cohort of 1,022 established in the pilot study, the cases and controls were selected based on maternal hair mercury concentration. The case group of 112 children whose mothers had hair mercury concentrations of 10 to 20 ppm was matched to children with prenatal exposure below 3 ppm (control). Age, sex, time of examination, and maternal Raven score

were matching criteria. The median maternal hair mercury concentrations in the two groups were 1.8ppm for the control group and 12.5 ppm for the cases, a sevenfold difference. The median cord blood mercury concentrations for the control and cases were 11.9 and 59.0 μg/L, respectively. Neuropsychological tests evaluated were these: NES2 FT Test, NES2 HEC Test, NES2 CPT, WISC-R Similarities, WISC Block Designs, Bender Visual Motor Gestalt Test, CVLT, and BNT. The case group performed less satisfactorily than those in the control. On 6 of the 18 test outcomes, the inferior scores achieved by the case group were statistically significant. In particular, the case group showed a deficit on the Finger Tapping condition and the overall hand-eye coordination. Girls and boys scored differently on the Bender Gestalt Test, California Verbal Learning Test, all three Finger Tapping conditions, CPT reaction time, and the average hand-eye coordination score. No differences were reported between girls in the cases versus controls, but boys in the case group scored poorer in the Finger Tapping reaction time than the boys in the control group. The deficit in motor coordination, especially in Finger Tapping with both hands, was highly significant for boys only. The author noted that the findings of this matched case-control study are in accordance with regression analyses performed on all 900 children at the 7-year evaluation; methylmercury effects appear in the several domains of the brain, focusing on motor function, language, and memory.

Benchmark Modeling (Budtz-Jorgesen et al., 2000)

Benchmark modeling of the data from the Faroese children at 7 years of age was reported by Budtz-Jorgesen et al. (2000). The exposure was modeled both as mercury concentration in cord blood and in maternal hair. The number of children that completed neuropsychological tests varied between 837 and 901. One neuropsychological test was selected for evaluation of each of the five domains of brain function:

1. Motor speed (NES FT Test)
2. Attention: NES2 CPT
3. Visuospatial performance: Bender Visual Motor Gestalt Test
4. Language: BNT
5. Short-term memory: CVLT

For tests of motor function, language, and memory, a logarithmic dose-response model tended to show a better fit than a linear dose model using cord blood mercury concentration as the dose parameter. The default p_0 is 5%, which equates to the level (x_0) of abnormal test performance as defined by a probability

of 5% in the unexposed population. The Faroese cohort does not include an unexposed control group; thus the performance level for an unexposed child is obtained by fitting a dose-response curve to all data points, followed by extrapolating to zero exposure. Four different dose-response models were employed: *K* power, linear, square root, and logarithmic.

The results from this analysis indicate that BMDs and BMDLs vary substantially. Of the four models, the logarithmic dose-response model provided the best fit for some of the outcome variables that showed the closest association with the cord blood mercury concentration. The lowest BMDLs averaged approximately 5 μg/L cord blood, which is equivalent to approximately 1 ppm in maternal hair. Most BMDLs for hair mercury concentrations were higher. However, the results for a BMR of 5% are the same order of magnitude as the cord blood results at a BMR of 10%. The authors concluded that the results of the benchmark calculation are highly dependent on the assumed dose-response model. Results of this analysis are discussed further in the Risk Assessment chapter (Chapter 4). (For a description of modeling terms see Section 4.3).

Second Cohort (Steurwald et al., 2000)

During a period from 1994 to 1995, a second cohort of 182 singleton term births was generated from consecutive births at the National Hospital in Thorshavn, Faroe Islands (Steurwald et al., 2000). Maternal hair, serum, breast milk, and umbilical cord blood were analyzed for contaminants, while selenium, thyroid hormones, and fatty acids were measured in cord blood. In addition to methylmercury, PCBs were examined as a possible confounder in test outcome. At 2 weeks of age, infants were administered a neurological examination. Assessment of functional abilities, reflexes and responses, and stability of behavioral status during examination were completed with a score of optimal, questionable, or suboptimal performance. The Neurologic Optimality Score (NOS) was the number of items rated as optimal out of a total of 60. Results from this study indicate that prenatal exposure to methylmercury and PCBs increased from maternal intake of seafood. After adjustment for confounders, a tenfold increase of the cord blood mercury concentration was associated with a decreased NOS of 2.0. This effect corresponds to a decrease in gestational age of about 3 weeks. The authors conclude that prenatal exposure to methylmercury from contaminated seafood was associated with an increased risk of neurodevelopmental deficit. No evidence for a protective or beneficial effect with respect to neurological optimality score (the number of main items rated optimal out of 60) was observed for essential fatty acids or selenium.

3.2.1.8 Germany

Cross-Sectional Study (Altmann et al., 1998)

From a larger comparative environmental screening study, 384 children between the ages of 5 and 8 years were selected to participate in a smaller field experiment to investigate the effects of low-level lead and mercury exposure on the functions of the developing visual system. Blood lead levels and urinary excretion of lead and mercury were used as exposure indices. Neurophysiological and psychophysical measurements were administered to the children. Visual functions were assessed for neurophysiological measurements, while psychophysical measurements were assessed by visual-evoked potentials and contrast sensitivity. Linear regression analyses were used to analyze the possible relationship between exposure to lead and mercury and outcome variables. Adjustments were made for potential confounding factors such as parental education, birth weight, length of lactation, and premature birth.

After adjustment for potential confounding factors, contrast sensitivity values were significantly reduced with increasing urinary mercury levels; four of the ten contrast sensitivity values tested showed a statistically significant decrease with increasing urinary mercury. Very subtle changes in the visual system function were noted at very low levels of urinary mercury. However, no significant associations were found between urinary mercury output and any visually evoked potential outcome variables.

3.2.1.9 Nambija, Ecuador

Cross-Sectional Study on Neurosensory Dysfunction (Counter et al., 2000)

A cross-sectional study was conducted in the remote Andean settlement of Nambija, Ecuador, to investigate whether blood mercury levels are associated with auditory neurosensory dysfunction. Participants in this study included 36 children and 39 adults living in Nambija , an area known to have extensive gold-mining operations where mercury is used in the extraction process. Mercury exposure was measured in whole blood. The mean blood mercury level was 17.5 µg/L. A group of 34 subjects (15 children and 19 adults) from a non-gold-mining area were selected as the control group. Their mean blood mercury level was 3.0 µg/L. A neuro-otological examination was administered; a neurological examination of the cranial nerves was administered using standard procedures and an audiological test was administered to 21 children and 19 adults.

Of those examined, 45% of the group complained of headaches and/or memory loss, three cases involved severe neurological impairment and four cases involved middle ear pathology. A statistically significant relationship was identified between blood mercury level and hearing level in children at 3 kHz in the right ear only. Adults were not affected. BAEP responses showed a significant correlation between blood mercury and the I-III interpeak latency on the left side. The authors conclude that the findings of this study suggest that overall auditory sensory-neural function and neural conduction time at the brain stem level were generally unaffected by elevated blood mercury levels in either children or adults.

3.2.1.10 Amazonian Basin

The conditions in the Amazon—extremely high temperatures and humidity with seasonal fluctuation of water during rainy and dry seasons—are conducive for mercury methylation because of high quantities of suspended organic matter, high temperature, acidity, and redox potential. These elements influence the availability of fish as a food resource. In 1996, Lebel and colleagues published results from a small preliminary study on individuals from the Amazonian basin to determine the relationship between mercury exposure and neurological outcomes and reported the decrease of visual and motor functions with increasing hair mercury levels. In 1998, Lebel and colleagues published another study to determine the neurofunctional and clinical manifestations of nervous system dysfunction in relation to hair mercury levels below 50 ppm. In 1999, Grandjean et al. published results from a study of populations living in four comparable Amazonian riverine communities located upstream of gold-mining fields, while in 2000, Dolbec et al. published results from a cross-sectional study in a village on the Tapajos River.

Lebel et al. (1996)

Lebel et al. (1996) published a study of 29 adult residents living in two villages located on the Tapajos River, a tributary of the Amazon, located approximately 200 kilometers from several gold-mining sites. Total hair mercury concentration ranged from 5.6 to 38.4 ppm; methylmercury constituted between 72.2% and 93.3% of the total mercury measured in hair samples. A quantitative behavioral neurophysiological battery was modified for administration to persons with minimal formal education living in an area without electricity. Women exhibited a decrease in manual dexterity, as measured in the Santa Ana Test (Helsinki version) that was correlated with increased mercury concentration in hair. For both men and women, there was a statistically significant decrease in color discrimination capacity with

increasing hair mercury concentrations. Near visual contrast sensitivity profiles and peripheral visual field profiles were both reduced in the individuals with the highest hair mercury concentrations. The authors note that constriction of the visual field has been observed in other instances of mercury intoxication and that changes in contrast sensitivity have been noted in nonhuman primates exposed to methylmercury (Rice and Gilbert 1982,1990).

Lebel et al. (1998)

A later study was conducted in a Tapajos River village that depends on fish as its main source of protein. A total of 91 adults (45 men and 46 women between the ages of 15 and 81) of the 98 voluntary participants were examined. Four measures of hair mercury concentrations were used: (1) mean total hair mercury, (2) total hair mercury, (3) total hair mercury in the highest value obtained out of all centimeters analyzed, and (4) total hair mercury in the first centimeter and methylmercury in the first centimeter. Several tests were administered to score for neuropsychological dysfunction. Motor strength was determined with a dynameter for grip test; manual dexterity was measured with the Santa Ana Test (Helsinki version); and visual functions, color vision, and contrast sensitivity were assessed with a battery of sensitive neurofunctional tests. Results were analyzed by multiple regression.

There was no difference between genders for all tests except the grip strength test. Women also exhibited decreased grip strength with increasing peak mercury levels. Intermediate and higher frequencies of near visual contrast sensitivity and manual dexterity (measured with the Santa Ana Test) varied with the level of mercury in hair. Gender-nonspecific muscular fatigue was also noted with increasing mercury levels. The authors suggest that there appears to be a dose-effect relationship for certain motor and visual functions. Manual dexterity, alternating hand coordination, and muscular fatigue were associated with hair mercury levels, while near visual contrast sensitivity and restricted visual fields were dose-dependently altered.

Cross-Sectional Study (Grandjean et al., 1999)

A cross-sectional study was conducted in four comparable Amazonian riverine communities located upstream toward gold-mining fields. Fish is consumed as a large part of the population's staple diet. Of the 420 eligible children between the ages of 7 and 12, 351 were examined for neurobehavioral dysfunction. Mercury exposure was measured through children's hair mercury levels because only 37% of the participants had maternal hair mercury samples. Children's hair mercury concentrations had an

overall geometric mean of 11.0 ppm and a median of 12.8 ppm, while mothers had geometric mean hair mercury levels of 11.6 ppm and a median value of 14.0 ppm. Maternal hair mercury concentrations were highly correlated with those of their children. Several neuropsychological tests of motor function, attention, and visuospatial capability were administered. These included Finger Tapping, Santa Ana form board, WISC-III Digit Spans Test, and two subtests of the Stanford-Binet Intelligence Scale (the copying test and memory condition). The relation between mercury exposure and neurobehavioral function was analyzed by multiple regression analyses with adjustment for covariates including, age, sex, health status, maternal education, and maternal marital status.

The Santa Ana form board and Stanford-Binet copying test showed the clearest associations with the hair mercury concentration. The authors note that the effect of mercury was significantly greater in younger children only for the nonpreferred hand condition of the Santa Ana Test. In interpreting these results, the authors caution that there were no data for the level of prenatal exposure experienced in the test children because of the lack of maternal hair samples. Additional sources of uncertainty in this study include nutritional deficiencies that occurred in the past and possible infection of tropical diseases that may have influenced the capabilities of these children at the time of neurological evaluation.

Cross-Sectional Study (Dolbec et al., 2000)

A cross-sectional study was conducted in May of 1996 in a village on the banks of the Tapajos river in the Amazonian Basin, Brazil (Dolbec et al., 2000). This study was conducted on 84 fish-eating adults between the ages of 15 and 79, to evaluate the effect of mercury exposure on motor performance. The mean hair total mercury level was 9 ppm. Pychomotor performance was evaluated using the Santa Ana Test for manual dexterity, the Grooved Pegboard Fine to test fine motor skills and NES Finger Tapping Test for motor speed. Motor strength was measured by dynamometry for grip and pinch strength.

Multivariate analysis of the variance indicated that the hair mercury levels were inversely associated with overall performance on the psychomotor tests, whereas an association was reported with blood mercury. Semipartial regression analyses reported that hair total mercury accounted for 8%-16% of the variance of psychomotor performance. The authors conclude that the findings of this study demonstrated neurobehavioral manifestations of subtle neurotoxic effects on motor functions associated with low-level methylmercury exposure.

3.2.1.11 Madeira

Cross-Sectional Study (Murata et al., 1999b)

A cross-sectional study (Murata et al., 1999) was conducted in the Madeiran community to determine possible—mercury exposure-related effects on evoked potentials in 149 children between the ages of 6.4 and 7.4 years. Children's hair mercury concentrations were used to reflect current exposure levels, while maternal hair levels from mothers who had followed consistent diets since pregnancy represented prenatal mercury exposures 7 years ago. The use of maternal hair concentration as a substitute for exposure during pregnancy is based on the assumption that mercury exposure has changed very little over time. The authors acknowledge, however, that current maternal hair mercury levels provide an imprecise indication of exposure during pregnancy and any recent dietary change would tend to weaken the association with the outcome variables. The 149 children were administered physical and functional neurological examinations, with an emphasis on motor coordination and perceptual motor performance. Tests included these:

- NES2 FT
- NES2 HEC
- NES2 CPT
- WISC-R subtests: Digit Spans forward condition and Block Designs
- Stanford-Binet Bead Memory Test

Evoked potentials were determined with a four-channel electromyograph, while pattern reversal visual-evoked potentials with binocular full-field stimulation were conducted in a darkened room. Associations between these outcomes and exposure to methylmercury were assessed by multiple regression analysis and were adjusted for possible confounding variables: age, sex, maternal and paternal education and employment, maternal alcohol use and smoking during pregnancy, numbers of older and younger siblings, school, and the level of the child's computer acquaintance.

Increased exposure to methylmercury was associated with delays in evoked potential latencies; peak III on the BAEP at 40 Hz, and N145 on the pattern reversal visual-evoked potentials at the 15-minute condition. When the maternal hair mercury concentration exceeded 10 ppm, the increase of the N145 visual-evoked potential latency at 15 minutes was 3.16 milliseconds (ms). The N75-N145 and P100 and N145 interval latencies showed similar regression coefficients for mercury, although

significance was evident only for the 15-minute condition. The authors suggest that this may indicate that there is a mercury-associated delay occurring between P100 and N145. Weak associations were also evidenced between maternal hair mercury levels and deficits on Digit Spans and Bead Memory tests.

3.2.1.12 French Guiana

Case-Control Study (Cordier and Garel, 1999)

High-exposure areas were selected in the Amerind villages in the Upper Maroni, with two other Amerind villages with less mercury contamination to serve as reference groups (Cordier and Garel, 1999. 261 children participated in the study, 69 from the village of Camopi (control), 82 from Awala (control) a total of and 110 in the Upper Maroni (cases). Hair samples were collected from both children and mothers to represent exposure indices. Maternal hair mercury levels ranged from 2.5 to 6.7 ppm. This was used as a surrogate for prenatal exposure. Children had slightly lower hair mercury levels than adults, but this did not vary with age. Neurological examinations were administered to children from 9 months to 6 years of age with special emphasis on neuromotor examination of the upper and lower limbs, axis of the body, deep reflexes, postural reactions, examination of the effects on neuromotor functions, neurosensory examination, and cranial growth. The battery of tests was selected to measure the child's abilities outside of educational or cultural influences; these include the NES FT Test to measure fine motor function, coordination, and speed of execution; and the Stanford-Binet Intelligence Scale, with subtests of immediate memory (bead memory) and ability to assess visuospatial and visuoconstructional function (block-copying). In addition, the McCarthy memory test for digits (backward and forward) and the McCarthy leg coordination test were utilized. Associations were analyzed by linear regression, adjusting for potential confounding factors (alcohol consumption during pregnancy, parity, place of birth of the child, and illnesses during childhood).

Within the case group, there is a significant decrease in the scores with exposure category for the Leg Coordination test and close to significance for the Copying test. When boys and girls were examined separately for the FT test, boys had higher scores than the girls, while a significant decrease is observed in the score on the Block Design test correlated with exposure in girls. Boys also exhibited greater incidence of increased reflexes correlated with maternal hair mercury concentrations. The authors conclude that results of this study suggest a link between exposure to mercury and perturbations of the child's neurological and intellectual development.

3.2.2 Animal Studies

Substantial information on the neurotoxicity of methylmercury has been generated from animal studies that support neurological effects reported in humans. Relatively brief, high-level exposures in rats have been shown to cause characteristic signs of neurotoxicity (flailing and hindlimb crossing when the animal is lifted by the tail), as well as neuronal degeneration in the cerebellum, cerebral cortex, and dorsal root ganglia (Inouye and Murakami, 1975; Leyshon and Morgan, 1991; Magos et al., 1985; Yip and Chang, 1981). As observed in humans, there is a latency period before onset of neurological symptoms. Toxic effects may not be observed or may not show maximal severity until several days after the initiation of dosing. In short-term studies, toxicity may not become evident until after the cessation of dosing. This section summarizes a few selected animal studies on neurotoxicity. For additional detail, please refer to Volume V of the *MSRC* (U.S. EPA, 1997e) and the *Toxicological Effects of Methylmercury* (NRC, 2000).

3.2.2.1 Acute Toxicity

In an acute study, exposure of rats to a single gavage dose of 19.9 mg mercury/kg as methylmercuric chloride resulted in impaired open-field tests such as decreases in standing upright, area traversed, and activity compared with the control group (Post et al., 1973). Animals were lethargic and ataxic initially, but symptoms disappeared within 3 hours.

3.2.2.2 Chronic Toxicity

Longer term, low-level exposures revealed that evidence of neuronal degeneration may be observed before the onset of overt signs of toxicity. Degeneration in the cerebellum was found in rats given 10 mg mercury/kg as methylmercuric chloride once every 3 days for 15 days (Leyshon and Morgan, 1991). Severe degenerative changes in the dorsal root fibers were observed in rats given 1.6 mg mercury/kg-day as methylmercuric chloride for 8 weeks (Yip and Chang, 1981). Munro et al. (1980) observed demyelination of dorsal nerve roots and damage in sciatic nerves with oral exposure to 0.25 mg mercury/kg-day as methylmercuric chloride for up to 26 months. In mice given 1.9 mg mercury/kg-day as methylmercury, cerebellar lesions were observed as early as 8 days after the start of dosing, but changes in motor activity did not develop until after 24 weeks of exposure (MacDonald and Harbison, 1977). Similarly, cats receiving methylmercury in the diet for 11 months displayed degenerative changes

in the cerebellum and cerebral cortex, but uncoordinated movements or weakness were observed only in a small number of animals with histopathological changes (Chang et al., 1974).

A 2-year feeding study of methylmercuric chloride was conducted in B6C3F1 mice (60 mice/sex/group) at doses of 0, 0.4, 2, and 10 ppm (0, 0.03, 0.15, and 0.73 mg mercury/kg-day in males; 0, 0.02, 0.11, and 0.6 mg mercury/kg-day in females) to evaluate chronic toxicity and carcinogenic effects (Mitsumori et al., 1990). Mice were examined clinically during the study, and neurotoxic signs characterized by posterior paralysis were observed in 33 males after 59 weeks and in 3 females after 80 weeks in the 0.6 mg mercury/kg-day group. A marked increase in mortality and a significant decrease in body weight gain were also observed in the high-dose males, beginning at 60 weeks. Postmortem examination revealed toxic encephalopathy consisting of neuronal necrosis of the brain and toxic peripheral sensory neuropathy in both sexes of the high-dose group. An increased incidence of chronic nephropathy was observed in the 0.11- and 0.6-mg mercury/kg-day males.

Groups of Wistar rats (50/sex/group) were administered daily doses of 0.002, 0.02, 0.05, and 0.25 mg mercury/kg-day as methylmercuric chloride for 26 months (Munro et al., 1980). Female rats that received 0.25 mg/kg-day had reduced body weight gains and showed only minimal clinical signs of neurotoxicity. Male rats that received this dose did show overt clinical signs of neurotoxicity, had decreased hemoglobin and hematocrit values and reduced weight gains, and showed increased mortality. Histopathologic examination of rats of both sexes receiving 0.25 mg/kg-day revealed demyelination of dorsal nerve roots and peripheral nerves. Males showed severe kidney damage and females had minimal renal damage. This study identified a NOAEL of 0.05 mg/kg-day and a LOAEL of 0.25 mg/kg-day, based on the observed demyelination effect.

Bornhausen et al. (1980) reported a decrease in operant behavior performance in 4-month-old rats whose dams had received methylmercuric chloride on gestation days 6 to 9. A statistically significant effect was seen in offspring whose dams had received 0.01 and 0.05 mg/kg five times during gestation. The authors postulated that more severe effects of *in utero* exposure would be seen in humans because the biological half-life of mercury in the brain of humans is five times longer than in the rat. In addition, much longer *in utero* exposure to mercury would occur in humans because gestation is much longer.

In a study of prenatal coexposure to metallic mercury vapor and methylmercury and their effects on the developing central nervous system, Fredriksson et al. (1996) reported interactive behavioral effects following exposure of pregnant female Sprague-Dawley rats to methylmercury and metallic mercury

vapor. Between 4 and 5 months, testing of behavioral function, spontaneous motor activity, spatial learning in a circular bath, and instrumental maze learning for food were performed. Exposure to mercury vapor at 1.8 mg/m^3 for 1.5 hours per day on gestation days 14 to 19 was related to hyperactivity and decreased spatial learning. Although exposure to methylmercury at 2 mg/kg per day on gestation days 6 to 9 was not related to adverse behavioral effects, coexposure to methylmercury and mercury vapor potentiated the activity and spatial learning effects observed with mercury vapor alone. The results of this study indicate that mercury vapor causes central nervous system functional disturbances in offspring after both prenatal and postnatal exposure. The authors also suggest that coexposure to methylmercury served to significantly aggravate the changes, whereas methylmercury alone did not cause any significant functional alterations in this study.

Ramussen and Newland (1999) studied the acquisition of Multiple Differential Reinforcement of High-Rate Extinction (MULT DRH-N:T EXT) schedules of reinforcement in female rats exposed to methylmercury during development. Female rats were administered methylmercury (0, 0.5, or 6.4 ppm) in drinking water from 4 weeks premating to postnatal day 16. Postnatal methylmercury concentrations in the brain at birth were 0.49 and 9.8 ppm for two exposure groups. In the MULT DRH-N:T EXT, female offspring were trained to press levers under schedules of reinforcement. Whenever a response occurred within a specific time measured in seconds, a food pellet was given. Two acquisition protocols were examined; one imposed three successive sessions in a 3:1, 5:2, and 9:4 ratio. Values were chosen so that the same rate of response was required by the schedules. The second acquisition protocol required lever repressing as reestablished and the three schedules were continued until the behavior became stable, which required more than 10 sessions. This study was not able to replicate the finding of abnormal response patterns using the DRL paradigm used by Bornhausen (1980).

Cholinergic systems also play an important role in learning and memory. Coccini et al. (2000) investigated the effect of low-level methylmercury exposure on muscarinic cholinergic receptor (mAChR) binding characteristics in adult female Sprague-Dawley rats. The rats (4/dose) were administered methylmercury in the drinking water at nominal concentrations of 0, 2.5, and 10 µg/L for 16 days. Mean daily intake in the methylmercury-exposed groups was 0.45 and 1.8 mg/kg-day, respectively. mAChR binding was assessed using the muscarinic antagonist [^{3}H]quinuclidinyl benzilate (QNB) to label receptors in excised brain tissues (cerebral cortex, hippocampus, and cerebellum). Exposure to methylmercury selectively increased mAChR density in the hippocampus and cerebellum by 20% to 44%. This response was characterized by a 2-week latency period before onset. Receptor affinity was

unaffected, as indicated by values for the dissociation constant. No significant effect on mAChR in cerebral cortex was observed.

Nonhuman Primates—Macaca Fascicularis Monkeys

Monkeys appear to be more sensitive to the neurotoxic effects of methylmercury than are rodents. The primate model is particularly useful for studies of developmental exposures because monkeys, like humans, have relatively prolonged periods of gestation, infancy, and adolescence (Burbacher and Grant, 2000). Long-term studies in primates have shown neurological impairment at doses as low as 0.05 mg mercury/kg-day. Exposure of monkeys to 0.03 mg mercury/kg-day as methylmercury for approximately 4 months caused no detectable changes in motor activity or effects on vision or hearing, but degenerative changes were observed in neurons of the calcarine cortex and sural nerve when these were examined by electron microscopy (Sato and Ikuta, 1975). At higher doses (0.08 mg mercury/kg-day), slight tremor, lack of motor coordination, and blindness were observed in monkeys after 4 months of exposure (Burbacher et al., 1988).

Gunderson et al. (1986) administered daily doses of 0.04–0.06 mg mercury/kg as methylmercuric hydroxide to 11 crab-eating macaques (*Macaca fascicularis*) throughout pregnancy. This dosing protocol resulted in maternal blood levels of 1,080–1,330 µg/L in mothers and 1,410–1,840 µg/L in the offspring. Infants of treated mothers exhibited visual recognition deficits when tested 35 days after birth.

Rice (1989b) dosed five cynomolgus monkeys (*Macaca fascicularis*) with 0.05 mg mercury/kg-day as methylmercuric chloride from birth to 7 years of age. Clinical and neurological examinations were performed during the dosing period and for an additional 6 years. Impairment of spatial visual function was observed after 3 years. In the later stages of the observation period, monkeys dosed with methylmercury were clumsier and slower to react when placed in the exercise cage than were unexposed monkeys. Decreased fine motor performance, touch, and pinprick sensitivity, and impaired high-frequency hearing were observed 6-7 years after cessation of dosing (Rice 1989a; Rice and Gilbert, 1982, 1990).

Rice (1998) did auditory testing of *Macaca fascicularis* monkeys exposed to methylmercury chloride at 10, 25, or 50 ug/kg per day *in utero*, throughout gestation, plus 4 years postnatally at 11 and 19 years of age. Results from this study indicated that at 19 months of age, all five *Macaca fascicularis* monkeys experienced deterioration in auditory function and elevated pure-tone thresholds throughout the

full range of frequencies tested (0.125 to 31.5 kHz) when compared with age-matched controls. The elevation of thresholds was in some cases 50 dB or higher. Because the auditory deficits are experienced approximately 7 to 15 years after cessation of methylmercury exposure, they are considered irreversible and permanent. The author concluded from this study that the high-dose monkeys experience an earlier onset of effect on the auditory function than do low-dose monkeys. The group of monkeys that showed delayed neurotoxicity at 15 years also had visual deficits identified at 3 years, as well as auditory and somatosensory impairment. The high-dose monkeys were also impaired at 11 years, and relatively more impaired than controls at 19 years, thus providing evidence for accelerated aging. These results provide evidence for the accelerated impairment of auditory function during aging as a consequence of developmental methylmercury exposure.

In another study by Rice (1998), monkeys with robust methylmercury-induced deficits in visual, auditory, and somatosensory function were tested on a series of tasks assessing central processing speed. This task is thought to be similar to tests measuring human intelligence. Five *Macaca fascicularis* monkeys were dosed with 50 μg/kg per day methylmercuric chloride from birth until 7 years of age. Blood mercury levels ranged from 0.8 to 1.1 μg/g until cessation of dosing. At 20 years of age, the monkeys and four age-matched and rearing-matched controls were tested on a series of simple and complex reaction-time tasks. In the simple reaction-time test, the monkeys were required to press a button when it changed from off to on (bright red light). The monkeys then performed a sequence of complex reaction-time tasks: two-button pressing, four-button pressing, and several tasks of increasing complexity using four buttons and multiple colors. The results indicated no differences between groups on any aspect of the experiment. The author concluded that the data provide further evidence for the absence of cognitive impairment in monkeys exposed developmentally to methylmercury.

In 1999, Burbacher et al. published a study that assessed visual and auditory functions in adult *Macaca fascicularis* monkeys exposed to methylmercury *in utero*. Maternal doses were 0, 50, 70, or 90 μg/kg per day; this resulted in infant blood mercury levels that ranged from 1.04 to 2.45 ppm. When the monkeys reached 15 years of age, they were tested on spatial visual contrast sensitivity tasks at spatial frequencies of 1, 4, 10, and 20 cycles per degree of visual angle and auditory pure tone detection tasks at frequencies of 125, 500, 1,000, 4,000, 10,000, 25,000, and 31,500 Hz. The results of these tests indicated that *in utero* exposure to methylmercury has long-term effects on visual contrast sensitivity thresholds. Preliminary results from the auditory task suggest that auditory thresholds are not affected by methylmercury exposure. The authors suggest that results from this study point to the postnatal period as a possible critical window for methylmercury induced auditory neurotoxicity.

3.3 CARDIOVASCULAR TOXICITY

3.3.1 Human Studies

3.3.1.1 Cardiovascular Effects From the Faroe Islands (Sorensen et al., 1999)

Sørensen et al. (1999) evaluated the relationship between prenatal exposure to methylmercury and occurrence of cardiovascular effects at 7 years of age in a birth cohort (n = 1,000) of children from the Faroe Islands. Prenatal exposure was assessed by analysis of cord blood and maternal hair collected at parturition. More than 80% of the hair samples exceeded a methylmercury concentration of 2 ppm, which corresponded to a cord blood concentration of approximately 10 μg/L. The cardiovascular endpoints evaluated at 7 years included systolic and diastolic blood pressure, heart rate, and heart rate variability. Weight, height, body mass index, sex, and maternal hypertension were examined as predictors of blood pressure and heart rate in approximately 900 children. Birth weight and placental weight were also examined as predictors of blood pressure. Following adjustment for body weight, diastolic and systolic blood pressure increased by 13.9 mm mercury (95% confidence limits [CL] = 7.4, 20.4) and 14.6 mm mercury (95% CL = 8.3, 20.8), respectively, as cord blood mercury concentrations increased from 1 to 10 μg/L. No further increase was noted at higher concentrations of mercury. Low-birth-weight children were more likely to experience methylmercury-related increase in blood pressure. A gender-specific decrease in heart rate variability was also noted with increasing mercury exposure. This effect was most pronounced in boys, where a 47% reduction in heart rate variability was observed when cord blood mercury concentrations increased from 1 to 10 μg. The authors concluded that the findings suggest that prenatal exposure to methylmercury may influence the development of cardiovascular regulatory mechanisms.

3.3.1.2 Cross-Sectional Study (Salonen et al., 1995)

Salonen et al. (1995) examined the relationship between dietary intake of fish and mercury and risk of acute myocardial infarction (AMI), death from coronary heart disease (CHD), and other cardiovascular diseases (CVD). Participants of this study included 1,833 men in eastern Finland between the ages of 42 and 60 with no clinically diagnosed CHD, claudication, stroke, or cancer. Baseline examinations were administered between March 1984 and December 1989. Fish consumption was assessed at time of blood sampling with an interview-verified 4-day food record. The food recording was repeated approximately 12 months after the baseline examination in a random sample of 50 men in the

cohort. Daily fish intake ranged from 0 to 619.2 g (mean of 46.5 g/day). Mercury in hair and urine was determined by flow injection analysis-cold vapor atomic absorption spectrometry and amalgamation. Hair mercury concentrations ranged from 0 to 15.67 ppm (mean of 1.92 ppm) while dietary mercury intake ranged from 1.1 to 95.3 μg /day (mean of 7.6 μg per day). In 2 to 7 years, 73 of the 1,833 men experienced an AMI; 18 of the 73 patients with AMI died of CHD, while 24 of the 73 died of CVD. Covariates included these: age; examination year; family history of CHD; place of residence (rural vs. urban); diabetes; socioeconomic status; iron intake; number of cigarettes, cigars, and pipefuls of tobacco currently smoked daily; duration of regular smoking in years; alcohol consumption; history of myocardial infarction; angina pectoris and other ischemic heart disease; presence of hypertension; and current antihypertensive medication. The Cox models reported dietary intakes of fish and mercury associated with increased risk of AMI and death from CHD, CVD, and any death. Results from this study indicated that eastern Finnish men with hair mercury levels exceeding 2 ppm had a twofold age- and CHD-adjusted risk of AMI and a 2.9-fold adjusted risk of cardiovascular death compared with those having lower hair mercury content.

3.3.1.3 Nested Case-Control Study (Salonen et al., 1995)

A nested case-control study was also conducted using a subsample of the original study participants. Serum immune complexes containing oxidized LDL were measured in a subsample of 187 control subjects using an ELISA assay with copper-oxidized LDL as the antigen. Pearson correlation coefficients adjusted for age and year of baseline examination were used to determine the association between hair mercury content and dietary intakes of fish and mercury. Partial associations of hair and urinary mercury with titers of immune complexes against oxidized LDL were estimated by SPSS step-up least-squares regression analysis. A multivariate logistic model included the following covariates: cigarette-years, serum ferritin concentration, ischemic exercise ECG, serum apolipoprotein, family history of CHD, maximal oxygen uptake, and serum HDL2 cholesterol. There was a statistically significant association between urinary mercury excretion and the risk of AMI was reported. For each microgram of mercury excreted daily, the risk of AMI increased by 36%. From the immunotoxicity test, both the hair and urinary excretion mercury levels were associated with immune complex titers measured with a rabbit antiserum against oxidized LDL and the γ-globulin fraction of a rabbit antiserum against oxidized LDL. Overall, hair mercury was the strongest predictor of both immune complex titers.

On the basis of these data, the authors concluded that a high intake of mercury from nonfatty freshwater fish, and the consequent excess risk of AMI as well as death from CHD and CVD in eastern Finnish men, may be due to the promotion of lipid peroxidation by mercury.

3.3.2 Animal Studies

Data on cardiovascular effects following oral methylmercury exposure were obtained from two studies in rats. Rats given two daily doses of methylmercuric chloride exhibited decreases in heart rates following two daily doses of methylmercury at 12 mg/kg per day (Arito and Takahashi, 1991). Wistar rats (n = 80) treated by subcutaneous injection with 0.5 mg/kg-day methylmercuric chloride for 1 month had increased systolic blood pressures beginning 42 days after cessation of dosing (Wakita, 1987). This effect persisted for more than a year.

Mitsumori et al. (1983, 1984) fed Sprague-Dawley rats diets containing methylmercuric chloride (males 0, 0.011, 0.05, or 0.28 mg/kg/day; females 0.014, 0.064, or 0.34 mg/kg/day) for up to 130 weeks. Polyarteritis nodosa and calcification of the arterial wall were seen at the highest dose. Histological examination revealed evidence of hemosiderosis and extramedullary hemotopoiesis of the spleen.

In a study on 7-week-old, hypertensive SHR/NCrj rats, Tamashiro et al. (1986) reported an increase in blood pressure resulting from exposure to methylmercury chloride once a day at 2 mg/kg/day for 26 consecutive days. Body weight loss, an early sign of methylmercury intoxication, was more marked in males than females. All male rats died by the 29th day posttreatment. Neurological signs, hindleg crossing, disturbed righting movement and abnormal gait always preceded death. No mortality was reported for the female rats. However, increase in blood pressure was sex-specific, being observed only in females. The authors noted that considerable variation was observed in blood pressure for both the methylmercury-exposed and the control rats; and that these findings suggest strain differences in male-female toxicity of methylmercury chloride.

3.4 IMMUNOTOXICITY

3.4.1 Human Studies

At this time, there are no studies published on the effect of methylmercury on the human immune system. In occupational exposure studies, elemental mercury has been found to affect particular immune

parameters. A study by Queiroz and Dantas (1997) evaluated B-lymphocyte, T-helper, T-suppressor, and T-cell proliferative response to phytohemagglutinin in 33 male workers in a Brazilian mercury production facility. These workers had a mean age of 29 and a mean mercury exposure period of 19 months. All of the workers had urinary mercury concentrations below 50 µg/g of creatinine. Analysis of the T-cell populations found a reverse CD4+ to CD8+ ratio that was characterized by a reduction in the number of CD4 lymphocytes. B-lymphocytes were also significantly reduced. Analysis of serum antibody levels found increased immunoglobulin E levels but did not detect anti-DNA or anti-nucleolar antibodies. No changes were observed in the proliferative response to phytohemagglutinin of lymphocytes from exposed individuals. The authors reported a negative correlation between the length of exposure to mercury and IgE levels, and no correlations between lymphocyte changes and urinary mercury concentrations, time of exposure, or the age of the workers. (Queriroz and Dantas, 1997)

Another occupational exposure study by Moszczynski et al. (1995) examined the lymphocyte subpopulation of T-cells, T-helper cells, T-suppressor cells, and natural killer cells in the peripheral blood of 81 men exposed to metallic mercury vapors and 36 unexposed men. The average workplace exposure to mercury in air was 0.0028 mg/m^3. Urinary mercury concentrations ranged from 0 to 240 µg/L and concentrations in the blood varied from 0 to 30 µg/L. Stimulation of the T-lymphocytes manifested by an increased number of T-cells, T-helper cells, and T-suppressor cells was observed.

3.4.2 Animal Studies

Data on the potential immunotoxic effects of methylmercury are available from several animal studies. Suppression of humoral and cellular immune responses has been observed in animals after oral exposure to methylmercury or methylmercuric chloride. Decreases in the production of antibody-producing cells and/or decreased antibody titer following inoculation with immune-stimulating agents (such as sheep red blood cells) have been observed in mice and rabbits (Blakley et al., 1980; Koller et al., 1977; Ohi et al., 1976). Decreases in natural killer T-cell activity and reduced thymus weight have been observed in female mice after 14 weeks of exposure to methylmercury (Ilback, 1991). Bernaudin et al. (1981) observed IgG deposits along the glomerular capillary wall of Brown Norway rats treated with methylmercury for 2 months and noted that these deposits were suggestive of autoimmune disease. The following sections include summaries of selected studies.

Wild et al. (1997) evaluated immune function in the offspring of Sprague-Dawley rats exposed to methylmercuric chloride (5 or 500 µg/L) or methylmercury sulfide (5 µg/L) via drinking water. There

were three exposed groups and one control group. The control group was fed plain tap water. Rats of both sexes were treated for 8 weeks prior to mating and treatment of female rats continued throughout pregnancy and nursing. The total duration of indirect exposure of the offspring to methylmercury was 42 days. Immunological function was assessed in six offspring per treatment group at 6 and 12 weeks of age (3 and 9 weeks after termination of methylmercury exposure at weaning, respectively). At 6 weeks, total body weights, splenic weights, and thymic weights were increased in the methylmercury chloride-exposed rats, whereas the rats exposed to methylmercury sulfide experienced only an increase in thymic weight at 6 weeks. At 12 weeks, natural killer cell activity was markedly depressed (56%) for rats exposed to methylmercury chloride in comparison with controls. Methylmercury sulfide appeared to have different effects on the immune system than did methylmercury chloride. For example, the sulfide form affected only thymic weight and had no significant effect on NK or splenocyte cell activity or splenocyte LPR. Whether this result reflects differential distribution of the sulfide form or affinity for different targets in the immune system is unknown. The authors concluded that methylmercury chloride seems to have an effect on splenocytes and natural killer cell activity.

Inorganic mercury has been observed to induce a variety of immune effects in mice. However, until recently there has been limited investigation of the ability of methylmercury to induce similar immune responses. Hultman and Hansson-Georgiadis (1999) investigated the ability of subcutaneously injected methylmercury to induce systemic autoimmunity in five genetically susceptible and resistant strains of mice. Female SJN/L, A.SW, B10.S (H-2^s), BALB/C, DBA/2 (H-2^d), A.TL, and B10.TL (H-2^u) mice were administered subcutaneous injections of 1 mg/kg methylmercury every third day for 4 weeks. This treatment protocol resulted in an average daily dose of approximately 350 μg mercury/kg-day. The immune response to methylmercury differed qualitatively and quantitatively from the response to inorganic mercury. Treatment with methylmercury induced at most a small increase in serum Ig concentrations after 4 weeks of treatment. The observed increases during the treatment period were generally marginal when compared with increases induced by mercuric chloride. Treatment with methylmercury induced development of antinucleolar antibodies (ANoA) targeting the nucleolar protein fibrillarin in the susceptible SJL, A.SW, and B10.S strains. Susceptibility to development of ANoA was linked to the mouse major histocompatibility complex H-2. However, background genes determined the strength of the response in susceptible strains. Serum IgE concentration and ANoA titer increased 2 to 3 weeks after cessation of treatment with methylmercury. In H-2^s mice, methylmercury induced a weaker general (polyclonal) and specific (ANoA) response when compared to mercuric chloride. Unlike mercuric chloride-treated mice, animals administered methylmercury did not develop systemic or renal immune system deposits.

3.5 REPRODUCTIVE TOXICITY

3.5.1 Human Studies

There are no studies of reproductive deficits in humans exposed to low-dose methylmercury.

3.5.2 Animal Studies

There are no two-generation reproductive assays for methylmercury.

3.6 GENOTOXICITY

3.6.1 Human Studies

Data from several studies in humans suggest that ingesting methylmercury may cause chromosomal aberrations and sister chromatid exchanges (SCE) (Skerfving et al., 1970; Wulf et al., 1986; Franchi et al., 1994).

A study of nine Swedish subjects who consumed mercury-contaminated fish and four controls showed a statistically significant rank correlation between blood mercury and percentage of lymphocytes with chromosome breaks (Skerfving et al., 1970). An extension of this study (Skerfving et al. 1974) included 23 exposed (5 females and 18 males) and 16 controls (3 females and 13 males). The authors reported significant correlations between blood mercury level and frequency of chromatid changes and "unstable" chromosome aberrations; there was no correlation with "stable" chromosome aberrations.

The Wulf et al. (1986) study was of 92 Greenlander Eskimos. Subjects were divided into three groups based on intake of seal meat (six times per week; two to five times per week, once a week, or no consumption of seal meat). Higher frequency of SCE in lymphocytes was correlated with blood mercury concentration; an increase of 10 µg mercury per liter of blood was associated with an increase of 0.3 SCE/cell. Positive correlations were also found for smoking, diet, living district, and cadmium exposure.

Franchi et al. (1994) evaluated formation of micronuclei in peripheral blood lymphocytes of Mediterranean fishers, a group with presumed high exposure to methylmercury. Fifty-one subjects were

interviewed on age, number of seafood-based meals/week, and habits such as smoking and alcohol consumption. Total blood mercury was measured; the range was 10.08-304.11 ng/g with a mean of 88.97 ± 54.09 ng/g. There was a statistically significant correlation between blood mercury concentration and micronucleus frequency and between age and micronucleus frequency (U.S. EPA, 1997e)

3.6.2 Animal Studies

In a study with cats (Charbonneau et al. 1976), methylmercury did not induce dose-related unscheduled DNA synthesis in lymphocytes or chromosomal aberrations in bone marrow cells after oral exposure for up to 39 months (Miller et al., 1979). Statistically significant decreases in unscheduled DNA synthesis and increases in chromosomal aberrations were observed, but there was no dose-response.

Strain-specific differences exist with respect to the ability of methylmercury to produce dominant lethal effects in mice (Suter, 1975). When (SEC × $C57B_1$)F_1 males were injected with 10 mg/kg methylmercury hydroxide, there was a slight reduction in the total number of implantations and a decrease in the number of viable embryos. This was not observed when (101 × C3H)F_1 males were exposed in a similar fashion. When female (10 × C3H)F_1 mice were treated with methylmercuric hydroxide, no increase in the incidence of dead implants was observed (unlike the case for mercuric chloride). Changes in chromosome number, but no increase in chromosome aberrations, were observed in oocytes of Syrian hamsters treated with one interperitoneal injection of 10 mg/kg methylmercuric chloride (Mailhes, 1983). Methylmercury was administered subcutaneously to golden hamsters at doses of 6.4 mg or 12.8 mg mercury/kg/body weight. Polyploidy and chromosomal aberrations were increased in bone marrow cells, but there was no effect on metaphase II oocytes. There was an inhibitory effect on ovulation, which the authors noted was not as severe as that induced by mercuric chloride in the same study (Watanabe et al., 1982). Nondysjunction and sex-linked recessive lethal mutations were seen in *Drosophila melanogaster* treated with methylmercury in the diet (Ramel, 1972).

As reviewed in WHO (1990), methylmercury is not a point mutagen but is capable of causing chromosome damage in a variety of systems. In vitro studies have generally shown clastogenic activity but only weak mutagenic activity. Methylmercuric chloride and dimethylmercury were both shown to induce chromosome aberrations and aneuploidy in primary cultures in human lymphocytes; methylmercuric chloride was the more potent clastogen at equally toxic doses (Betti et al., 1992). Both methylmercury and mercuric chloride induce a dose-dependent increase in SCE in primary human

lymphocytes and muntjac fibroblasts; methylmercury was about five times more effective in this regard (Verschaeve et al., 1984; Morimoto et al., 1982).

Methylmercury has been shown to inhibit nucleolus organizing activity in human lymphocytes (Verschaeve et al., 1983). Methylmercury can induce histone perturbation and has been reported to interfere with gene expression in cultures of glioma cells (WHO, 1990). Impaired growth and development was noted in cultured mouse embryonic tissue treated in vitro with methylmercuric chloride, but there was no increase in SCE (Matsumoto and Spindle, 1982). Costa et al. (1991) showed that methylmercuric chloride caused DNA strand breaks in both V79 and rat glioblastoma cells treated in vitro. Methylmercuric chloride produced more strand breaks than did mercuric chloride.

Evidence of DNA damage has been observed in the *Bacillus subtilis* rec-assay (Kanematsu et al., 1980). These authors reported negative results for methylmercury in spot tests for mutagenicity in the following bacterial strains: *E. coli* B/r WP2 and WP2; and *Salmonella typhimurium* strains TA1535, TA1537, TA1538, TA98, and TA100. Jenssen and Ramel (1980) indicated in a review article that methylmercury acetate was negative in both micronucleus assays and mutagenicity tests in Salmonella; the article referred to Heddle and Bruce (1977) and provided no experimental details. Weak mutagenic responses for methylmercuric chloride and methoxyethyl mercury chloride were observed in Chinese hamster V79 cells at doses near the cytotoxic threshold (Fiskesjo, 1979), and methylmercury produced a slight increase in the frequency of chromosomal nondysjunction in *Saccharomyces cerevisiae* (Nakai and Machida, 1973). Methylmercury, however, caused neither gene mutations nor recombination in *S. cerevisiae* (Nakai and Machida, 1973). Methylmercury retarded DNA synthesis and produced single-strand breaks in DNA in L5178Y cells (Nakazawa et al., 1975).

3.7 CARCINOGENICITY

3.7.1 Human Studies

At this time, no human studies have reported an association between methylmercury exposure and overall cancer rates. Three studies were identified that examined the relationship between methylmercury exposure and cancer. No persuasive evidence of increased carcinogenicity attributable to methylmercury exposure was observed in any of the studies. Interpretation of these studies, however, was limited by poor study design and incomplete descriptions of methodology and/or results.

3.7.2 Animal Studies

The results from three dietary studies in two strains of mice indicate that methylmercury is carcinogenic. Interpretation of two of the positive studies was complicated by observation of tumors only at doses that exceeded the Maximum Tolerated Dose (MTD). Therefore, only one positive animal study is appropriate for consideration. A fourth dietary study in mice, three dietary studies in rats, and a dietary study in cats failed to show carcinogenicity of methylmercury. Interpretation of four nonpositive studies was limited because of deficiencies in study design or failure to achieve an MTD.

Methylmercuric chloride was administered in the diet at levels of 0, 0.4, 2, or 10 ppm (0, 0.03, 0.14, and 0.69 mg Hg/kg-day in males and 0, 0.03, 0.13, and 0.60 mg Hg/kg-day in females) to B6C3F1 mice (60/sex/group) for 104 weeks (Mitsumori et al., 1990). In high-dose males, a marked increase in mortality was observed after 60 weeks (data were presented graphically; statistical analyses not performed). Survival at study termination was approximately 50%, 60%, 60%, and 20% in control, low-, mid-, and high-dose males, respectively, and 58%, 68%, 60%, and 60% in control, low-, mid-, and high-dose females, respectively. The cause of the high mortality was not reported. At study termination, the mean body weight in high-dose males was approximately 67% of controls and in high-dose females was approximately 90% of controls (data presented graphically; statistical analyses not performed). Focal hyperplasia of the renal tubules was significantly ($p<0.01$) increased in high-dose males (14/60; the incidence was 0/60 in all other groups). The incidence of renal epithelial carcinomas (classified as solid or cystic papillary type) was significantly ($p<0.01$) increased in high-dose males (13/60; the incidence was 0/60 in all other groups). The incidence of renal adenomas (classified as solid or tubular type) was also significantly ($p<0.05$) increased in high-dose males; the incidence was 0/60, 0/60, 1/60, and 5/60 in control, low-, mid-, and high-dose males, respectively, and 0/60, 0/60, 0/60, and 1/60 in control, low-, mid-, and high-dose females, respectively. No metastases were seen in the animals. The incidences of a variety of nonneoplastic lesions were increased in the high-dose rats including these: sensory neuropathy, neuronal necrosis in the cerebrum, neuronal degeneration in the cerebellum, and chronic nephropathy of the kidney. Males exhibited tubular atrophy of the testis (1/60, 5/60, 2/60, and 54/60 in control, low-, mid-, and high-dose, respectively) and ulceration of the glandular stomach (1/60, 1/60, 0/60, and 7/60 in control, low-, mid-, and high-dose males, respectively). An MTD was achieved in middose males and high-dose females. High mortality in high-dose males indicated that the MTD was exceeded in this group.

Mitsumori et al. (1981) administered 0, 15, or 30 ppm of methylmercuric chloride (99.3% pure) in the diet (0, 1.6 and 3.1 mg Hg/kg-day) to ICR mice (60/sex/group) for 78 weeks. Interim sacrifices of up to 6/sex/group were conducted at weeks 26 and 52. Kidneys were microscopically examined from all animals that died or became moribund after week 53 or were killed at study termination. Lungs from mice with renal masses and renal lymph nodes showing gross abnormalities were also examined. Survival was decreased in a dose-related manner; at week 78 survival was 24/60, 6/60, and 0/60 in control, low-, and high-dose males, respectively, and 33/60, 18/60, and 0/60, in control, low-, and high-dose females, respectively (statistical analyses not performed). The majority of high-dose mice (51/60 males and 59/60 females) died by week 26 of the study. Examination of the kidneys of mice that died or were sacrificed after 53 weeks showed a significant ($p<0.001$) increase in renal tumors in low-dose males (13/16 versus 1/37 in controls). The incidence of renal epithelial adenocarcinomas in control and low-dose males was 0/37 and 11/16, respectively ($p<0.001$). The incidence of renal epithelial adenomas in control and low-dose males was 1/37 and 5/16, respectively ($p<0.01$). No renal tumors were observed in females in any group. No metastases to the lung or renal lymph nodes were observed. Evidence of neurotoxicity and renal pathology was observed in the treated mice at both dose levels. The high mortality in both groups of treated males and in high-dose females indicated that the MTD was exceeded in these groups.

A followup study to the Mitsumori et al. (1981) study was reported by Hirano et al. (1986). Methylmercuric chloride was administered in the diet to ICR mice (60/sex/group) at levels of 0, 0.4, 2, or 10 ppm (0, 0.03, 0.15, and 0.73 mg Hg/kg-day in males and 0, 0.02, 0.11, and 0.6 mg Hg/kg-day in females) for 104 weeks. Interim sacrifices (6/sex/group) were conducted at 26, 52, and 78 weeks. Complete histopathological examinations were performed on all animals found dead, killed *in extremis*, or killed by design. Mortality, group mean body weights and food consumption were comparable to controls. The first renal tumor was observed at 58 weeks in a high-dose male, and the incidence of renal epithelial tumors (adenomas or adenocarcinomas) was significantly increased in high-dose males (1/32, 0/25, 0/29, and 13/26 in the control, low-, mid-, and high-dose groups, respectively). Ten of the 13 tumors in high-dose males were adenocarcinomas. These tumors were described as solid type or cystic papillary types of adenocarcinomas. No invading proliferation into the surrounding tissues was seen. The incidence of renal epithelial adenomas was not significantly increased in males, and no renal adenomas or adenocarcinomas were observed in any females. Focal hyperplasia of the tubular epithelium was reported to be increased in high-dose males (13/59; other incidences not reported). Increases in nonneoplastic lesions in high-dose animals provided evidence that an MTD was exceeded. Nonneoplastic lesions reported as increased in treated males included the following: epithelial

degeneration of the renal proximal tubules; cystic kidney; urinary cast and pelvic dilatation; and decreased spermatogenesis. Epithelial degeneration of the renal proximal tubules and degeneration or fibrosis of the sciatic nerve were reported in high-dose females.

No increase in tumor incidence was observed in a study using white Swiss mice (Schroeder and Mitchener 1975). Groups of mice (54/sex/group) were exposed from weaning until death to methylmercuric acetate in the drinking water at two doses. The low-dose group received 1 ppm methylmercuric acetate (0.19 mg Hg/kg-day). The high-dose group received 5 ppm methylmercuric acetate (0.95 mg Hg/kg-day) for the first 70 days and then 1 ppm, thereafter, due to high mortality (21/54 males and 23/54 females died prior to the dose reduction). Survival among the remaining mice was not significantly different from controls. Significant ($p<0.001$) reductions in body weight were reported in high-dose males (9–15% lower than controls) and high-dose females (15–22% lower than controls) between 2 and 6 months of age. Mice were weighed, dissected, gross tumors were detected, and some sections were made of heart, lung, liver, kidney, and spleen for microscopic examination. No increase in tumor incidence was observed. This study is limited because complete histological examinations were not performed, and pathology data other than tumor incidence were not reported.

Mitsumori et al. (1983, 1984) conducted a study in Sprague-Dawley rats. They administered diets containing 0, 0.4, 2, or 10 ppm of methylmercuric chloride (0, 0.011, 0.05, and 0.28 mg Hg/kg-day in males; 0, 0.014, 0.064, and 0.34 mg Hg/kg-day in females) to Sprague-Dawley rats (56 animals/sex/group) for up to 130 weeks. Interim sacrifices of 10/group (either sex) were conducted at weeks 13 and 26 and of 6/group (either sex) at weeks 52 and 78. Mortality was increased in high-dose males and females. At week 104, survival was approximately 55%, 45%, 75%, and 10% in control, low-, mid-, and high-dose males, respectively, and 70%, 75%, 75%, and 30% in control, low-, mid-, and high-dose females, respectively (data presented graphically). Body weight gain was decreased in high-dose animals (approximately 20–30%; data presented graphically). No increase in tumor incidence was observed in either males or females. Noncarcinogenic lesions that were significantly increased ($p< 0.05$) in high-dose rats included the following: degeneration in peripheral nerves and the spinal cord (both sexes); degeneration of the proximal tubular epithelium of the kidney (both sexes); severe chronic nephropathy (females); parathyroid hyperplasia (both sexes); polyarteritis nodosa and calcification of the abdominal arterial wall (females); bone fibrosis (females); bile duct hyperplasia (males); and hemosiderosis and extramedullary hematopoiesis in the spleen (males). In addition, mid-dose males exhibited significantly increased degeneration of the kidney proximal tubular epithelium and hyperplasia

of the parathyroid. An MTD was achieved in mid-dose males and in high-dose females; the MTD was exceeded in high-dose males.

No increase in tumor incidence or decrease in tumor latency was observed in another study using rats (strain not specified) (Verschuuren et al., 1976). Groups of 25 female and 25 male rats were administered methylmercuric chloride at dietary levels of 0, 0.1, 0.5, and 2.5 ppm (0, 0.004, 0.02, and 0.1 mg Hg/kg-day) for 2 years. No significant effects were observed on growth or food intake except for a 6% decrease (statistically significant) in body weight gain at 60 weeks in high-dose females. Survival was 72%, 68%, 48%, and 48% in control, low-, mid- and high-dose males, respectively; and 76%, 60%, 64%, and 56% in control, low-, mid- and high-dose females, respectively (statistical significance not reported). Increases in relative kidney weights were observed in both males and females at the highest dose. No effects on the nature or incidence of pathological lesions were observed, and tumors were reported to have been observed with comparable incidence and latency among all of the groups. This study was limited by the small sample size and failure to achieve an MTD.

No tumor data were reported in a study using Wistar rats (Munro, 1980). Groups of 50 Wistar rats/sex/dose were fed diets containing methylmercury; doses of 2, 10, 50, and 250 micrograms Hg/kg-day were fed for 26 months. High-dose female rats exhibited reduced body weight gains and showed minimal clinical signs of neurotoxicity; however, high-dose male rats showed overt clinical signs of neurotoxicity, decreased hemoglobin and hematocrit values, reduced weight gains and significantly increased mortality. Histopathologic examination of the high-dose rats of both sexes revealed demyelination of dorsal nerve roots and peripheral nerves. Males showed severe dose-related kidney damage, and females had minimal renal damage.

No increase in tumor incidence was observed in a multiple generation reproduction study using Sprague-Dawley rats (Newberne et al., 1972). Groups of rats (30/sex) were given semisynthetic diets supplemented with either casein or a fish protein concentrate to yield dietary levels of 0.2 ppm methylmercury (0.008 mg Hg/kg-day). Another group of controls received untreated rat chow. Rats that received diets containing methylmercury during the 2-year study had body weights and hematology comparable to controls. Detailed histopathologic analyses revealed no lesions of the brain, liver, or kidney that were attributable to the methylmercury exposure. Mortality data were not presented. Interpretation of these data is limited by the somewhat small group sizes and failure to achieve an MTD.

No increase in tumor incidence was observed in a study using random-bred domestic cats (Charbonneau et al., 1976). Groups of cats (4–5/sex/group) were given doses of 0.0084, 0.020, 0.046, 0.074 or 0.176 mg Hg/kg-day either as methylmercury-contaminated seafood or as methylmercuric chloride in the diet for up to 2 years. Controls were estimated to have received 0.003 mg Hg/kg-day. Food consumption and body weight were not affected by treatment with methylmercury. Due to advanced signs of neurotoxicity (loss of balance, ataxia, impaired gait, impaired reflexes, weakness, impaired sensory function, mood change and tremor), cats at the highest dose tested were sacrificed after approximately 16 weeks, and cats at the next highest dose were sacrificed after approximately 54–57 weeks. Cats at the next highest dose generally exhibited mild neurological impairment (altered hopping reaction and hypalgesia). One cat at this dose was sacrificed after 38 weeks because of neurotoxicity, and one cat died of acute renal failure after 68 weeks. Cats at the two highest doses had pathological changes in the brain and spinal cord, but no histopathological changes were noted in other tissues examined. Interpretation of the results of this study is limited because of the small group sizes, early sacrifice of cats at the two highest dose levels and no available data regarding pathological changes in cats at the three lowest dose levels. This study was also limited by its short duration when compared to the lifespan of a cat.

Blakley (1984) administered methylmercuric chloride to female Swiss mice (number/group not specified) in drinking water at concentrations of 0, 0.2, 0.5 or 2.0 mg/L for 15 weeks. This corresponded to approximately 0, 0.03, 0.07 and 0.27 mg Hg/kg-day. At the end of week 3, a single dose of 1.5 mg/kg of urethane was administered intraperitoneally to 16–20 mice/group. No effects on weight gain or food consumption were observed. Lung tumor incidence in mice not administered urethane (number/group not specified) was less than 1 tumor/mouse in all groups. Statistically significant trends for increases in the number and size of lung adenomas/mouse with increasing methylmercury dose were observed; the tumor number/mouse was 21.5, 19.4, 19.4, and 33.1 in control, low-, mid- and high-dose mice, respectively, and the tumor size/mouse was 0.70, 0.73, 0.76 and 0.76 mm in control, low-, mid- and high-dose mice, respectively. The study authors suggest that the increase in tumor number and size may have been related to immunosuppressive activity of methylmercury. It should be noted that this is considered a short-term assay and that only pulmonary adenomas were evaluated.

4.0 RISK ASSESSMENT FOR METHYLMERCURY

4.1 BACKGROUND

Methylmercury is highly toxic to mammalian species and causes a variety of adverse effects. It is a developmental toxicant in humans and animals. It causes chromosomal effects but does not induce point mutations. The *Mercury Study Report to Congress* (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. There is no two-generation study of reproductive effects, but shorter term studies in rodents, guinea pigs, and monkeys have reported observations consistent with reproductive deficits. There are no data to indicate that methylmercury is carcinogenic in humans, and it induces tumors in animals only at highly toxic doses. Application of the revised Guidelines for Cancer Risk Assessment leads to a judgment that methylmercury is not likely to be carcinogenic for humans under conditions of exposure generally encountered in the environment.

The quantitative health risk assessment for a noncarcinogen is the reference dose (RfD). This is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious health effects during a lifetime.

EPA has published two 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 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 criteria 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.

For this document the following definitions apply. These reflect usage in the National Research Council publication *Toxicological Effects of Methylmercury* (NRC, 2000) (see Section 1.5).

NOAEL No-observed-adverse-effect level. An exposure level at which there are no statistically or biologically significant increases in the frequency or severity of adverse effects in a comparison between an exposed population and a control group. Effects may be seen at this level of exposure, but they are not considered to be adverse. For risk assessment the NOAEL is generally the highest level at which no adverse effects are seen.

LOAEL Lowest-observed-adverse-effect level. The lowest exposure level at which there are statistically or biologically significant increases in frequency or severity of adverse effects in a comparison between an exposed population and a control group.

BMD Benchmark dose. In common parlance this term refers to a quantitative assessment for noncancer health effects that uses a curve-fitting procedure to determine a level functionally equivalent to a NOAEL. In this chapter, BMD will be used to mean an estimated dose that corresponds to a specified risk above the background risk.

BMDL Benchmark dose lower limit, a statistical lower limit on a calculated BMD. In this document that will be the 95% lower confidence limit. The BMDL will be used as the starting point for the calculation of the methylmercury RfD.

4.1.1 Other RfDs Published by EPA

Two RfDs based on human studies have been published as consensus values for EPA. In addition, the MSRC (EPA, 1997) describes an RfD that could be estimated from animal data.

4.1.1.1 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 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 NOAEL. An additional uncertainty factor (UF) 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.

4.1.1.2 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, 1997e).

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 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 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 μg mercury/L blood using a 250:1 ratio as described in the MSRC (U.S. EPA, 1997e, pp. 6-22 to 6-23):

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

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 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:

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

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

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

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, 1997e) 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, 1997e). 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, 1997g).

4.1.1.3 Reference Values Derived From Animal Data

There are issues inherent to epidemiological studies, including the possibility of coexposure to other potential toxicants, that are not of concern in controlled experimental animal studies. It is therefore informative to compare RfDs that may be derived from animal studies to those derived from the epidemiological literature. RfDs derived from monkey studies are particularly relevant, as the neurotoxic effects produced by developmental methylmercury exposure in monkeys are similar to those identified in humans (Burbacher et al., 1990a; Gilbert and Grant-Webster, 1995). The studies at the University of Washington were of a relatively large cohort of macaque monkeys whose mothers were exposed throughout pregnancy to 50 µg/kg/day of methylmercury. The studies revealed deficits on cognitive tests during infancy, which may represent retarded development (Burbacher et al., 1986; Gunderson et al., 1986, 1988). These methylmercury-exposed monkeys also displayed aberrant play and social behavior (Burbacher et al., 1990b). Studies at the Canadian Health Protection Branch in the same species of monkey, dosed with 50 µg/kg/day from birth to 7 years of age, revealed visual, auditory, and somatosensory deficits, including evidence of delayed neurotoxicity identified in middle age (Rice and Gilbert, 1995, 1992, 1982; Rice, 1989a). Research in a cohort of monkeys dosed beginning *in utero* and continuing until 4 years of age revealed similar sensory system impairment (Rice, 1998; Rice and Gilbert, 1995, 1990). Three individuals dosed at 10 or 25 µg/kg/day all exhibited impaired function in at least

one sensory system in addition to evidence of delayed neurotoxicity (Rice, 1998). In none of these studies was a NOAEL identified.

Calculation of an RfD from these data according to the method typically used by the EPA would include application of a number of UFs, including dividing the LOAEL by a factor of 10 (because no NOAEL was identified), division by 10 again for extrapolation from animal to human data, and division by another factor of 10 in consideration of individual variation in sensitivity. Monkeys and humans have approximately the same brain:blood mercury ratio following chronic exposure (Burbacher et al., 1990a), although the ratio in humans may be slightly higher than in monkeys (Rice, 1989b). However, the half-life of mercury in the blood of monkeys is about 15 days (Rice, 1989c), whereas clearance times for humans averaged 45-70 days in several studies, with some individuals having even longer clearance times (see Section 4.2.3). The shorter clearance time in monkeys would result in an UF of at least 5 based on pharmacokinetic considerations alone; therefore an overall factor of 10 appears appropriate for interspecies extrapolation. This calculation would yield an RfD of 0.05 µg/kg/day from the *in utero* and postnatal exposure studies, and an RfD as low as 0.01 µg/kg/day based on combined *in utero* and postnatal exposure (Rice, 1996). Gilbert and Grant-Webster (1995) suggested an RfD of 0.025 µg/kg/day based on the same data.

4.1.2 Risk Assessments Done by Other Groups

Quantitative estimates of hazards of oral exposure to methylmercury 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.

4.1.2.1 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 converted from a mercury standard to one based on methylmercury (Federal Register *49*; November 19, 1984).

The action level takes into consideration the tolerable daily intake (TDI) for methylmercury as well as information on seafood consumption and associated exposure to methylmercury. The TDI is the amount of methylmercury that can be consumed daily over a long period of time with a reasonable certainty of no harm. 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 methylmercury. These amounts are equivalent to 5 and 3.3 μg, respectively, per kilogram of body weight. Using the values of methylmercury, this tolerable level would correspond to approximately 230 μg/week for a 70-kg person, or 33 μg/person/day (0.47 μg/kg bw/day). The TDI was calculated from data developed in part by Swedish studies of Japanese individuals poisoned in the Niigata episode, 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 later poisoning event in Iraq, FDA has acknowledged that the fetus may be more sensitive than adults to the effects of mercury (Federal Register *44*, 3990, January 19, 1979; U.S. FDA Consumer, September 1994). In recognition of these concerns, FDA has provided advice to pregnant women and women of childbearing age to limit their consumption of fish known to have high levels of mercury (U.S. FDA Consumer, 1994). 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, because of the uncertainties associated with the Iraqi study, FDA has chosen not to use the Iraqi study as a basis for revising its action level. Instead, FDA has chosen to wait for findings of prospective studies of fish-eating populations in the Seychelles Islands.

4.1.2.2 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 μg 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.

4.1.2.3 ATSDR

In 1993, ATSDR first published a Minimal Risk Level (MRL) for methylmercury. An MRL is derived in a manner similar to the RfD; it is defined as an estimate of the daily human exposure to a hazardous substance that is likely to be without appreciable risk of adverse noncancer health effects over a specified duration of exposure. In 1999 ATSDR published a revised methylmercury MRL using the Seychelles Islands study (SCDS) (Davidson et al., 1998) as the starting point (ATSDR, 1999). In this study (described in detail in Section 3.2.2.5 and summarized in Section 4.2.13), the investigators examined the correlation between subtle neurological effects and low-dose chronic exposure to methylmercury. No correlation between maternal hair mercury concentrations and neurological effects was seen in the SCDS 66-month-old children. ATSDR determined a minimal risk level of 0.3 μg/kg per day, based on a dose of 1.3 μg/kg per day, which reflects the average concentration of the upper quintile of the exposed population but does not necessarily correspond to a NOAEL. ATSDR used a UF of 1.5 to account for pharmacokinetic variability within the human population; they made their choice based on the analyses of Clewell et al. (1998). An additional factor of 1.5 was applied to account for any other individual variability (e.g., pharmacodynamics) as well as a modifying factor of 1.5 to account for the possibility that domain-specific tests used in the Faroe Islands study might have allowed detection of subtle neurological effects that were not evaluated in the Seychelles cohort. Although the conventional risk assessment approach is to multiply UFs, ATSDR summed these factors to develop an overall safety factor of 4.5.

4.1.3 SAB Review of the Mercury Study Report to Congress

The Science Advisory Board (SAB) is a public advisory group providing extramural scientific information and advice to the Administrator and other officials of the EPA. The SAB is structured to provide balanced, expert assessment of scientific matters relating to problems facing the Agency. The SAB reviewed a draft of the eight-volume MSRC (U.S. EPA, 1997a-h) in the context of a public meeting held February 13 and 14, 1997. A panel of 33 scientists reviewed the entire MSRC. A subgroup focused on the health effects data, and in particular EPA's use of those data to derive the methylmercury RfD of 0.1 μg/kg/day, based on effects observed in Iraqi children exposed *in utero*.

The SAB report was published in October 1997 (EPA-SAB-EC-98-001). It made the following statement:

> In general, from the standpoint of looking at human health effects and the uncertainties, the draft report [MSRC] is a very good document and an important step forward in terms of bringing the relevant information together into one place for the first time. The current RfD, based on the Iraqi and New Zealand data, should be retained at least until the on-going Faeroe and Seychelles Islands studies have progressed much further and been subjected to the same scrutiny as has the Iraqi data.

The SAB report continued:

> Investigators conducting two new major prospective longitudinal studies—one in the Seychelles Islands, the other in the Faeroe Islands—have recently begun to publish findings in the literature and are expected to continue releasing their findings during the next 2-3 years. These studies have advantages over those cited in the previous paragraph in that they have much larger sample sizes, a larger number of developmental endpoints, potentially more sensitive developmental endpoints, and control a more extensive set of potential confounding influences. On the other hand, the studies have some limitations in terms of low exposures (to PCBs in the Faeroes) and ethnically homogenous societies. Since only a small portion of these new data sets have been published to date and because questions have been raised about the sensitivity and appropriateness of the several statistical procedures used in the analyses, the Subcommittee concluded that it would be premature to include any data from these studies in this report until they are subjected to appropriate peer review. **Because these data are so much more comprehensive and relevant to contemporary regulatory issues than the data heretofore available, once there has been adequate opportunity for peer review and debate within the scientific community, the RfD may need to be reassessed in terms of the most sensitive endpoints from these new studies.** [Emphasis theirs]

4.1.4 Interagency Consensus Process

Among the many reviews of the MSRC was one by scientists and policy-makers from interested Federal agencies, sponsored by the Committee on Environment and Natural Resources (CENR), Office of Science and Technology (OSTP). This review highlighted many divergent points of view as to the appropriate basis for quantitative assessment of the low-dose effects of methylmercury exposure. It was decided that an interagency process with external involvement would be undertaken to review new methylmercury data and evaluate new and existing data. EPA committed to participate in this process and, at its conclusion, to assess its 1995 RfD for methylmercury to determine if a change was warranted. Subsequently a workshop was organized by an interagency committee at the request of OSTP. The organizing committee was chaired by the National Institute of Environmental Health Sciences (NIEHS) and included representatives from several agencies:

Department of Health and Human Services (DHHS)

Office of the Assistant Secretary for Planning and Evaluation

Centers for Disease Control and Prevention (CDC)

Agency for Toxic Substances Disease Registry (ATSDR)

Food and Drug Administration (FDA)

Environmental Protection Agency (EPA)

National Oceanic and Atmospheric Administration (NOAA)
Office of Science and Technology Policy (OSTP)
Office of Management and Budget (OMB)

The Methylmercury Workshop was a response to the suggestion that the emerging Seychellois and Faroese data undergo a level of scrutiny beyond journal peer review if they were to be used in policy setting.

The Workshop on the Scientific Issues Relevant to Assessment of Health Effects from Exposure to Methylmercury was held in Raleigh, North Carolina, November 18–20, 1998. The purpose of the workshop was to discuss and evaluate the major epidemiologic studies associating methylmercury exposure with an array of developmental measures in children. The workshop did not attempt to derive a risk assessment, but it was assumed by participants that the workshop evaluation would facilitate agreement on risk assessment issues. The major studies considered were those that have examined populations in Iraq, the Seychelles, the Faroe Islands, and the Amazon, along with the most relevant animal studies. Study authors made detailed presentations to respond to a series of questions on study exposures, potential confounders, measurements of effect, and other related topics. Five expert panels discussed the presentations and published data; panels covered the following areas: exposure, neurobehavioral endpoints, confounders and variables, design and statistics, and experimental (animal and *in vitro*) data. The results of their deliberations were published in the Spring of 1999 (NIEHS, 1999). Conclusions of the report were reviewed by workshop panelists and by Federal scientists who had attended the workshop. The conclusions are quoted below.

1. Methylmercury is a developmental neurotoxin, but effects at low doses encountered by eating fish are difficult to evaluate.

2. All the studies reviewed were considered of high scientific quality, and the panel recognized that each of the investigations had overcome significant obstacles to produce important scientific information. The panel also stated that continued funding of the studies in the Seychelles, Faroes, and Amazon is necessary for the full potential of those studies to be realized. This is particularly the case for the Faroes and Seychelles studies, which have assessed and are currently assessing the potential developmental neurotoxic effects of methylmercury in fish-eating populations. The developmental studies would benefit by evaluation of common endpoints using similar analytical methods. It is important to note that the Amazon study did not assess developmental endpoints but assessed effects in adults.

3. Results from the Faroes and Seychelles studies are credible and provide valuable insights into the potential health effects of methylmercury.

4. Some differences are clearly present in results from the Faroes, Seychelles, and Amazon, but the panel was not able to clearly identify the sources of these differences. Among possible sources are the different effects of episodic versus continuous exposure, ethnic differences in methylmercury responses, lack of common

endpoints in the Faroes and Seychelles studies, and several other confounders or modifying factors such as those found in diet and lifestyle, as well as in chemicals present in seafood, which is the source of methylmercury to these populations. The other chemical constituents of seafood that may be explanatory include those that may be beneficial to fetal neurodevelopment (i.e., omega-3 fatty acids) and those that may be harmful to fetal neurodevelopment (e.g., PCBs).

5. These studies have provided valuable new information on the potential health effects of methylmercury, but significant uncertainties remain because of issues related to exposure, neurobehavioral endpoints, confounders and statistics, and design.

The interagency organizing committee agreed unanimously that the deliberations of the panels and the workshop report will be a key factor in subsequent public health policy actions taken by each of the participating agencies.

4.1.5 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 (NRC, 2000). Conclusions of that report are summarized below.

The report concludes that methylmercury is a highly toxic substance; a number of adverse health effects associated with methylmercury exposure have been 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. (NRC, 2000 p. 9)

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 (NRC, 2000 p. 11).

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. 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 recommended use of data from the Faroe Islands study for derivation of the RfD (NRC, 2000 p. 11).

NRC recommended 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 ($K \geq 1$) 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, 2000, pp. 11-12).

NRC recommended use of the Boston Naming Test (BNT) 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 BNT 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 reviewed several sources of variability and uncertainty and recommended that an uncertainty factor of at least 10 be used. NRC recommended a factor of 2 to 3 for biological variability in dose estimation. They also recommended 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 (NRC, 2000, p. 327).

4.1.6 External Peer Review of Draft RfD

A draft EPA RfD document was submitted for external scientific peer review in late October 2000; the reviewers are listed at the front of this document. At the same time the draft RfD document was circulated for comment to other Federal Agencies through CENR and OSTP. A public scientific review meeting was held November 15, 2000; the final peer review report was delivered to EPA on December 7, 2000, and is available in the docket. The external peer reviewers supported the use of the Faroes data, derivation of a BMD as described by NRC, and application of a tenfold uncertainty factor to the BMDL. They agreed with EPA's use of a one-compartment model for dose conversion as well as with most of the parameter estimates; they commented correlation among some of the parameters. The peer reviewers disagreed with NRC's recommendation to set the RfD on the BNT results from the full Faroese cohort. They felt that the BNT scores showed an effect of concomitant PCB exposure in some analyses. They preferred a PCB-adjusted BMDL of 71 ppb mercury in cord blood for the BNT. They also offered suggested alternatives to use of the BNT test reslts. The peer reviewers validated a final RfD of 0.1 µg/kg bw /day.

4.1.7 Revised RfD

The development of this RfD considered the NRC recommendations and followed them for the most part. Most recommendations of the peer-review panel were incorporated as well. The following sections provide rationales for choices made by EPA in determining the basis for the RfD.

4.2 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 (summarized in NRC, 2000, pp. 190-191). 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.

4.2.1 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 4.2.1.1 describes the evidence for effects of methylmercury on neurological status; Section 4.2.1.2 describes the effects on attainment of

developmental milestones during infancy; Section 4.2.1.3 describes other effects during infancy and early childhood; Section 4.2.1.4 presents evidence for cognitive deficits during childhood (school age); and Section 4.2.1.5 describes sensory and other effects of methylmercury.

For more detailed study descriptions refer to Section 3 of this document or to the MSRC.

4.2.1.1 Status on Neurological Examination

Cree Population—McKeown-Eyssen et al. (1983)

McKeown-Eyssen et al. (1983) studied a population of 234 12- to 30-month-old Cree Indian 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 neurological findings were different from those described in previous reports of neurological abnormalities after prenatal exposure to higher levels of methylmercury.

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 methylmercury 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 were 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.

SCDS Pilot Study—Myers et al. (1995b)

In the cross-sectional or pilot study of the SCDS (Myers et al., 1995b), 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 hair mercury at 0 to 3 ppm to 11.7% in the group with Hair mercury 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.

SCDS Main Study—Myers et al. (1995c)

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.

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 mercury concentration than children with automatic performance (465 children) (23.9 versus 21.8 μg/L, $p = 0.04$) (Grandjean et al., 1997).

Faroes Population—Steurwald et al. (2000)

A cohort of 182 singleton, full-term infants born in the Faroe Islands between 1994 and 1995 was recruited. The cohort represented 64% of all births in the study area. Data were collected on maternal hair mercury, cord whole-blood mercury, and cord serum mercury. A total of 15 maternal hair measurements exceeded 10 ppm. Measurements were also taken of 18 pesticides or metabolites and 28 polychlorinated biphenyl (PCB) congeners in maternal serum. At 2 weeks of age infants were given a neurological examination designed to assess functional abilities, reflexes and responses, and stability of behavioral status during examination. Responses were categorized as optimal, questionable, or suboptimal. The neurological optimality score (NOS) was the number of items rated as optimal out of a total of 60. Two subscores were generated (muscle tone and reflexes) and a variety of thyroid-function indices were also assessed. 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.

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. Neurological 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.

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.

4.2.1.2 Age at Achievement of Developmental Milestones

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 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.

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.

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. Breastfeeding 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 breastfeeding.

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 breastfed 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.

4.2.1.3 Infant and Preschool Development

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.

New Zealand population—Kjellstrom et al. (1986)

Kjellstrom et al. (1986) studied a cohort of New Zealand children for whom prenatal methylmercury 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-mercury 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.

SCDS pilot study—Myers et al. (1995b)

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).

SCDS main study—Myers et al. (1995c)

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. The researchers 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.

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 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.

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 (NRC 2000, p. 200).

The NRC panel noted that the BSID is currently considered to be the best available instrument for infant assessment and is useful for measurement of prenatal exposures to neurotoxicants (NRC 2000, p. 200). 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.

4.2.1.4 Childhood Development

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 [WISC-R]), 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); 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

breastfeeding. 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. 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.

SCDS pilot study— Myers et al. (1995a), Davidson et al. (2000), Davidson et al. (1998), Myers et al. (2000).

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 (Myers et al., 1995a). 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.

Further evaluation was performed on a portion of the Seychelles pilot cohort at 108 months of age (Davidson et al., 2000). Eighty-seven children were tested on five subtests of the WISC-III (Information, Block Design, Vocabulary, Digit Span, and Coding), CVLT, BNT, Beery-Buktenica Development Test of Visual Motor Integration (VMI) (copying geometric figures), Finger Tapping, grooved pegboard, Trailmaking (tracing the correct route through a form with a pencil), and the design memory subtest of the Wide Range Assessment of Memory and Learning (WRAML) (drawing each of four geometric designs from memory). Performance on BNT, VMI, and grooved pegboard showed a positive association related to mercury exposure in males, whereas there were trends toward poorer performance related to mercury exposure for grooved pegboard in females ($p = 0.07$). Given the small number of subjects, the power of the study was probably quite low; these largely negative results should be interpreted with caution.

No effect of mercury was identified on the Child Behavior Check List (CBCL) at 66 months of age in the main cohort of Seychelles study as determined by the total T score (Davidson et al., 1998). The CBCL is a report inventory scored by the caregiver that assesses eight domains: withdrawn, somatic complaints, anxious/depressed, social problems, thought problems, attention problems, delinquent behavior, and aggressive behavior. An analysis of these subscales was performed on the 711 children assessed on this test (Myers et al., 2000). No effect of mercury was identified on individual subscales.

SCDS Main Study—Davidson et al. (1998), Axtell et al. (2000), Palumbo et al. (2000)

As part of the main SCDS, 711 children 66 months of age (from the original cohort of 779) were evaluated with 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 exposure. The following were assessed: general cognitive ability (McCarthy Scales of Children's Abilities), expressive and receptive

language (Preschool Language Scale, PLS), 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 (CBCL). 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 mercury concentrations were associated with better scores. Among boys, increased postnatal mercury concentrations were associated with fewer errors on the Bender Gestalt Test.

The investigators published additional analyses of the 66-month data evaluating the possibility of non-linear relationships associated with mercury exposure (Axtell et al., 2000). Endpoints included the six primary variables analyzed previously: McCarthy GCI, PLS, Woodcock-Johnson (WJ) applied problems, WJ letter/word recognition, Bender copying errors, and CBCL total T score. Generalized additive models, which make no assumptions about the relationship between exposure and test score, were used. Nonlinearities were identified between prenatal exposure and PLS and CBCL, and between postnatal exposure and McCarthy GCI. For the PLS the trend involved a decrement of 0.8 points (poorer performance) from 0 to 10 ppm and an increase of 1.3 points above 10 ppm. For the CBCL there was an increase (representing a poorer score) between 0 and 15 ppm and a decrease above 10 ppm. The GCI increased (improved) by 1.8 points through 10 ppm in the child's hair and declined by 3.1 above 10 ppm. Although these results are difficult to interpret, they provide limited evidence of an adverse effect of mercury exposure below 10 ppm maternal hair on two measures, and are associated with child's hair mercury concentration above 10 ppm on the GCI. As pointed out by the authors, there are fewer data points above 10 ppm (this is especially true for child's hair mercury), and therefore trends above this level are estimated less precisely.

The SCDS investigators used multiple linear regression to assess the results of the McCarthy GCI administered at 66 months (Palumbo et al., 2000). They analyzed the standard MSCA subscales and also constructed specific subscales to approximate the domains of cognitive functioning assessed in the Faroe Islands study: attention, executive function, expressive language, receptive language, nonverbal memory, visuospatial, and gross motor visuomotor development. They found a positive association between the child's hair mercury at 66 months and the standard memory subscale, with no other associations identified. As with all the previous analyses of these variables, the raw scores were converted to "normative" scores. As pointed out by the OSTP panel (NIEHS, 1999, Section 3.5 of the Confounders

and Variables Section), the applicability of U.S. norms to this population is unclear, and the use of standardized scores may decrease sensitivity by collapsing different raw scores to one standard score.

Faroes 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; CVLT; the BNT; 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 (CPT) (in the first year of data collection), WISC-R digit span, BNT, and CVLT. 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 CPT 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 mercury 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 blood 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 CVLT, all three Finger Tapping conditions, CPT reaction time, and average hand-eye coordination score. For all scores, adverse mercury effects were noted for boys but not girls.

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. It was noted that hair mercury concentrations and village residence were so highly confounded, however, that adjustment for village might be inappropriate.

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 with poorer block-design scores in girls.

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 (cross-sectional) 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 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 (NRC 2000, pp. 203, 205).

In the Faroes population, mercury exposure measured in cord blood was associated with deficits on several measures: Finger Tapping, preferred hand; CPT (first year of data collection, two scores); mean reaction time, WISC-R digit span; BNT (with and without cues); and CVLT (short-term and long-term reproduction). The 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 analysis, 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.

4.2.1.5 Sensory, Neurophysiological, and Other Endpoints in Children

Faroes 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.

Madeira population—Murata et al. (1999b)

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.

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.

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.

4.2.2 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.

4.2.2.1 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 (NRC, 2000 p. 251) 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 was peer reviewed. 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. 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 (NRC, 2000, p. 209) 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. The section below discussed controls for possible confounders in the SCDS and Faroes studies. An important variable is the concomitant exposure to organochlorine compounds such as PCBs and pesticides that could have neurotoxic effects. There is essentially no information on the extent of such exposures in the New Zealand study population, either in the original report or in follow-up analyses (e.g. Crump, 1998).

4.2.2.2 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 (NIEHS, 1999).

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 (NIEHS, 1999). 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) (NRC, 2000, p. 261).

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.

Test administration

The neuropsychological test examiner was routinely controlled for in the Faroe Islands study (see NIEHS, 1999, Section 3.5), but not 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 (NIEHS, 1999). 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 (NRC, 2000 p. 263).

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, 1999). 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 (NRC, 2000, p. 263). 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 could 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.

Some members of the scientific community have noted the possibility that the most important difference in the design of the two studies is the age of the child at assessment; 7-year-olds were tested in the Faroes as opposed to children 5.5 years of age in the SCDS. Developmental assessments are likely to be less sensitive in detecting subtle neurotoxic effects when they are administered during a period of rapid developmental change. Individual differences in the rate of neurocognitive maturation may mask subtle differences in function attributable to toxic exposures. NRC (2000, pp. 257-258) also noted that

infant assessments in the SCDS (namely the 19 and 29 month Bayley Scale examinations) were not given at optimal age points for detecting effects, particularly in this developmentally robust population.

Selection bias from exclusion of individuals with severe impairments

The OSTP workshop Confounders and Variables Panel (NIEHS, 1999) 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.

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 (NIEHS, 1999). 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 ($p<0.1$) with log transformed PCB levels in cord tissue before adjustment for mercury. These four endpoints were also significantly related to mercury cord-blood concentrations. These were CPT reaction time, BNT with and without cues, and CVLT long-term reproduction (Table 4-1). When PCBs were included in the regression analysis, only the CPT reaction time remained significantly associated with mercury. CVLT and BNT with no cues were not significantly associated with either agent, whereas BNT with cues was about equally associated with both ($p \leq 0.10$). It is important to recognize that such an analysis removes the shared variance related to both mercury and PCBs, thereby reducing the p value associated with either agent.

The Faroes investigators considered CPT reaction time to be a test of attention, BNT to assess language, and CVLT to assess memory (Grandjean et al., 1997). Deficits in overall cognitive functioning and verbal comprehension have been found to be associated with *in utero* PCB exposure in a study of 4.5-year-old children in the Netherlands (Patandin et al., 1999a), whereas deficits on a vigilance task similar to the CPT were associated with cord PCB levels (commission errors) as well as the child's concurrent PCB exposure (reaction time) (Patandin et al., 1999b). In the Patandin et al. study, PCB and dioxin exposure was through diet unrelated to fish consumption. Another study reported effects of exposure to children through their mothers' consumption of contaminated Lake Michigan fish. Deficits in attention, language processing (reading comprehension), and memory related to prenatal PCB

Table 4-1. Regression coefficients (betas) for effects of logarithmic transformations of mercury before and after adjustment for PCB concentrations on Faroese neuropsychological tests: results from 7-year-old children from the first year of testing.

	Before Adjustment		After Adjustment for PCB			
				p-Values		
Neuropsychological Test	Beta	p-Value	Beta	Mercury	PCB	Both
Continuous Performance Test						
Average reaction time (ms)	39.3	<0.001	37.8	0.002	0.64	0.001
Boston Naming Text						
No cues	-1.58	0.04	-1.04	0.21	0.16	0.05
With cues	-2.03	0.007	-1.36	0.10	0.08	0.008
California Verbal Learning Test (Children)	-0.99	0.03	-0.78	0.11	0.26	0.05
Long-term reproduction						

From Grandjean et al., 1997.

exposure were identified in 11-year-old children (Jacobson and Jacobson, 1996). Other contaminants undoubtedly present in the fish, including methylmercury, were not assessed in this study; the potential contribution of methylmercury exposure to the observed effects could not be evaluated.

It is informative to compare PCB levels in other studies reporting adverse effects associated with PCBs with PCB levels in the Faroese women. No breast milk or blood PCB levels from the mothers or infants in the Faroe Islands cohort have been published. However, a recent study compared levels of PCB congener 153 in human blood in pregnant women from the Faroe Islands consuming 0-1 blubber meals/month ("low") or 2-3 blubber meals/month ("high") with other populations (Fängström et al., 2000). "Low" Faroese exposure was comparable to blood PCB levels in an unspecified number of pregnant women in the Netherlands, whereas "high" Faroese blood PCB levels were comparable to those in an identified highly exposed population in the Quebec Arctic. The Faroese samples in the Fängström et al. (2000) analysis were collected in 1994-1995, and the cohort for the Faroe study of developmental neurotoxicity was recruited in 1986-1987. It is unclear when the Dutch samples in the Fängström et al. (2000) study were collected; the cohort in the Dutch developmental study was recruited in 1990-1992. Blood levels cannot be directly compared between the Dutch study and the Fängström et al. (2000) data because one was on lipid-adjusted serum and the other on non-lipid-adjusted plasma. Similarly, breast

milk levels cannot be directly compared (Grandjean et al., 1995a; Steurwald et al., 2000; Lanting et al., 1998). In general, human body burdens of PCBs have decreased by about 50% over the past decade, so it is possible that blood levels in the Dutch study were higher than those reported in the Fängström et al. (2000) paper. It is also quite probable that PCB levels in the Faroe Islands were higher in the mid-1980s than the mid-1990s, suggesting that the "low" Faroe exposure is comparable to levels in the Dutch study. It is important to reiterate that whereas there may have been effects of PCBs in addition to those of methylmercury, statistical analyses indicated that the effects were independent in this population (Budtz-Jørgensen et al., 1999).

The Confounders and Variables Panel at the OSTP meeting (NIEHS, 1999) concluded that both PCB and mercury had adverse effects on the CVLT score and on the BNT scores with and without cues. They felt 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 reached a similar conclusion to the Confounders and Variables Panel; a likely explanation is that both PCB and mercury adversely affect some test outcomes, but their relative contributions cannot be determined given their co-occurrence in the Faroes population. NRC states it is unlikely that a difference in PCB exposure between the two populations explains the lack of developmental neurotoxic effects in the Seychelles (NRC, 2000, pp. 220 and 223).

In a second set of analyses, Budtz-Jørgensen et al. (1999) found that the 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. Calculations of benchmark doses and lower limits (BMDLs) were done using the whole cohort, after a PCB correction and for the portion of the cohort with the lowest PCBs (NRC 2000 , Table 7-4, reproduced here as Table 4-2). In this table results are reported separately for methylmercury measured in hair and cord blood and are calculated using the K-power model described in Section 4.3.4. NRC commented on the results for the low-PCB-exposed subset for the two endpoints that were related to PCB exposure, the BNT and the CVLT. They noted that the BMDs for these outcomes did not differ from the BMDs for the total sample by any more than the BMDs for the two endpoints that were not related to PCB exposure. NRC opined that the variability seen in Table 4-2 is no more than that expected

by chance; the BMDs and BMDLs for both the PCB-adjusted and the low-PCB subset analyses are within the intervals defined by the BMDs and corresponding BMDLs derived for the full cohort. The difference between the BMDs based on the full cohort and the low PCB subset is less than one standard error of the low PCB subset (NRC, 2000, p. 288). These analyses support a conclusion that there are measurable effects of methylmercury exposure in the Faroese children that are not attributable to PCB toxicity.

PCB body burdens in the Seychellois are very low by comparison to 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, the panel felt that it may not be the most reliable indication of total PCB body burden (NIEHS, 1999). The cord samples were analyzed for a small subset of PCB congeners that were used to represent the biologically significant PCB exposure. In an earlier publication (Grandjean, et al., 1995) it was shown that these congeners predominate in samples from the Faroes cohort; comprise these three congeners comprise approximately 50% of the PCBs in breast milk lipid. These same three congeners, along with one other, were used to quantify PCB body burdens in milk and plasma in a study of children in the Netherlands (Lanting et al., 1998). The approach taken in the Faroes for quantifying PCB exposure (adding three key congeners together and multiplying by 2) appears to be a reasonable approach for estimating total PCB exposure and is not expected to introduce a bias into the analysis.

4.2.2.3 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 4.2.2.1), 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 homogeneity in the Faroes 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.

Table 4-2. BMD (BMDL) Estimates from the Faroe Islands Study with and without adjustment for PCBs and in the subset of Low PCB-exposed children (reproduced from NRC 2000)

		Full Cohort	Adjusted for PCBs	Low PCB subset
Exposure	**Endpoint**	**BMD (BMDL)[a]**	**BMD (BMDL)**	**BMD (BMDL)**
Hair	Finger Tapping	20 (12)	17 (9)	7 (4)
	CPT Reaction Time	18 (10)	27 (11)	13 (5)
	Boston Naming Test	15 (10)	24 (10)	21 (6)
	CVLT: Delayed Recall	27 (14)	39 (12)	32 (7)
Cord Blood	Finger Tapping	140 (79)	149 (66)	41 (24)
	CPT Reaction Time	72 (46)	83 (49)	53 (28)
	Boston Naming Test	85 (58)	184 (71)	127 (40)
	CVLT: Delayed Recall	246 (103)	224 (78)	393 (52)

[a] BMDs are calculated 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).
Source: E. Budtz-Jørgensen, Copenhagen University, N. Keiding, Copenhagen University, and P. Grandjean, University of Southern Denmark, unpublished material, April 28, 2000.

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.

The point was made in Section 4.2.2.2 that recruitment in the SCDS was limited to children with no severe debilitating conditions. In the opinion of some scientists this may contribute to making the Faroes sample more representative of the population at risk in the United States in that it includes infants with some degree of initial perinatal risk.

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 are 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 adverse effects of prenatal Hg exposure. Statistical power to find an adverse effect is discussed in Section 4.2.2.8.

4.2.2.4 Assessment of Prenatal Mercury Exposure

In the Faroes study, mercury in cord blood and maternal hair was measured; in the Seychelles, maternal hair mercury was the biomarker of exposure. 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 (presumably reflecting the entire pregnancy).

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 (NRC, 2000, p. 252). 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.

4.2.2.5 Level of Exposure

In their analyses the SCDS authors used maternal hair mercury as the biomarker of exposure; the Faroes investigators used both cord blood and maternal hair mercury. A comparison of maternal hair mercury levels indicates that exposure in the two studies was in the same range. For the main SCDS, the median hair mercury was 5.9 ppm with a range of 0.5 ppm to 26.7 for the whole cohort. In the Faroes birth cohort (n = 1,020), the median hair mercury was 4.5 ppm with a range of 2.7 to 42.6 ppm (Grandjean et al., 1992) . That the Seychelles Islands study may entail a lower exposure level than the Faroe Islands study could be concluded from two lines of evidence: the hair:blood ratio from the

Seychelles Islands and laboratory studies suggesting that dietary factors can influence tissue levels of methylmercury.

The ratio of hair mercury to blood mercury in the Seychelles study was estimated to be 416, a value that is higher than ratios reported elsewhere, which span 190 to 367 (Stern, 1997). The hair: cord blood ratio for the Faroes cohort was 191 (Grandjean et al., 1992). The value commonly used in dose conversion models is 250 (Stern, 1997; U.S. EPA, 1997e). If the value of 416 is used in estimating maternal or fetal blood mercury then estimates of the dose experienced by the Seychellois fetuses would be lower, by almost twofold, than assumed.

The hair: blood ratio of 416 is plausible for the Seychellois population considering their high fish diet and suggestions in the literature that diet can influence tissue levels of mercury. Average fish consumption in that population is 12 fish meals/week, which is likely to result in comparatively high levels of n-3 fatty acids and selenium. Such a diet may alter the kinetics of mercury by lowering blood or organ levels of mercury associated with a certain level of intake.

4.2.2.6 Episodic Versus Continuous Exposure

Exposure to methylmercury in the Seychelles is through daily consumption of fish. 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, 1999). There is no evidence to indicate that methylmercury bioavailability from the muscle of pilot whale is any different from that of fish tissue.

In the New Zealand study, there was the assumption of regular consumption of a relatively high-mercury fish (shark) in fish and chips, the major fast food of the area; the actual frequency and pattern of exposure are unavailable.

The degree to which differences in exposure pattern among studies account for differences in outcome is uncertain. It has been suggested 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. The Faroese investigators did segmental analyses of a small number of long hair strands from cohort mothers. Their results indicated a few instances of hair mercury peaks that implied temporal variation or spiking. They noted, however, that the peak level was only about twice the lowest hair mercury concentration (Budtz-Jørgensen et al., 1999).

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 episodic and continuous 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 (U.S. EPA, 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 (see also Section 5.4.4 of this document). It 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.

4.2.2.7 Endpoints Assessed

As described in Section 4.2.1, there have been inconsistent indications of adverse effect in newborns or preschool children of mothers experiencing low-dose, long-term exposure to methylmercury. The lack of consistent positive findings using standard newborn neurological tests has been considered unsurprising. Neurological examination of the newborn and young infant presents testing challenges that are difficult to meet in large-scale studies. The state of the newborn determines to a significant degree the quality and intensity of response to stimulation during an examination. "The state of an infant is usually dependent upon factors that are often outside the examiner's control, such as hunger, hydration, illness, and the temporal location of an infant in its sleep-wake cycle. The recognition that state is a key variable in newborn behavior can be found in the fact that neonatal behavioral and

neurologic assessments usually indicate what state the newborn should be in before a given item series is administered..." (K. Deitrich, in U.S. EPA, 2000f).

It has been observed that most of the deficits associated with low-level prenatal exposure to developmental toxicants would not be revealed in a pediatric neurological examination and that gross neurological findings are unlikely in such studies. It has also been shown in studies not related to methylmercury that minor neonatal neurological deviations from the norm are not predictive of later neurobehavioral morbidity (U.S. EPA, 2000f).

Screening tests such as the Denver Developmental Screening Test have been used with highly variable results in methylmercury studies. Section 4.2.1 reports the differences in results among the New Zealand, SCDS pilot, and SCDS main cohorts. Recent research suggests that screening tests are not as sensitive as once believed and are no longer recommended for use in studies of low-level environmental chemical exposures to the fetus or infant (U.S. EPA, 2000f).

In the opinion of most developmental scientists, the Faroes and Seychelles studies used very different neurobehavioral test batteries. 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. The OSTP Neurobehavioral Endpoints Panel was similarly disposed. They noted that it is plausible that prenatal exposure to methylmercury may not affect IQ, but rather domain-specific areas such as memory deficits, motor delays, or effects on so called "executive functions" – the complex domains that involve planning and cognitive flexibility (NIEHS, 1999). The Faroese test battery consisted of highly focused tests selected from those commonly used in clinical neuropsychology (e.g., CVLT and BNT) and did not include an apical test of global function. They observed effects in areas of language, memory, motor skills, visual-spatial abilities, and attention.

Many of the subscales of the McCarthy Scales might be expected to provide measures comparable to some tests administered to the Faroese children. However, there was no evidence from the McCarthy subtests of domain-specific effects in the Seychelles. These included verbal, perceptual-performance, quantitative memory, and motor scores. One conclusion is that if there were actually domain-specific effects occurring in the 5-year-old Seychellois, they should have been observed in the analyses of the McCarthy Scales results. The NRC panel came to a different conclusion: "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." (NRC, 2000, pp. 256-257).

One test was administered to both populations: the Bender-Gestalt Test. The investigators used different scoring systems; the SCDS used the Koppitz system whereas the Faroes used the Gottingen system. 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 able to detect 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) (NRC, 2000 pp. 257-258).

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 (NRC, 2000, p. 258).

4.2.2.8 Power of Studies

NRC commented on the power to detect subtle effects in the admittedly large human studies (NRC, 2000, pp. 266-267). 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 noted 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, multi-determined neurobehavioral endpoints" (NRC, 2000, p. 267).

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)" (NRC, 2000, p. 267).

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.

4.2.2.9 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 peer reviewed and intensively analyzed. Some results from these studies of large populations are in apparent conflict. The previous sections 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.

The NRC (NRC, 2000, p. 221) suggests that the finding of a low-dose methylmercury effect in a culturally and genetically heterogenous population in New Zealand study decreases the importance of population sensitivity issues in comparing the Seychelles and Faroes studies. The New Zealand study had a higher baseline rate of abnormal and questionable DDST scores in the test (8%-17% in controls) than did the Seychelles study (8% in the complete pilot cohort, 1.9% of the complete main cohort). This observation is consistent with the suggestion that the lack of effects in the Seychelles population is related to its relatively higher level of neurological performance at critical early life stages. Another possibility is that the manner in which the tests were given in the Seychelles led to better test performance, resulting in a less sensitive measure (i.e., an easier test for children to pass). The SCDS may also have had reduced power because of the small number of maternal-child pairs with methylmercury over 15 ppm. A comparison of the numbers in the relatively high-exposure range is instructive. If one uses 10 ppm maternal hair mercury as the high-exposure cutoff, there are about 150 Faroes subjects, at least 100 Seychelles subjects, and only 16 New Zealand subjects in this category (see Fig. 5-6, p. 166, NRC report).

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. However, EPA acknowledges and shares the NRC reservations about using the New Zealand study as the basis for the methylmercury RfD. The New Zealand study is relatively small, with 237 subjects, by comparison with the population of up to 900 for the Faroes tests. Moreover, 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 these:

- large sample size;
- good statistical power as calculated by conventional means;
- the use of two different biomarkers of exposure;
- comprehensive and focused neuropsychological assessment;
- assessment at an age and state of development when effects on complex neuropsychological functions are most likely to be detectable;
- statistically significant observations that remain after adjusting for potential PCB effects; 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 (1999) workshop and by NRC (2000). 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 (NRC, 2000, p. 6). Moreover, although one can model the nonpositive results of the SCDS, the resulting estimates of no effect level are difficult to interpret.

The study selected by EPA as the basis of the methylmercury RfD is the report of developmental neurotoxicity in 7-year-old children in the Faroes. The next section discusses issues in choice of endpoint for the RfD calculation. Many of the arguments in study selection pertain to choice of endpoint as well.

4.2.3 Choice of Critical Effect (endpoint)

EPA considered recommendations of NRC and the external peer reviewers in making the choice of a critical effect or endpoint from the Faroese data on neuropsychological effects in children. Rather than choosing a single measure for the RfD critical endpoint, EPA considers that this RfD is based on several Faroese test scores. These test scores are all indications of neuropsychological processes that are involved with the ability of a child to learn and process information. The issues and decision points in coming to this choice are described in the following sections.

4.2.3.1 Endpoints Suitable for RfD Derivation

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.

As is shown in Table 4-3, the tests used in the Seychelles and New Zealand studies in general were apical tests, assessing broad functional categories. These tests are widely used clinically and have been validated and normed for the U.S. population (but not the populations in which they were used). In contrast, the tests used in the Faroe Islands study were chosen to assess specific behavioral domains. The global clinical instruments such as the McCarthy, WISC-R, and CBCL have manuals that describe the tests and domains assessed, as well as the predictive validity of scores on these instruments to "real-world" behavior such as school performance. For the tasks used in Faroe Islands, Finger Tapping is a commonly used assessment of motor speed (Letz, 1990), and the Bender is a standardized test of childhood development. The other three endpoints also have demonstrated clinical relevance and predictive value As outlined in the table, most of these endpoints are predictive of ability in various academic skills, and therefore school performance. These tests, whether designed to be relatively global or domain-specific, were adversely affected by methylmercury exposure in the Faroe Islands and New Zealand, but not the Seychelles Islands, studies. In addition, motor performance was adversely affected in both New Zealand and the Faroe Islands. The only study that assessed social and adaptive behavior was the SCDS. BMD analysis performed by the NRC committee identified adverse effects on the CBCL at maternal hair levels comparable to those at which effects were observed in the Faroe Islands study (NRC, 2000, Table 7-5, p. 291). As concluded by the NRC (NRC, 2000, p. 325), the deficits observed in the New Zealand and Faroe Islands study can be considered predictive of problems in cognitive and academic performance associated with methylmercury exposure.

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 4-4), 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. (NRC, 2000, p. 282).

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 4.2.2.9). 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 ($p = 0.0001$)

Table 4-3. Tests modeled by NRC, functions assessed, and potential societal relevance

Study	Test	Domain/Function Assessed	Societal Relevance
Seychelles	Bender Copying Errors	Visuospatial	Math performance
	McCarthy GCI	Full-scale IQ	School performance, intelligence
	WJ Applied Problems	Ability to solve problems	Academic skills
	CBCL	Social and adaptive behavior	Antisocial behavior, need for therapeutic services
	Preschool Language Scale	Broad-based language	Learning, intelligence, school performance
	WJ letter/word recognition	Word recognition	Reading ability, school performance
Faroes	Finger Tapping	Motor performance	Motor speed/neuropathy
	CPT Reaction Time	Vigilance, attention, information processing speed	Intelligence, school behavior and performance
	Bender Copying Errors	Visuospatial	Math performance
	Boston Naming Test	Expressive vocabulary	Reading, school performance
	CVLT: Delayed Recall	Memory	Learning ability, school performance
New Zealand	TOLD Language Development	Broad-based language	Literacy skills, learning, school performance
	WISC-R: PIQ	Performance IQ, e.g. visuospatial, sustained attention, sequential memory	Learning, school performance
	WISC-R: FSIQ	Full-scale IQ, e.g. PIQ + verbal processing, expressive vocabulary	Learning, school performance
	McCarthy Perceptual Performance	Performance IQ, e.g. visuospatial, audition, memory	Learning, school performance
	McCarthy Motor Test	Gross and fine motor skills	Motor system integration

Abbreviations: WJ, Woodcock-Johnson Tests of Achievement; CBCL, Child Behavior Check List; 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.

Table 4-4. 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
	CVLT: 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.

- 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 4-5; this presents calculations, in terms of cord-blood mercury concentrations, of BMDs and BMDLs for five Faroese endpoints.

4.2.3.2 Comparison of Endpoints

Boston Naming Test (BNT)

The BNT was the endpoint of choice of the NRC panel (NRC, 2000, p. 327). This test assesses word retrieval and formulation abilities in children, adults, and brain-injured patients. In the test, 60 line drawings are shown to the subject one at a time, and the subject is asked to name each of them. Familiarity (frequency of occurrence of the target names) decreases as the test progresses. Responses of the patient are scored for latency and correctness. When the subject misses an item, two kinds of cues may be given. A "stimulus cue" is a short phrase that gives additional information about the target item (e.g., something to eat). A "phonetic cue" is the first sound of the target word. Scores are summarized according to the number of spontaneously given correct responses, the number of correct responses following stimulus cues, and the number of correct responses following phonetic cues. The number of stimulus cues and the number of phonetic cues given by the examiner also is recorded. The peer-review panel noted that there is not much normative data on the BNT but that it is often used by child clinical neuropsychologists because of its documented validity in various child studies (EPA, 2000e). The BNT

Table 4-5. 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).

has been useful as a measure of confrontation naming and word retrieval skills and can be used to differentiate between children with and without language-based learning disabilities; moreover, it is a predictor of related cognitive and academic skills, especially reading achievement (Yeates, 1994, as quoted in U.S. EPA 2000e).

Continuous Performance Test (CPT)

The endpoint from the Faroe Islands study that yielded the lowest BMDL in the NRC analysis was the CPT reaction time. This test was modified from the Neurobehavioral Evaluation System (NES) version, which is a standardized battery used mainly in occupational settings in adults. In the Faroe Islands study, the child was required to respond as quickly as possible when a silhouette of a cat appeared on a computer screen, but not when the silhouettes of other animals (number not specified) appeared (Grandjean et al., 1997). Dependent variables included number of missed responses (omission errors) and average reaction time for the last 3 minutes of a 4-minute task. False positives (errors of commission) apparently were not analyzed. Reaction time in a task that includes decision making (respond to cat, don't respond to others) is a measure of the speed of information processing. The investigators found an increase in reaction time correlated with cord blood using all data; this correlation was still seen when only data were used from children whose mothers had hair concentrations below 10 ppm (low-level exposure). In addition, there was an association between cord blood mercury levels and an increase in omission errors in the full group and low-level exposure group. This finding indicates poorer attention to the task as a function of methylmercury exposure.

Speed of information processing as measured by reaction time is highly correlated with IQ in humans (Jensen and Munro, 1979; Matthews and Dorn, 1989; Vernon, 1983; Vernon et al., 1985; Western and Long, 1996). It has been argued that speed of information processing is a measure of *g*, the highest order common factor in all tests of cognitive ability (Jensen, 1993b). Reaction time in complex reaction time tasks is consistently observed to be correlated with psychometric *g* in studies in several cultural groups (Buckhalt and Jensen, 1989; Ja-Sòng and Lynn, 1992; Lynn et al., 1991; Lynn and Wilson, 1990; Shigehisa and Lynn, 1991). Generally, the association between *g* and decision reaction time increases with increasing task complexity (Beh et al., 1994; Jensen, 1987). It is estimated that the correlation between reaction time and *g*-loaded psychometric tasks is 0.3-0.5, whereas the correlation based on several reaction time and psychometric tasks approaches 0.7 (Jensen, 1993a; Vernon, 1989), which is similar to the correlation among different IQ tests (Jensen, 1993a). Reaction time tasks also discriminate between brain-injured and other individuals (Western and Long, 1996) and identify children with attention deficits (Zahn et al., 1991).

The NRC chose not to rely on CPT reaction time as the critical endpoint because results were from only half the cohort. The Faroe investigators reported that effects on CPT reaction time were significant for the first year of testing but not the second, with combined effects for the 2 years significant at $p = 0.01$. The authors stated that "[b]ecause supervision was stringent only during the first year, these data were chosen for development of the final regression model" (Grandjean et al., 1997, pp. 422-423). The NRC felt that measures from the full cohort would be more reliable than those based on half the cohort; their report did not state any concerns regarding elimination of the second year data per se (NRC, 2000, p.286).

Advantages of the choice of the CPT reaction time as the critical endpoint would be that there was no evidence of an effect of PCBs on this measure, and the correlation of complex reaction time with measures of intelligence such as IQ. The disadvantage is that the analysis is based on half the cohort. However, this limitation also holds true for the BNT corrected for PCB exposure. Therefore, there is little or no reason to choose one over the other in this regard.

California Verbal Learning Test for Children (CVLT)

The California Verbal Learning Test for Children is a word-list-learning task that measures acquisition of information following repeated exposure to verbal stimuli. Of principal interest are the variables of learning, delayed recall, and perseveration. The test has good test-retest reliability as well as internal consistency. The theoretical foundations of the CVLT are based upon several decades of cognitive science research in brain/behavior relationships. The test discriminates clinical groups such as those with hyperactivity/attention deficit disorders, children with learning disabilities, and children suffering prenatal insults such as fetal alcohol syndrome.

4.2.3.3 Consideration of Potential PCB effect

EPA agrees with NRC that analyses of the Faroese test results show that there are real mercury-related adverse effects that cannot be attributed to concomitant PCB exposure. This was noted in Section 4.2.2.2. The external peer review panel for the methylmercury RfD agreed with that conclusion. However, they disagreed with the NRC choice of the BNT results from the full cohort because of the potential effect of PCB exposure. They thought that the BNT results were the most sensitive to PCB influence of any evaluated in the Faroe Islands. The peer review panel pointed to the analyses presented by NRC (reproduced in this document as Table 4-6) as presenting an opportunity to consider the use of benchmark estimates corrected for any potential PCB influence. The Faroes investigators calculated a

PCB-adjusted BMD and BMDL for the BNT using cord blood as the exposure biomarker; these were considerably greater than the BMD/BMDL for either the full cohort without PCB adjustment or that from the low-PCB tertile. Similar increases after adjusting for PCBs were not seen for Finger Tapping, CPT reaction time, or CVLT delayed recall tests, when cord blood was the exposure metric. NRC noted that the PCB measurements were done on cords from only about one-half of the Faroese cohort (about 450 children) and that the use of data from only the low-PCB tertile further reduces *n* to about 150 children. NRC reported that the reduced sample sizes in these analyses increased the variability in the results. They saw no clear pattern as to how the PCB-adjusted analyses differed from the original results. The NRC concentrated its focus on the low-PCB subset BMDs and BMDLs. They compared results from two tests with no PCB effect (CPT and Finger Tapping) with those with potential for PCB influence (BNT and CVLT). They reported that the BMDs for the low-PCB subset for the BNT and CVLT did not differ from the BMDs for the whole cohort any more than did the BMDs for the two tests with no influence of PCBs. The NRC authors felt that the variability seen in Table 4-6 is no more than that which would be expected by chance alone (NRC, 2000, p. 288).

Table 4-6. BMD (BMDL) Estimates from the Faroe Islands Study With and Without Adjustment for PCBs and in the Subset of Low PCB-Exposed Children (calculated using the K-power model)

		Full Cohort	Adjusted for PCBs	Low-PCB subset
Exposure	Endpoint	BMD (BMDL)[a]	BMD (BMDL)	BMD (BMDL)
Hair	Finger Tapping	20 (12)	17 (9)	7 (4)
	CPT Reaction Time	18 (10)	27 (11)	13 (5)
	Boston Naming Test	15 (10)	24 (10)	21 (6)
	CVLT: Delayed Recall	27 (14)	39 (12)	32 (7)
Cord Blood	Finger Tapping	140 (79)	149 (66)	41 (24)
	CPT Reaction Time	72 (46)	83 (49)	53 (28)
	Boston Naming Test	85 (58)	184 (71)	127 (40)
	CVLT: Delayed Recall	246 (103)	224 (78)	393 (52)

[a]BMDs are calculated 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).
Source: E. Budtz-Jørgensen, Copenhagen University, N. Keiding, Copenhagen University, and P. Grandjean, University of Southern Denmark, unpublished material, April 28, 2000, in Table 7-4, p. 289, NRC 2000.

4.2.3.4 Supporting Studies

A second Faroese cohort was recruited from children born between 1994 and 1995. In the study reported by Steurwald et al. (2000), decreases in neurologic optimality score (NOS) were associated with increasing cord blood mercury. This association remained statistically significant after adjustment for confounders (including cord and maternal serum PCB levels). Inspection of data plotted in the paper indicate that a decrease in NOS was observed in the two highest quartiles; that is, at cord blood mercury levels greater than 20 ppb. This indicates a dose-dependent effect at levels as low as (or lower than) those for which neuropsychological deficits were reported in the main study of 7-year-old children (Grandjean et al, 1997). The size of this study is rather small (N = 182) and involves subtle changes at a very early developmental period, the clinical implications of which are less clear than the changes found in the main study of 7-year-olds.

NRC conducted an analysis that combined results from the SCDS, New Zealand, and Faroes studies (NRC, 2000, pp. 290-294). Their approach was to use a hierarchical random-effects model that followed a method proposed by Dominici et al. (in press). To inform their analyses, NRC plotted BMDs and BMDLs (as ppm mercury in maternal hair) for measures from all three studies. For outcomes in the SCDS for which there were no BMDs, the analysis used an arbitrary value of 150. They concluded from the plot (Figure 7-3, NRC, 2000, p. 285) that study-to-study variability was large relative to outcome-to-outcome variability. NRC felt that use of a hierarchical model would allow one to borrow strength from the different studies to achieve greater precision in BMD and BMDL estimates. The NRC results are seen in their Table 7-5 (NRC, 2000, p. 291). They present what they refer to as smoothed results, which reflect reduced random variability. For the Faroes data, the BMDL estimates are not much changed from the original values; the unsmoothed range of BMDLs is 10 to 15 ppm mercury in maternal hair, while the smoothed results range from 12 to 15 ppm. The NRC notes that all smoothed BMDLs are closer to their BMDs; they also concluded that the hierarchical modeling reduced much variability among outcomes but not among studies.

NRC estimated a central tendency measure, equivalent to a BMD, across all three studies and all endpoints. They also determined a lower limit based on a theoretical distribution of BMDs, which is the logical equivalent of a BMDL. These values as well as other estimates derived from the Faroes and New Zealand studies are in Table 4-7.

Table 4-7. Central tendency estimates, ppm mercury in maternal hair[a]

Approach	Original values		Smoothed values	
	BMD	(BMDL)	BMD	(BMDL)
Most sensitive endpoint from New Zealand	8	(4)	12	(7)
Median endpoint from New Zealand	12	(6)	13	(8)
Mean of endpoints from New Zealand	12	(6)	13	(8)
Most sensitive endpoint from Faroes	15	(10)	17	(12)
Median endpoint from Faroes	20	(12)	20	(13)
Mean of endpoints from Faroes	22	(12)	21	(13)
Mean of all endpoints		(14)		(15)
Integrative analysis			21[b]	(8[c])

[a] Source: Table 7-6, NRC 2000, p. 294.

[b] Logically equivalent to a BMD.

[c] Logically equivalent to a BMDL.

The external review panel for the methylmercury RfD suggested that a reasonable alternative to using a single test result as the basis for the RfD would be to develop a composite index from several test outcomes. Their recommendation was to evaluate mercury-associated endpoints for any potential PCB effect. The next step would be to use either PCB-adjusted results or only those results with no PCB effect in some compositing approach to provide a multiendpoint BMDL. The most appropriate compositing approach would be one with a weighting scheme to account for different sample sizes for the individual tests.

A second way to proceed would be to use factor analysis to create a composite factor that accounts for the majority of the variance among the individual test results. The resulting estimate would serve as the basis for RfD calculation. The peer review panel that suggested this approach noted that it is novel and would require substantial effort to reanalyze the data (U.S. EPA, 2000f).

EPA has decided that the two suggestions have a great deal of merit. We will pursue some of these analyses for the extant Faroes and New Zealand data and for the SCDS data on 7-year-old children as they become available. We felt, however, that the integrative analysis reported by NRC serves as substantial support for the choice of an endpoint from the Faroese test data. We felt that at this time the use of NRC's integrated BMD /BMDL or one derived form the suggested alternatives as the sole basis for an RfD would introduce an unacceptable degree of model uncertainty into the RfD.

4.2.3.5 Choice of Endpoint

The lowest of the BMDLs from the Faroese tests is 46 μg/L mercury in cord blood for the CPT 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; thus the *n* was about half that for the other tests. The NRC panel suggested that a more appropriate choice would be to select the second most sensitive endpoint, the BNT BMDL of 58 ppb mercury in cord blood (NRC, 2000, p. 300). Interestingly, the BNT had the lowest BMDL in the analyses based on maternal hair mercury.

The external peer reviewers of the methylmercury RfD disagreed with the NRC choice. The felt that the use of a single neuropsychological endpoint to form the basis for making a risk assessment is problematic. They felt that the use of the BNT data from the whole Faroese cohort was not warranted, as the BMDL thus derived could reflect an effect of PCB exposure. The peer reviewers preferred the BNT BMDL adjusted for PCB exposure of 71 ppb mercury in cord blood. In their report they noted that the adverse effect of methylmercury reflected in the BNT scores is not isolated, but rather occurs at levels not far removed from effects on other neuropsychological tests, providing some assurance of its credibility. A difficulty with the use of the PCB-adjusted BMDL is that this BMDL is based on scores from only about one-half of the total cohort. As noted in Section 4.2.3.3, NRC felt it was more appropriate to use the BMDL from analyses with the larger *n*.

The peer review panel described three other options for RfD derivation. One option would be to use the BMDL from the CVLT. The panel noted the clinical relevance and predictive value of this test as the well as likelihood that there is no influence of PCB exposure on this measure. The major drawback to this choice is that the BMDL from this test for the full cohort is the highest (103 ppb mercury in cord blood or 14 ppm mercury in maternal hair) of those listed in Table 4-6. One could easily argue that the RfD based on this measure is not public health protective. In the light of analyses that indicate that mercury correlations with test measures remain when the highest exposure subset is eliminated (10 ppm or more mercury in maternal hair), this would seem a poor choice.

A third option would be to develop a composite index across several measures in the Faroes study. The peer reviewers suggested that the BMDLs from the statistically significant tests could be developed, evaluated for effects of PCBs, and composited in some way, such as a geometric mean. The compositing

method should consider a weighting scheme to deal with varying sample sizes for the different tests. NRC essentially did a composite measure with the integrative analysis; for all endpoints in all three large studies, the BMDL is 8 ppm mercury maternal hair, or 32 ppb cord blood mercury (Table 4-7). Geometric means for the Faroese measures are in Table 4-8 below. These were calculated separately for the whole cohort, PCB-adjusted BMDLs, and lowest PCB subset. EPA will pursue the suggestion of a weighted composite index at a future time.

A final longer term option of the peer review panel was to devise a within-study integrative multivariate approach using factor analysis for analytical derivation of a composite factor that combines results across tests with overlapping functional domains. The panel acknowledged that this would require some statistical methodology development.

EPA prepared a comparison of the NRC and peer-reviewer-recommended approaches, which also includes the BMDLs from the NRC integrative analysis and geometric means of four scores from the Faroes. Table 4-8 presents BMDLs in terms of cord blood mercury. These are converted (using a one-compartment model as in Section 4.4.2) to an ingested dose of methylmercury that would result in the cord blood level. The last column of Table 4-8 shows the corresponding RfD from application of a UF of 10 (see Section 4.5.6). The calculated RfD values converge at the same point: 0.1 μg/kg/day. Among all the endpoints listed, there are few deviations from 0.1 μg/kg/day: 0.2 μg/kg/day for the CVLT entire cohort and 0.05 μg/kg/day for CPT and Finger Tapping, lowest PCB subset. For comparative purposes several measures from the New Zealand data analyses were also included in Table 4-8; the median BMDL from the New Zealand study would give an RfD of 0.05 μg/kg/day. If one were to use the NRC integrative analysis BMDL equivalent value, the resulting RfD would be 0.05 μg/kg/day.

Rather than choosing a single measure for the RfD critical endpoint, EPA considers that this RfD is based on several scores from the Faroes measures. These test scores are all indications of neuropsychological processes involved with a child's ability of a child to learn and process information. The BMDLs for these scores are all within a relatively close range. In subsequent sections, one endpoint is carried through the dose conversion and application of the UF to calculation of the RfD; namely, the NRC-recommended BMDL of 58 ppb mercury in cord blood from the BNT.

Table 4-8. Comparison of BMDLs–endpoint from Faroes, New Zealand and NRC Integrative Analysis[a]

Test[b]	BMDL ppb mercury cord blood	Ingested dose µg/kg bw /day[c]	RfD µg/kg bw /day[d]
BNT Faroes			
Whole cohort	58	1.081	0.1
PCB adjusted	71	1.323	0.1
Lowest PCB	40	0.745	0.1
CPT Faroes			
Whole cohort	46	0.857	0.1
PCB adjusted	49	0.913	0.1
Lowest PCB	28	0.522	0.05
CVLT Faroes			
Whole cohort	103	1.920	0.2
PCB adjusted	78	1.454	0.1
Lowest PCB	52	0.969	0.1
Finger Tap Faroes			
Whole cohort	79	1.472	0.1
PCB adjusted	66	1.230	0.1
Lowest PCB	24	0.447	0.05
Geometric mean			
Whole cohort	68	1.268	0.1
PCB adjusted	65	1.212	0.1
Lowest PCB	34	0.634	0.1
Median values			
Faroes	48	0.895	0.1
New Zealand	24	0.447	0.05
Smoothed values			
BNT Faroes	48	0.895	0.1
CPT Faroes	48	0.895	0.1
CVLT Faroes	60	1.118	0.1
Finger Tap Faroes	52	0.969	0.1
MCCPP New	28	0.522	0.05
MCMT New	32	0.596	0.1
Integrative			
All endpoints	32	0.596	0.1

[a]BMDLs from NRC (2000), Tables 7-4, 7-5, 7-6. Hair mercury was converted to blood mercury using a 250:1 ratio and an assumption of equivalent maternal and cord levels.
[b]Abbreviations: BNT, Boston Naming Test; CPT, Continuous Performance Test; CVLT, California Verbal Learning Test; MCCPP, McCarthy Perceived Performance; MCMT, McCarthy Motor Test.
[c]Calculated using a one-compartment model as in Section 4.4.2.4.
[d]Calculated using an UF of 10 as in Section 4.5.6.

4.3 CHOICE OF DOSE-RESPONSE APPROACH

4.3.1 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 (U.S. EPA, 2000g).

NRC also made comments on the applicability or preference for BMDs over NOAELs (NRC, 2000, pp. 272-273). 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 over the LOAEL/NOAEL approach. The traditional method does not incorporate information on the shape of the dose-response curve, but rather uses only a single point (NOAEL or LOAEL). This point depends on the number of doses and spacing of those doses in the experiment. The possible LOAEL/NOAELs are limited to the discrete values of the experimental doses, whereas the "real" value of the NOAEL could be any value between the experimental NOAEL and the LOAEL.

The determination of a NOAEL is dependent on the background incidence of the effect in controls. Statistically significant differences between treatment groups and controls are more difficult to detect if background incidence is relatively high, even if biologically significant effects are noted.

The peer reviewers of the methylmercury RfD provided comment on the appropriateness of the BMD methodology for the methylmercury human data:

> Derivation of LOAELs and NOAELs from the data would require disaggregation of the data based upon artificial cutpoints (e.g., quartiles) to determine which range of exposure appears to be different from the baseline group. While this approach provides a useful profile of effect with dose (e.g. Fig. 1 of the 1997 Faroes paper), it uses a grouping of the data that makes specifying the threshold less exact than with the more statistically robust and inclusive benchmark dose approach. The LOAEL/NOAEL approach also does not factor variability into the estimation of the threshold dose in the health protective way that the BMDL concept accomplishes. In the LOAEL/NOAEL approach, the more variable the data the higher the LOAELs and NOAELs tend to become because it is more difficult to define a statistical difference from the control group. In contrast, greater variability will tend to drive down the estimate of the BMDL since it is the lower 95% confidence limit estimate on the BMD. (G. Ginzberg in U.S. EPA, 2000f)

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

4.3.2 Choice of Exposure Metric

NRC discussed at length in its Chapter 4 the suitability of both hair and blood mercury as biomarkers of exposure. 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 a 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 (Table 6-1, NRC, 2000, p. 253) 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.

4.3.3 Choice of BMD

In applying a BMD approach to data that are continuous in effect, there are several interdependent 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 4.3.4). The second step is to define the cutoff for normal versus abnormal response. This cutoff point (x_0) is defined statistically. 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. In other words, the BMD is the dose that results in an increased probability of an abnormal test performance by a benchmark response; that is, from P_0 for an unexposed person to P_0 + BMR for a person exposed to the BMD. The last step is to calculate the BMDL or 95% lower limit on the BMD. Choices for P_0 and BMR are described below.

One could set P_0 based on clinical definitions of adverse response or other information. For example, long experience with birth weight in a population could prompt a choice of 2500 g as a cutoff for normal. Alternatively P_0 can be set as a fixed percentile of performance in the unexposed population. For a linear model and random error normally distributed with variance, this has the effect of setting P_0 at a specified number of standard deviations below the mean for the unexposed group. Generally the larger the P_0, the lower the BMD. For the analysis of the behavioral data, including the Faroe study, the NRC panel (NRC, 2000, p. 298) 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. This means that the cutoff point (x_0) is defined by a probability of 5% in an unexposed population. It should be noted that 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 (Budtz-Jørgensen et al., 1999). Support for P_0 of 0.05 is found in Crump et al. (2000); the authors note that this choice is "suggested by the convention of considering 95% of the clinical responses in healthy individuals to define the normal range." EPA agrees that $P_0 = 0.05$ is a reasonable choice.

BMR is the benchmark response, the specific risk above background risk. In other risk assessments (mostly on quantal data) it has been set at 0.1, 0.05, or 0.01. In the MSRC, BMDs and BMDLs were calculated for BMRs of 0.1, 0.05, or 0.01. EPA chose to apply a BMR of 0.1 to the Iraqi data (MSRC volume V, pp. 6-27-6-28; U.S. EPA, 1997e). This was based on publications by Allen et al. (1994) that indicated that a 10% risk level roughly correlated with a NOAEL for developmental toxicity data from controlled animal studies. For a methylmercury RfD based on the Faroese data, NRC 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 (NRC, 2000, pp. 283, 298).

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 an acceptable risk level for the effects reported in the Faroese children. EPA is in the process of publishing guidance on benchmark dose methodology and processes. Most of the experience that supports this guidance comes from assessment of toxicological

(animal) data. The guidance acknowledges that choices of model, and inputs such as P_0 and BMR, should be informed by a consideration of the type of data and the ancillary information on which the assessment is based. 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.

4.3.4 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. In response to NRC, Budtz-Jørgensen and colleagues provided some additional analyses. These were sensitivity analyses that repeated the regression models after omitting some of the highest observations (E. Budtz-Jørgensen, Copenhagen University, N. Keiding, Copenhagen University, and P. Grandjean, University of Southern Denmark, unpublished material, April 28, 2000, quoted in NRC, 2000, p. 293). Their results suggested that the influence of the extreme observations did not explain the model-to-model variability (NRC, 2000, p. 293).

NRC concluded that the most reliable and defensible results for the purpose of risk assessment are those based on the K-power model. (NRC, 2000, pp. 293-298). This model takes the following form, as presented in Budtz-Jørgensen et al. (2000):

$$\mu(d) = \beta \cdot d^K$$

where d is the child's mercury dose and K and β are parameters to be estimated. The K-power model was fit under the constraint that $K \geq 1$, so that supralinear models were ruled out. A power of 1 generally provided the best fit to the Faroese data (Budtz-Jørgensen et al., 2000). With K = 1, the above model is linear.

NRC 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 do linear or K-power models. The mechanisms by which methylmercury exerts its neurotoxic effects in developing systems are speculative. However, 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.

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) responded 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 mercury data for the linear, square-root, and log transformations. Budtz-Jørgensen et al. (2000) provided some information on model fit. They did not present goodness-of-fit statistics *per se*, but rather tested each model against an expanded model that included both the linear and logarithmic term. The authors observed that for $P_0 = 0.05$, and with cord blood as the exposure metric, the logarithmic transformation tended to show a better fit than the linear model for the following tests: CPT, BNT, and CVLT. There was no difference in fit for the Finger Tapping and Bender Gestalt test or for any of the five tests when maternal hair mercury was the biomarker. The 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 its conclusion on model choice, NRC concluded that biologically based arguments were needed. The 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. (NRC, 2000 p. 297).

4.3.6 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. Our choice is a benchmark approach using the results of the Faroese tests with significant associations with cord-blood mercury. As an example, the BNT results for the whole cohort are used. The K-power model ($K \geq 1$ 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.

The result for the example calculation is a BMD of 85 ppb and a BMDL of 58 ppb; other BMDs and BMDLs are given in Table 4-8.

4.4 DOSE CONVERSION

The biomarker of choice for the Faroes data was cord blood and the BMDLs were presented in units of ppb mercury in cord blood. In order to calculate an RfD, it is necessary to convert this figure to an ingested daily amount that would result in exposure to the developing fetus at the BMDL level in terms of ppb mercury in blood. NRC (2000) offered advice on the use of these dose-conversion procedures.

4.4.1 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. 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 (NRC, 2000, p. 84). 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. This model has shown reasonably good fit to data on mercury blood level changes in human subjects during and after consumption of methylmercury-contaminated fish (Ginsberg and Toal, 2000). It has been used by other public health agencies such as WHO and ATSDR (1999).

4.4.2 One-Compartment Model for Methylmercury

4.4.2.1 Description of Model

The model is described by the formula below:

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

where

d = daily dietary intake (expressed as μg of methylmercury)
c = concentration in blood (expressed as 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 following form of the equation expresses d in units of μg/kg body weight/day.

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

where

bw = body weight (expressed in kg).

In this one-compartment model, all maternal compartments are compressed to one: namely, 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 higher than in maternal blood (for example, Dennis and Fehr, 1975: Pitkin et al., 1976; Kuhnert et al., 1981). Other publications show that there is no difference in concentration (for example, 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. This is discussed in Section 4.5.4.1.

4.4.2.2 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 (NRC, 2000, pp. 83-95). 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 4-9 reproduces NRC's compilation of those analyses. In this table NRC also presented 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 this 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

Table 4-9. 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

Study	Maternal medium	50th percentile[a] (μg/kg-d)	50th percentile/ 5th[b] percentile	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.

conversion, it is necessary to include a UF in the final RfD calculation to ensure that pharmacokinetic variability is appropriately factored into the consideration of sensitive subgroups.

The choice of UF 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, rather than blood mercury was the biomarker used. 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 UF. This UF considers the probability distribution that relates biomarker concentration and ingested dose (see Section 4.5). This approach was recommended in the NRC report. 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" (NRC, 2000, pp. 94-95).

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 a UF in the calculation of the RfD.

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

NRC recommended (NRC, 2000, p. 95), 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 (U.S. EPA, 1997e). 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 in the example). 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; it probably includes some inorganic mercury formed from the ingested methylmercury and subsequently excreted. 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 (U.S. EPA, 1997e).

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 per 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 although the Smith et al. (1994) and Kershaw et al. (1980) data could be fit by a log-normal distribution, the data sets were 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).

ATSDR (1999) used a factor of 0.05. They noted that estimates of f for the 6 women from the study by Sherlock et al. (1984) had an average value of 0.048, as compared with the value of 0.059 for the 14 men in the same study. ATSDR offered the opinion that these data suggest f may be lower for women than men. Apparently the study by Miettinen et al. (1971) included six female volunteers (in addition to nine males), though ATSDR did not comment on whether these data similarly provided any indication that the fraction daily intake taken up by blood was lower for females. It is not likely that any of the female subjects were pregnant. Sherlock et al. (1984) published a negative correlation between f and body weight; thus, if this is generalizable, one would expect f to decrease (as V increases) throughout pregnancy.

EPA chooses to use the median value of 0.059 published by Swartout and Rice (2000) 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 (U.S. EPA, 1997e) used an average elimination constant from 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-Shahristani data were removed. The author observed that the half-lives reported by Al-Shahristani 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-Shahristani data set by comparison to the others. Stern says that an alternative explanation is that the Al-Shahristani 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-Shahristani 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-Shahristani 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 by 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 (U.S. EPA, 1997e), 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 (U.S. EPA, 1997e). 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, 2000a) 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 on the basis of the relatively current data from NHANES III. This is the value used for body weight in the dose conversion.

4.4.2.4 Dose Conversion Using the One-Compartment Model

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 0.95, unitless decimal fraction)
f = fraction of daily intake taken up by blood (0.059, unitless)
bw = body weight (expressed as 67 kg)

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

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

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

rounded to 1.0 μg/kg/day. Other BMDLs expressed as ingested maternal dose can be found in Table 4-8.

4.5 CHOICE OF UNCERTAINTY FACTOR

4.5.1 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 (U.S. EPA, 2000g).

In the MSRC, EPA published qualitative discussions and quantitative analyses of uncertainty and variability in the RfD based on the Iraqi data (U.S. EPA, 1997e,g). 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 UF of 10 was applied to account for these factors and the EPA policy choice to use a UF 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 UFs. NRC (NRC, 2000, p. 319) suggests that given the state of the human data on methylmercury, UFs 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 a UF of at least 10 be applied to a BMD calculated from the BNT results from the Faroe Islands study (NRC, 2000, pp. 321-322). EPA is in general agreement with NRC's conclusions and recommendations and considered them in the choice of the numerical UF. EPA's choice is to consider the RfD to be based on the group of Faroese neuropsychological measures associated with cord-blood mercury; the areas of uncertainty and variability are the same for the choice of one test result (e.g., BNT whole cohort) or the group of test results. Descriptions of areas of uncertainty and variability and choice of UF are in the following sections.

4.5.2 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 heterogeneous 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, but that toxicodynamic variability must be considered in the determination of the overall uncertainty factor.

4.5.3 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. At this time there are not data to support a quantitative determination of this area of uncertainty.

4.5.4 Pharmacokinetic Variability

4.5.4.1 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.

Review of the literature identified 21 studies that reported cord blood mercury and maternal blood mercury data (Amin-Zaki et al., 1974; Baglan et al., 1974; Dennis and Fehr, 1975; Pitkin et al., 1976; Kuhnert et al., 1981; Nishima et al., 1977; Lauwerys et al., 1978; Fujita and Takabatake, 1977; Kuntz et al., 1982; Tsuchiya et al., 1984; Truska et al., 1989; Sikorski et al., 1989; Hansen et al., 1990; Soong et al., 1991; Soria et al., 1992; Ong et al., 1993; Akagi et al., 1997; Yang et al 1997; Ramirez et al., 2000; Bjerregaard and Hansen, 2000; Vahter et al., 2000). Twenty of the studies provided data in a format that could be compared with one another. The exception is Truska et al. (1989), whose published data were based on erythrocyte mercury concentrations without reported hematocrit values. Absence of these values precluded expressing mercury concentration on a μg/L or ppb whole-blood basis.

Data from 18 of the 20 studies (with a combined total of 2,676 maternal and 2,522 cord-blood samples) indicated that cord-blood mercury concentration exceeded maternal-blood mercury

concentration. Mean values ranged from a ratio of 1.04 (Fujita and Takabatake, 1977) to 2.63 (Amin-Zaki et al., 1974); the average of mean ratios was 1.55. Two studies reported cord:maternal blood ratios equal to or less than 1. Kuntz et al. (1982) (based on 57 maternal-cord blood pairs) and Sikorski et al. (1989) (based on 56 maternal-cord blood pairs) reported cord/maternal blood mercury concentration of 1.0 and 0.83, respectively.

Speciated mercury measurements were performed in 9 studies that included 550 maternal and 526 cord-blood samples. This permitted calculation of the ratios of cord blood methylmercury:maternal blood methylmercury that are presented in Table 4-10. In all nine studies, the mean values for methylmercury concentration was higher for cord blood than maternal blood. The number of subjects in these 9 studies ranged from 9 to 226 pregnant woman-fetal pairs. To deal with this variation in *n*, Table 4-10 reports both a simple average of mean ratios (cord methylmercury:maternal methylmercury = 1.68) and the mean ratio weighted by the number of subjects in the study (ratio =1.73).

Overall, these data indicate that cord-blood mercury is higher than maternal-blood mercury. The composite ratio from the studies reporting methylmercury concentrations indicates that the cord blood:maternal blood ratio is around 1.7. These values are ratios of means and do not reflect the full range of variability in the individual mother-fetal pairs. Vahter et al. (2000 reported the 5th and 95th percentiles of cord:maternal Hg to be 0.88 and 3.1. Individual data were available from Fujita and Takabatake (1997); ratios calculated from these data ranged from 0.78 to 4.36.

As indicated in Section 4.4.2.1, EPA chooses not to make a numerical adjustment between cord-blood and maternal-blood mercury. Such an adjustment factor would best be calculated after evaluation of data quality and variability within and between studies. EPA feels that this analysis would be an important contribution to reducing uncertainty in the RfD. At this time the relationship between cord blood and maternal-blood mercury is considered an area of uncertainty to be included in the determination of the UF.

Table 4-10. Ratio of Cord to Maternal Blood Methylmercury

Investigator	Number of Subjects	Ratio of Cord:Maternal Blood
Nishima et al., 1977	49 maternal, 49 fetal	2.17
Kuhnert et al., 1981	29 maternal, 29 fetal	1.34
Tsuchiya et al., 1984	226 maternal, 226 fetal	1.60
Hansen et al., 1990	37 maternal, 37 fetal	2.11
Soria et al., 1992	19 maternal, 19 fetal	1.08
Ong et al., 1993	29 maternal, 29 fetal	1.65
Akagi et al., 1997	21 maternal, 21 fetal	1.75
Yang et al., 1997	9 maternal controls, 9 fetal controls; 9 occupationally exposed mothers, 9 occupationally exposed fetuses.	1.67 - controls 1.39 - occupationally exposed
Vahter et al., 2000	112 maternal (gestation week 36), 98 fetal	1.92
Arithmetic mean of average ratios of cord:maternal methylmercury		1.68
Mean weighted by number of subjects for cord:maternal blood methylmercury		1.73

4.5.4.2 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-life 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 4.4.2 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 presented analyses of uncertainty around dose-conversion estimates, which are summarized in Table 4-9 in Section 4.5.2.2. 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 (NRC, 2000, pp. 91-95). 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 4.4.2.1. 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 of the distributions 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 4-9 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 4-9 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 uncertainty introduced by assuming cord-blood mercury is equivalent to maternal mercury provides additional justification for a toxicokinetic UF of 3. The choice of a factor of 3 is consistent with the standard EPA practice of a using a half-log to account for toxicokinetic variability.

4.5.5 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 most sensitive indicator of methylmercury effects.

4.5.5.1 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.

4.5.5.2 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. These present evidence that neurological dysfunction among people who have been exposed

to methylmercury becomes more pronounced 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 in 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 (1989a) 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 11 and 19 years. 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. Rats exposed to methylmercury *in utero* through 16 days of age exhibited a decline in performance in a task that required a substantial motor output at an earlier age than did control rats; high-dose rats exhibited a decline in performance at about 500 days of age compared with 950 days for controls (Newland and Rasmussen, 2000), with no differences between groups in survival time. 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.

4.5.5.3 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 intraperitoneally. 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 subcutaneously 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 1997e) concluded that because there are data for mammalian germ-cell chromosome aberrations 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.

4.5.6 Choice of Uncertainty Factor

For this methylmercury RfD the two major areas of uncertainty that can be addressed with a UF are interindividual toxicokinetic variability in ingested dose estimation and pharmacodynamic variability and uncertainty. 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. We chose not to make a numerical adjustment in the dose conversion for the potential differences in cord vs. maternal blood mercury level, but rather consider this an additional area of toxicokinetic uncertainty. A quantitative uncertainty analysis was not feasible for toxicodynamics. A common practice is to apply a threefold UF for toxicodynamic variability and uncertainty.

In the calculation of this methylmercury RfD, a composite UF 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.

- Pharmacodynamic variability and uncertainty. A factor of 3 is applied for this area.

There are additional areas of concern in this risk estimate that lend support to an overall factor of 10. These include the following: inability to quantify long-term sequelae, lack of a two-generation reproductive effects assay, and issues on selection of critical effect (concern that there may be observable methylmercury effects at exposures below the BMDL). Section 4.5.5 discusses some of the concerns on selection of the critical effect. In this context one must also consider the analyses of the Faroese neuropsychological data wherein the observations in the most highly exposed subgroup were excluded from the model. Associations remained significant when the part of the cohort with maternal hair mercury concentrations greater than 10 ppm was excluded from the analyses. This indicates that it would be reasonable to expect some percentage of the population to show effects at or below 10 ppm hair mercury or at levels at or below 40 ppb cord blood. Given the overall robustness of the methylmercury database, but in consideration of the above areas of uncertainty, a composite factor of 10 is warranted.

4.6 CALCULATION OF THE RfD

The critical endpoint is drawn from the series of neuropsychological test results reported from the Faroese cohort. The BMDLs calculated on these endpoints are in Table 4-8. The ingested doses in μg/kg bw/day that correspond to the BMDLs range from 0.447 to 1.92. The ingested dose for the BNT whole-cohort BMDL is 1.081 μg/kg bw/day, rounded to 1.0 μg/kg bw/day.

For methylmercury, the RfD is calculated as follows:

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

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

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

$$= 0.1\ \mu g/kg/day.$$

As shown in Table 4-5, an RfD of 0.1 μg/kg bw/day reflects the range of neuropsychological test results in the Faroese children exposed *in utero*. These test scores are all indications of neuropsychological processes that are involved with the ability of a child to learn and process information. In the studies so far published on subtle neuropsychological effects in children, there has been no definitive separation of prenatal and postnatal exposure that would permit dose-response modeling. That is, there are currently no data that would support the derivation of a child (vs. general population) RfD. This RfD is applicable to lifetime daily exposure for all populations including sensitive subgroups. It is not a developmental RfD per se, and its use is not restricted to pregnancy or developmental periods.

5.0 EXPOSURE ASSESSMENT

5.1 OVERVIEW OF RELATIVE SOURCE CONTRIBUTION ANALYSIS

When a water quality criterion is based on noncarcinogenic effects, anticipated exposures from sources other than drinking water and fish ingestion are taken into account so that the entire RfD is not attributed to drinking water and freshwater/estuarine fish consumption alone. The amount of exposure attributed to each source compared with total exposure is called the relative source contribution (RSC) analysis. The RfD used in calculating the criterion incorporates the RSC to ensure that the criterion is protective enough, given the other anticipated sources of exposure. The method of accounting for nonwater exposure sources is described in more detail in the revised 2000 Human Health Methodology (U.S. EPA, 2000a).

The method of determining the RSC differs depending on several factors, including (1) the magnitude of total exposure compared with the RfD, (2) the adequacy of the exposure data available, (3) whether more than one guidance or criterion is to be set for a contaminant, and (4) whether there is more than one significant exposure source for the chemical and population of concern. The population of concern for methylmercury is discussed in Section 5.2. The sources of exposure to methylmercury and estimates of exposure used to determine the RSC for the identified population are discussed in Sections 5.3 through 5.4. Section 5.5 summarizes the exposure uncertainties based on data adequacy. Finally, Section 5.6 provides the RSC estimates for methylmercury.

5.2 POPULATION OF CONCERN

Methylmercury is a highly toxic contaminant that can cause a variety of adverse health effects. Toxicity has been observed in adults exposed through consumption of contaminated food. Toxic effects and subtle neuropsychological effects have been seen in children exposed *in utero* when their mothers consumed contaminated food while pregnant. The RfD (see section 4) is based on changes in neuropsychological measures in children exposed *in utero*. The choice was made to use a developmental endpoint, as this appeared to be the most sensitive indicator of a methylmercury effect. As discussed in section 4, there is concern that other less-studied effects may occur at lower doses. There is also concern (based on recent reports on the Minamata, Japan, population) that exposure *in utero* or in childhood could result in subtle impairments that would not be detectable until middle age or older.

The RfD for methylmercury was not calculated to be a developmental RfD only. It is intended to serve as a level of exposure without expectation of adverse effects when that exposure is encountered on a daily basis for a lifetime.

In the studies on subtle neuropsychological effects in children published so far, there has been no definitive separation of prenatal and postnatal exposure that would permit dose-response modeling. That is, there are currently no data that would support the derivation of a child RfD versus a general population RfD.

Therefore, the population at risk evaluated for the methylmercury criterion is adults in the general population, not only the developing fetus or child.

5.3 OVERVIEW OF POTENTIAL FOR EXPOSURE

The sources and fate of methylmercury are discussed in detail in Volume III of the *Mercury Study Report to Congress* (MSRC) (U.S. EPA, 1997b). The MSRC exposure assessment is in Volume IV (U.S. EPA, 1997c). A brief summary of the information in that document is presented here. Methylmercury occurs naturally in the environment. It is readily produced from inorganic mercury in fresh and marine surface waters and sediments through the methylating action of certain microorganisms. Bacterial methylation rates appear to increase under anaerobic conditions, elevated temperatures, and low pH. Methylmercury generally constitutes no more than 25% of the total mercury in surface water; typically, less than 10% is observed (U.S. EPA, 1997b). According to the MSRC, mercury cycles in the environment as a result of natural and anthropogenic activities. Most of the mercury in the atmosphere is elemental mercury vapor, which can remain there for as much as 1 year and, due to atmospheric mobilization, can be widely dispersed and transported thousands of miles from likely sources of emission (U.S. EPA, 1997b). However, the MSRC also clearly states that methylmercury is the chemical species of concern due to its fate and transport to waterbodies and sediments, and its subsequent bioaccumulation in the aquatic food web.

Because the source of most mercury is deposition from atmospheric mercury emissions, ingestion is an indirect route of exposure. The MSRC included numerous computer-simulated estimates of mercury exposure for selected population scenarios, based on fate and transport models (see U.S. EPA, 1997b,c). These are summarized throughout this chapter in the *Predicted Concentrations* subsections. Further exposure assessment information is presented in Volumes III and IV of the MSRC (U.S. EPA, 1997b,c)

and a characterization of human health from methylmercury exposure is discussed in detail in Volume VII (U.S. EPA, 1997g). That exposure assessment information is summarized throughout this chapter. The primary source of human exposure to methylmercury is through consumption of contaminated fish and seafood. This reflects the tendency of aquatic organisms to rapidly absorb methylmercury and to store it for long periods of time in their muscle tissue, thus accumulating it to levels that are potentially toxic to humans who eat fish and shellfish. The concentrations of methylmercury in fish tissue are highly variable across water bodies. Within a water body, methylmercury concentration generally increases with fish size and trophic level.

Derivation of the water quality criterion requires that intake of methylmercury from other sources of exposure be evaluated for comparison with intake from water and/or freshwater and estuarine fish. In addition to its occurrence in water and freshwater and estuarine fish, methylmercury occurs in soil, air, marine fish and other seafood, and nonfish foods. Intake of these media thus represent potential pathways for exposure. Other potential routes include occupational exposure and erosion of dental amalgams. Estimates of intake from these sources are presented in Section 5.4 below. Assessment of these sources of methylmercury clearly indicates that substantially all exposure to methylmercury occurs from the ingestion of contaminated fish. The other sources of exposure (water, nonfish foods, air, and soil) are all several orders of magnitude less than exposures from fish consumption.

5.4 ESTIMATES OF OCCURRENCE AND EXPOSURE FROM ENVIRONMENTAL MEDIA

This section reports data available for the estimation of methylmercury intake from relevant exposure sources. Exposure may occur from several environmental sources including soil, sediment, ambient surface water, drinking water, food products, and air. Human exposures are estimated by combining information on the occurrence of methylmercury in environmental media with intake rates for these media. Information on intake assumptions, environmental concentrations, and estimated exposure are reported by medium below.

Table 5-1. Exposure parameters used in derivation of the water quality criterion

Parameter	Population			Source
	Children (0-14 years)	Women of Childbearing Age (15-44 years)	Adults in the General Population	
Body Weight, kg	30	67	70	U.S. EPA (2000a)
Drinking Water Intake, L/day	1.0	2.0	2.0	U.S. EPA (2000a)
Freshwater/Estuarine Fish Intake, gm/day	156.3[b]	165.5[b]	17.5[c]	U.S. EPA (2000a)
Inhalation, m^3/day	10.4	11	20	U.S. EPA (1994, 1997h)[d]
Soil Ingestion, g/day	0.0001, 0.01[a]	0.00005	0.00005	U.S. EPA (1997h)
Mean Marine Fish Intake, kg/day	74.9[b]	91.04[b]	12.46[c]	U.S. EPA (2000b)
Median Marine Fish Intake, kg/day	59.71[b]	75.48[b]	0[c]	U.S. EPA (2000b)
90th Percentile Marine Fish Intake, g/day	152.29[b]	188.35[b]	49.16[c]	U.S. EPA (2000b)

[a]Pica child soil ingestion
[b]For children and women of childbearing age, intake rates are estimates of "consumers only" data (as described in U.S. EPA, 2000b).
[c]For adults in the general population, intake rates are estimates of all survey respondents to derive an estimate of long-term consumption (U.S. EPA).
[d]Inhalation rates for children and women of childbearing age from U.S. EPA, 1997h. Inhalation rates for adults in the general population from U.S. EPA (1994).

5.4.1 Exposure Intake Parameters

Exposure parameters selected for derivation of the water quality criterion should reflect the population to be protected. Default values for most exposure parameters are provided in the 2000 Human Health Methodology (U.S. EPA, 2000a). Where necessary, values for parameters not specified in the Methodology were obtained from the Exposure Factors Handbook (U.S. EPA, 1997h). Parameter values used to estimate intake of methylmercury by children aged 0-14 years, women of childbearing age, and adults in the general population are summarized in Table 5-1.

5.4.2 Intake from Drinking Water/Ambient Water

In cases where the water quality criterion is based on fish intake only, drinking water intake is accounted for as a separate exposure. In these instances, information on treated drinking water, if available, is the relevant information to use when accounting for other sources of exposure. Measured concentrations for methylmercury in drinking water and raw surface and ground source waters have been reported in the MSRC (U.S. EPA, 1997c). Predicted concentrations and ingestion rates summarized in this section are based on computer simulation models described in Volume IV of the MSRC (U.S. EPA, 1997c).

5.4.2.1 Measured Concentrations in Water

Raw Surface Water. Studies in the United States and Europe suggest that the concentrations of methylmercury in raw surface water are highly variable (U.S. EPA, 1997b). Properties reported to influence the levels of methylmercury in water bodies include proximity to a point source of mercury, pH, anoxia, dissolved organic carbon, and the presence of wetlands (U.S. EPA, 1997b). Estimates of the percent of total mercury in surface waters that exists as methylmercury are available from a number of studies. The available data suggest that methylmercury generally constitutes less than 20% of the total mercury in the water column (Kudo et al., 1982; Parks et al., 1989; Bloom and Effler, 1990; Watras et al., 1995a). In lakes without point source discharges, methylmercury frequently constitutes 10% or less of total mercury in the water column (Lee and Hultberg, 1990; Bloom et al., 1991; Lindqvist, 1991; Porcella et al., 1991; Watras and Bloom, 1992; Driscoll et al., 1994, 1995; Watras et al., 1995b). U.S. EPA (1997b) reported the use of Monte Carlo simulation to derive a point estimate of 0.078 for the fraction of total mercury present as methylmercury in the epilimnion (water column above the thermocline) of lakes for the purpose of estimating a bioaccumulation factor (BAF) for trophic level 4. Speciation data used as input for the simulation are shown in Table 5-2.

Data for measured concentrations of methylmercury and total mercury in ambient water as presented in the MSRC (U.S. EPA, 1997b) are summarized in Table 5-3. Since publication of the MSRC, Krabbenhoft et al. (1999) reported concentrations of total mercury and methylmercury in surface water samples collected as part of a U.S. Geological Survey (USGS) national scale pilot study to examine relations for total mercury and methylmercury in water, sediment, and fish. Water samples were collected in the summer and fall of 1998 at 106 sites from 21 basins across the United States, including Alaska and Hawaii. The sampling sites spanned the dominant east-to-west mercury deposition gradient

Table 5-2. Data Used in the Monte Carlo Simulation to Estimate the Fraction of Total Dissolved Mercury in the Epilimnion Present as Methylmercury

Fraction of Total Mercury Present as Methylmercury	Location	Reference
0.046	Pallette Lake, WI	Bloom et al. (1991)
0.054	Oregon Pond, NY	Driscoll et al. (1995)
0.059	Lake Michigan	Mason and Sullivan (1997)
0.089	Clear Lake, CA	Suchanek et al. (1993)
0.089	Onondaga Lake, NY	Henry et al. (1995)
0.092	Iso Valkjarvi, Finland	Rask and Verta (1995)
0.15	22 lake aggregate, WI	Watras et al. (1995a,b)

Source: U.S. EPA (1997c, Appendix D)

and represented a wide range of environmental settings. The study authors reported that most (number not reported) samples were collected from streams. Total mercury was measured using U.S. EPA Method 1631 with detection by cold vapor atomic fluorescence spectroscopy (CVAFS). Methylmercury was analyzed by distillation and aqueous phase ethylation, with detection by CVAFS. The detection limits for total mercury and methylmercury were 0.04 ng/L and 0.025 ng/L, respectively (Olson and DeWild, 1999). Of the 106 total sites, 21 were classified as background or reference sites. The mean concentration for methylmercury at background sites was 0.13 ng/L, which represented 3.4% of the mean total mercury concentration. When all sites were considered, the mean methylmercury concentration (104 sites) was 0.15 $\pm$ 0.26 ng/L (range 0.01 to 1.481 ng/L). The median value was 0.06 ng/L. The difference in mean and median values was attributed to high mercury concentrations at sites impacted by mining activities, which resulted in a skewed distribution. Methylmercury constituted 1% to 11% of total mercury concentration in the 21 study basins.

Other measured concentrations of total mercury and methylmercury in fresh water as reported in the MSRC (U.S. EPA, 1997b) are summarized in Table 5-3. Reported values for methylmercury measured at two sites in the United States ranged from less than 0.004 ng/L to 0.06 ng/L. The New Jersey Department of Environmental Protection and Energy (NJDEPE) (1993) reported total mercury concentrations for lakes of 0.04 to 74 ng/L and values of 1 to 7 ng/L for rivers and streams. Based on the

Table 5-3. Measured Methylmercury Concentrations in Surface Fresh Water

Study Description	Total Mercury (ng/L)	Methylmercury (ng/L)	Methylmercury % of Total	Reference
Lake Crescent, WA	0.163	<0.004	<2.5	Bloom and Watras (1989)[a]
Little Rock Lake (reference basin)	1.0-1.2	0.045-0.06	mean of 5	Watras and Bloom (1992)[a]
Lake Michigan (total)	7.2 microlayer 8.0 at 0.3m 6.3 at 10m	NA	NA	Cleckner et al. (1995)[a]
Lake Champlain	(filtered) 3.4 microlayer 3.2 at 0.3m 2.2 at 15m	NA	NA	Cleckner et al. (1995)[a]
Lakes Rivers and Streams	0.04 - 74 1 - 7	NA	NA	NJDEPE (1993)[a]
USGS National Mercury Pilot Study (predominately streams)	3.43 Background 16.6 All sites	0.13 Background 0.15 All sites	3.4 1 - 11	Krabbenhoft et al. (1999)

[a] As reported in U.S. EPA (1997c)
NA Not available

U.S. EPA (1997b) Monte Carlo estimate for speciation (0.078), these values would correspond to approximate methylmercury concentrations of 0.003 to 6 ng/L for lakes and 0.078 to 0.55 ng/L for rivers and streams. The MSRC did not indicate whether the NJDEPE (1993) data represented measures of central tendency.

Ground Water. Nationally aggregated data for mercury or methylmercury concentrations in ground water were not reported in the MSRC (U.S. EPA, 1997b). Local estimates of concentration are available from three studies. Krabbenhoft and Babiarz (1992) reported mercury levels of 2 to 4 ng/L in near-surface ground water in remote areas of Wisconsin, with a maximum of 0.3 ng/L (roughly 7.5% to 15% of total mercury concentration) occurring as methylmercury. Bloom et al. (1989) reported a value of 0.3 ng/L for total mercury in a Washington state well. In contrast to these comparatively low concentrations, Dooley (1992) reported total mercury levels up to and exceeding 2,000 ng/L in southern New Jersey domestic wells.

Drinking Water. Much of the data reported for total mercury concentration in drinking water is below the detection limit of 100 ng/L associated with older methods of analysis (U.S. EPA, 1997b). Lindqvist and Rodhe (1985) estimated that the concentration range of mercury in drinking water is the same as rain, with an average level of total mercury in drinking water of 25 ng/L. NJDEPE (1993) reported a range of 0.3 to 25 ng/L for total mercury in U.S. drinking and tap water. Speciation data for mercury in drinking water are not available, but may be similar to those observed for rain water (U.S. EPA, 1997c). The percentage of total mercury that is methylmercury in rain water ranged from 0.1% to 6.3% in two studies reported by Lee and Iverfeldt (1991) and Fitzgerald et al. (1991). The high end of this range approaches the point estimate of 7.8% derived for the fraction of methylmercury in the water column of lakes using Monte Carlo simulation (U.S. EPA, 1997b). Assuming that 7.8% of the total mercury is methylmercury (U.S. EPA, 1997b), these data suggest a crude estimate of methylmercury concentration in drinking and tap water ranging from 0.023 ng/L to 1.95 ng/L.

5.4.2.2 Predicted Concentrations in Water

U.S. EPA (1997b) reported the results of watershed fate and transport modeling conducted to predict the background concentration of mercury in water bodies. Atmospheric concentrations and deposition rates were used as inputs to the IEM-2M model. The IEM-2M model is composed of two integrated models that simulate mercury fate using mass balance equations that describe processes in watershed soils and a shallow lake. Using this approach, background levels of total dissolved mercury concentrations in the water column of 0.9 and 0.2 ng/L were predicted for hypothetical Eastern and Western U.S. sites, respectively. More than 80% of the total mercury in the water column was predicted to occur as the inorganic divalent species. As indicated above, the fraction of the predicted background concentration occurring as methylmercury was 7.8% (U.S. EPA, 1997b).

In the MSRC, the background values reported above were used as inputs to a localized model analysis that examined the impact of a variety of anthropogenic emission sources (municipal waste combustors, hospital medical waste incinerators, utility boilers, chlor-alkali plant) on methylmercury concentrations in the water column at distances of 2.5, 10, or 25 km from the source. This effort was undertaken because some monitoring studies suggest that measured mercury concentrations may be higher in areas adjacent to stationary industrial and combustion sources known to emit mercury (U.S. EPA, 1997b). Results of this analysis are of relevance to derivation of the water quality criterion because they include data specifically for predicted methylmercury concentrations, and thus permit comparison with measured concentrations.

The Industrial Source Code air dispersion model (ISC3) was used for simulation. Hypothetical facilities were defined to represent actual emissions from existing industrial processes and combustion sources; these were situated in hypothetical locations intended to simulate a site in either the Western or Eastern United States (U.S. EPA, 1997b). Input values for air concentrations consisted of simulated concentration results (50th and 90th percentile values) obtained using the regional Lagrangian model of air pollution (RELMAP). The assumptions and inputs utilized in this modeling effort are described in detail in Volume III of the MSRC (U.S. EPA, 1997b).

Results for predicted methylmercury concentrations in water are illustrated in Table 5-4. Predicted concentrations for dissolved methylmercury in water across all scenarios ranged from 0.014 to 1.0 ng/L. The highest predicted concentrations occurred at a location 2.5 km from a chlor-alkali plant. The predicted contribution of the hypothetical emission sources to methylmercury concentration ranged from 0 to 99% across all modeling scenarios. Although these results are meant to describe events on a local (adjacent to emission source) rather than nationwide scale, they provide a general frame of reference for comparison with measured values. The predicted range compares to the measured concentration range of 0.01 to 1.481 ng/L reported by Krabbenhoft et al. (1999) for 104 surface water samples collected at sites across the United States. The range of predicted concentrations overlapped the methylmercury concentrations in ground water (less than or equal to 0.3 ng/L, based on one study) and drinking water (0.023 to 1.95 ng/L) estimated from measurement data presented in Section 5.4.2.1.

5.4.2.3 Intake Estimates for Drinking Water and Ambient Water

Using the methylmercury concentration data in treated drinking water, and in ambient water it is possible to estimate exposure from water ingestion. For methylmercury, data on measured concentrations in ground and treated drinking water are limited. The database for surface water is somewhat more extensive. Estimates of intake based on ingestion of drinking water and ambient water are provided below.

Ambient Surface Water

A central tendency value for methylmercury in ambient surface water based on national data is available from a pilot study conducted by the U.S. Geological Survey (Krabbenhoft et al., 1999). Concentrations of methylmercury in ambient surface water ranged from a mean background level of 0.13 ng/L (or 1.3×10^{-7} mg/L) to a mean concentration for all sites of 0.15 ng/L (or 1.5×10^{-7} mg/L).

Combining the mean for methylmercury concentrations at all sites with default exposure assumptions of a 30 kg child aged 0 to 14 years who consumes 1 L/day of ambient surface water yields an estimated exposure of 5.0×10^{-9} mg/kg-day. Combining the mean value for methylmercury concentrations at all sites with default exposure assumptions of 2 L/day for water ingestion rate and 67 kg for body weight yields an exposure estimate of 4.5×10^{-9} mg/kg-day for a woman of childbearing age (15-44 years old). Adults in the general population have an estimated exposure value of 4.3×10^{-9} mg/kg-day, based on a default body weight and water intake rate of 70 kg and 2 L/day, respectively. These values are summarized in Table 5-5.

Table 5-4. Range of Predicted Dissolved Methylmercury Concentrations in Water for Hypothetical Emissions Scenarios

Site	RELMAP Percentile	Methylmercury (ng/L)		Scenario	
		Min	Max	Min	Max
Eastern	50	0.077	1.0	Large hospital incinerator, 25 km	Chlor-alkali plant, 2.5 km
Eastern	90	0.11	1.0	Multiple scenarios	Chlor-alkali plant, 2.5 km
Western	50	0.014	1.0	Multiple scenarios	Chlor-alkali plant, 2.5 km
Western	90	0.034	1.0	Multiple scenarios	Chlor-alkali plant, 2.5 km

Source: U.S. EPA (1997c)

Table 5-5. Ambient Surface Water Intake Assumptions and Estimates

Population of Concern	**Methylmercury in Ambient Surface Water[a] (mg/L)**	**Ingestion Rate[b] (L/day)**	**Body Weight[b] (kg)**	**Daily Exposure Estimate (mg/kg-day)**
Children (0-14 yr)	1.5×10^{-7}	1.0	30	5.0×10^{-9}
Childbearing Women	1.5×10^{-7}	2.0	67	4.5×10^{-9}
Adults in the General Population	1.5×10^{-7}	2.0	70	4.3×10^{-9}

[a] Methylmercury concentration is the mean for all sites in the national pilot study as reported in Krabbenhoft et al. (1999)

[b] U.S. EPA (2000a)

Drinking Water

Although drinking water concentrations can be calculated based on surface water and ground-water concentrations (U.S. EPA, 2000a), the available ground-water data were not adequate for this purpose. Therefore, exposure from drinking water was roughly estimated for women of childbearing age, children aged 0-14 years, and adults in the general population based on existing drinking and tapwater concentration data (NJDEPE, 1993). For the purpose of this estimate, it was assumed that the reported data reflected contributions from both ground water and surface water. Combining the estimated range for methylmercury concentrations in drinking water (0.0234 to 1.95 ng/L, or 2.34×10^{-8} to 1.95×10^{-6} mg/L) with default values for a 30 kg child aged 0 to 14 years consuming 1 L/day of drinking water yields an exposure estimate ranging from 7.8×10^{-10} to 6.5×10^{-8} mg/kg-day. Combining the estimated range for methylmercury concentrations in drinking water with default values of 2 L/day for drinking water intake and 67 kg for body weight yields an exposure estimate that ranges from 7.0×10^{-10} to 5.8×10^{-8} mg/kg-day for a woman of childbearing age (15-44 years old). Exposure estimates from ingesting drinking water by adults in the general population range from 6.7×10^{-10} to 5.6×10^{-8} mg/kg-day, based on a default body weight and water intake rate of 70 kg and 2 L/day, respectively. These values and intake assumptions are summarized below in Table 5-6.

Table 5-6. Drinking Water Intake Assumptions and Estimates

Population of Concern	**Methylmercury in Drinking Water (mg/L)**	**Ingestion Rate[a] (L/day)**	**Body Weight[a] (kg)**	**Daily Exposure Estimate (mg/kg-day)**
Children (0-14 yr)	2.3×10^{-8} to 1.9×10^{-6}	1.0	30	7.8×10^{-10} to 6.5×10^{-8}
Childbearing Women	2.3×10^{-8} to 1.9×10^{-6}	2.0	67	7.0×10^{-10} to 5.8×10^{-8}
Adults in the General Population	2.3×10^{-8} to 1.9×10^{-6}	2.0	70	6.7×10^{-10} to 5.6×10^{-8}

[a] U.S. EPA (2000a)

5.4.3 Nonfish Dietary Exposures

5.4.3.1 Measured Concentrations in Food Other Than Fish

Historically, measurements of mercury have not been speciated in food items other than fish, primarily because of the lack of adequate methodology (Madson and Thompson, 1998). However, the limited data available suggest that nonfish foods such as dairy products, fruits, and vegetables may potentially contribute to intake of methylmercury. Furthermore, it is possible that the agricultural practice of using fishmeal in animal feeds may result in increased levels of methylmercury in nonfish foods (ATSDR, 1999). This section examines the available data on mercury and methylmercury concentrations in nonfish human food items.

Information on the concentration of total mercury in dietary items is available from the *Total Diet Study* (TDS) conducted by the U.S. Food and Drug Administration (U.S. FDA). The TDS is an on-going nationwide program that determines the levels of nutrients and selected contaminants in foods for the purpose of estimating intakes of these substances by the U.S. population. A total of 839 samples for 47 food items were collected and analyzed for total mercury during the period from 1991 to 1996 (U.S. FDA, 1999). Of the reported results, 756 (90%) were below the detection limit for mercury (0.01 to 0.02 mg/kg depending on food item) and 30 (3.6%) were considered to contain trace amounts of mercury. These trace values represent the best estimates of those who analyzed the data, but in all cases are below the nominal limit of quantitation.

Examination of the data for the 41 nonfish dietary items analyzed (6 items were fish) indicates that the total mercury concentration was below the detection limit for most samples. These samples were assigned a concentration of zero for statistical analysis (U.S. FDA, 1999). Trace amounts of total mercury were found in one sample each (out of 18 total samples for each item) of fried beef liver, cooked oatmeal, and boiled spinach. The maximum detected concentration of mercury in nonfish dietary items was 0.03 mg/kg in fried beef liver. The reported median concentrations for total mercury in all individual nonfish dietary categories were zero. Based on these data, the central tendency estimate for methylmercury intake from nonfish dietary items is zero. For comparison, the mean mercury concentration from all 47 food categories (containing both fish and nonfish dietary items) was 0.006 mg/kg (U.S. FDA, 1999).

The MSRC (U.S. EPA, 1997b) also summarized data for methylmercury concentrations reported in local studies. Measured concentrations of methylmercury in garden produce and crops are summarized in Table 5-7. Because the database for methylmercury content in these foods is limited, information is also presented from studies that report total mercury concentrations. In general, the level of methylmercury in agricultural produce is low, with the highest concentration (30 ng/g dry weight) observed in leafy vegetables. Plants grown in the presence of elevated soil or atmospheric concentrations of mercury are reported to contain elevated concentrations of total mercury (U.S. EPA, 1997b). Temple and Linzon (1977) sampled the mercury content of fresh fruits and vegetables around a large chlor-alkali plant in an urban-residential neighborhood. Among garden produce, leafy crops accumulated the highest levels of mercury. One lettuce sample contained 99 ng/g wet weight of mercury (background: <0.6 ng/g), and a sample of beet greens contained 37 ng/g wet weight (background: 3 ng/g). Tomatoes and cucumbers within 400 m of the chlor-alkali plant averaged 2 and 4.5 ng/g wet weight of mercury, respectively, compared with measured background levels of 1 ng/g.

Because the mercury content in plants tends to be low, livestock typically accumulate little mercury from forage or silage (U.S. EPA, 1997b). However, use of fishmeal as food for poultry and other livestock may result in increased mercury levels in these animals (ATSDR, 1999). Measured concentrations of mercury and methylmercury in meat products are summarized in Table 5-8. Although the database is limited, the available data suggest that methylmercury concentrations in meats are generally low in comparison with levels observed in fish (U.S. EPA, 1997b).

Pedersen et al. (1994) monitored the level of mercury in wine, beer, soft drinks, and various juices. Total mercury levels in these beverages were at or below the detection limit of 6 μg/L in all samples tested.

Infant postnatal exposure to methylmercury through ingestion of breast milk is a pathway of potential concern. As noted in Section 3.4, methylmercury is excreted in breast milk (Bakir et al., 1973; Sundberg and Oskarsson, 1992). The ratio of mercury in breast milk to mercury in whole blood was approximately 1:20 in women exposed to methylmercury via contaminated grain in Iraq between 1971 and 1972 (Bakir et al., 1973). Skerfving (1988) found that 16% of mercury in human breast milk is methylmercury. Note that the MSRC found the data on breast milk to be insufficient to support estimation of exposure by this route.

Table 5-7. Measured Mercury Concentrations in Garden Produce and Crops

Study Description	Total Mercury (ng/g dry wt)	Methylmercury (ng/g dry wt)	Methylmercury (mg/kg dry wt)	% Methylmercury	Reference
NY Garden Conditions: Leafy Vegetables	64-139	9.5-30	9.5×10^{-3} - 30×10^{-3}	15-23	Cappon (1987)
NY Garden Conditions: Tuberous Plants	11-36	0.3-6.6	0.3×10^{-3} - 6.6×10^{-3}	11-36	
NY Garden Conditions: Cole[a]	50-64	8.8-12	8.8×10^{-3}- 12×10^{-3}	18	
NY Garden Conditions: Fruiting vegetables	2.9-27	0-2.4	0 - 2.4×10^{-3}	0-9.1	
NY Garden Conditions: Beans	4.3	0	0	0	
Maize	1.7 - 7.3	NA	NA	NA	Szymaczak and Grajeta (1992)

NA Not available
[a] Members of the plant genus *Brassica* including cabbage, broccoli, and cauliflower.
Source: U.S. EPA (1997c)

Table 5-8. Measured Mercury Concentration in Meats

Study Description	Total Mercury (ng/g wet weight)	Approx. Total Mercury (ng/g mercury dry weight)[1]	Approx. Total Mercury (mg/kg mercury dry weight)	% Methyl-mercury	Reference
Saginaw River, MI "Roaster" Ducks (n=6)	48	124.7	124.7×10^{-3}	NA	U.S. EPA (1992a)
Wild Deer (Northern Wisconsin)	5-14	13-36	13×10^{-3} - 36×10^{-3}	11-57 %	Bloom and Kuhn (1994)
Beef: Raw	< 1	< 2.6	$<2.6 \times 10^{-3}$	> 10%	
Beef: Lunch Meat	21	54.5	54.5×10^{-3}	4%	
Beef: Frank	<1	< 2.6	$<2.6 \times 10^{-3}$	> 60%	
Beef Muscle: Control Group	2-3	5.2 - 7.8	5.2×10^{-3} - 7.8×10^{-3}	NA	Vreman et al. (1986)*
Beef Muscle: Exposed Group	1-4	2.6 - 10.4	2.6×10^{-3} - 10.4×10^{-3}	NA	
Beef Liver: Control Group	3000 - 7000	7800 - 18000	7.8 - 18.0	NA	
Beef Liver: Exposed Group	9000 - 26000	23400- 67000	23.4 - 67.0	NA	
Pork: Raw and Sausage	< 1	< 2.6	$<2.6 \times 10^{-3}$	0-70%	Bloom and Kuhn (1994)
Chicken: Raw and Lunch Meat	< 1 to 29	< 2.6 to 75.4	$<2.6 \times 10^{-3}$ - 75.4×10^{-3}	20-67%	
Turkey: Lunch Meat	< 1	< 2.6	$<2.6 \times 10^{-3}$	>20%	

* Exposed animals received 1.7 mg mercury/day as mercury acetate; intake for controls was approximately 0.2 mg mercury/day.
[1] Based on an assumed water content of 0.615, which is average for beef (Baes et al., 1984).
Source: U.S. EPA (1997c)

5.4.3.2 Predicted Concentrations in Foods Other than Fish

U.S. EPA (1997d) reported predicted concentrations in fruits, vegetables, beef, pork, poultry, dairy products, and eggs. As described in previous sections on predicted concentrations in various media, this effort was undertaken because some monitoring studies suggest that measured mercury concentrations may be higher in areas adjacent to stationary industrial and combustion sources known to emit mercury (U.S. EPA, 1997b). Results of this local study are of relevance to derivation of the water quality criterion because they include data specifically for predicted methylmercury concentrations, and thus permit comparison with measured concentrations.

The Industrial Source Code air dispersion model (ISC3) was used for the computer simulation to estimate nonfish dietary exposure. Model plants (defined as hypothetical facilities which were developed to represent actual emissions from existing industrial processes and combustion sources), were situated in hypothetical locations intended to simulate a site in either the Western or Eastern United States (U.S. EPA, 1997b). Input values for air concentrations consisted of simulated concentration results (50^{th} and 90^{th} percentile values) obtained using the regional Lagrangian model of air pollution (RELMAP). The assumptions and inputs utilized in this modeling effort are described in detail in Volume III of the MSRC (U.S. EPA, 1997b).

Predicted concentrations in a variety of nonfish foods are reported in Table 5-9. Because the computer models used to generate these concentrations incorporated a point source for mercury emissions, these predictions likely approach a worst-case scenario for methylmercury levels in foods. Based on a large hospital waste incinerator scenario in the Eastern United States (50th percentile), concentrations of methylmercury (expressed on a dry-weight basis) ranged from 0.095 ng/g to 7.1 ng/g in fruits and vegetables, with the highest concentration observed in leafy vegetables. Concentrations of methylmercury animal products ranged from 0.0013 ng/g to 4.2 ng/g, with the highest concentrations observed in beef and dairy products. The hypothetical facility was considered to contribute less than 10% to the total plant mercury concentration (U.S. EPA, 1997b). The local source was considered to contribute 7% to 11% of the total mercury in beef, dairy products, and pork and 41% of total mercury in poultry and eggs (U.S. EPA, 1997b).

5.4.3.3 Intake Estimates for Food Other Than Fish

Data from the U.S. FDA TDS (described in Section 5.4.3.1) suggest that nonfish dietary items generally account for a very small fraction of total mercury intake. For the purpose of estimating methylmercury intake from nonfish foods, the central tendency estimate of methylmercury concentration is assumed to be zero. Thus, the average daily intake is zero mg/kg-day for adults in the general

Table 5-9. Predicted Methylmercury Concentrations in Produce and Animal Products Based on a Large Hospital Waste Incinerator Scenario

Item	Total Mercury (ng/g dry wt.)	% Methylmercury	Methylmercury (ng/g dry wt.)
Produce			
Root vegetables	1.9	5	0.095
Fruits	35	5	1.7
Fruiting vegetables	35	5	1.7
Leafy vegetables	34	21	7.1
Animal Products			
Beef	8.6	19	1.6
Beef liver	22	19	4.2
Dairy	11	19	2.1
Pork	0.007	18	0.0013
Poultry	0.12	3	0.0036
Eggs	0.12	3	0.0036
Lamb	3.9	19	0.74

[a]Data based on ISC simulation for receptors at a humid site 2.5 km from a large hospital hazardous materials incinerator (HMI) and input from RELMAP (East 50th Percentile).
Source: U.S. EPA (1997b)

population, children, and women of childbearing age. This estimate is in agreement with WHO (1990), which reported that nonfish foods accounted for 0% of average daily intake of methylmercury.

Methylmercury intake from animal products and produce has been estimated by computer model simulation for four hypothetical high-end exposure scenarios: rural subsistence farmer (adult and child), rural home gardener (adult and child), urban high-end adult, and high-end fisher (adult and child) (U.S. EPA, 1997c). These predicted methylmercury intakes are presented in Table 5-10. Methylmercury intake from animal products was estimated only for the rural subsistence farmer. Intake from animal products and produce was not considered in the remaining scenarios. The subsistence farmer was anticipated to represent a very high-end exposure scenario. Simulation of intake for these scenarios employed a body-weight exposure assumption for children (i.e., 17 kg) that differs from the currently recommended value (i.e., 30 kg) for derivation of water quality criterion values (see Table 5-1). Estimated exposure from produce for several high-end scenarios ranged from 2.3×10^{-7} mg/kg-day for the

high-end urban adult to 5.8 x 10^{-5} mg/kg-day for the adult high-end fisher. Estimated exposures from animal products for the rural subsistence farmer scenario were 2.1 x 10^{-6} mg/kg-day and 5.3 x 10^{-6} mg/kg-day for an adult and child, respectively. These model-predicted estimates support the finding of generally low methylmercury intake from nonfish foods indicated by measurement data from the TDS (U.S. FDA, 1999) and the conclusion in the MSRC that substantially all exposure to methylmercury is from fish consumption.

5.4.4 Fish Consumption Estimates

The MSRC concluded that most human exposure to methylmercury is from food and that it is primarily from fish consumption (U.S. EPA, 1997g). Ingestion of contaminated fish is also reported by many other authors to be the only significant source of methylmercury exposure to the general human population (Stern, 1993; Swedish EPA, 1991; WHO, 1990). This conclusion is based on the observation that in many nonfish foods, the mercury content is typically near detection limits and is comprised mainly of inorganic species (WHO, 1990). In contrast, most of the mercury in fish is methylated.

This section provides information on measured and predicted tissue concentrations of methylmercury in freshwater fish and marine fish, and estimates of intake for several target populations. The MSRC presented data for freshwater fish and marine fish. The MSRC did not include a separate evaluation of estuarine fish, although the data on marine species presented in the MSRC (from the National Marine Fisheries Service) include some estuarine species. Sections 5.4.4.1 and 5.4.4.2, below, summarize the major studies presented in the MSRC for freshwater fish. Section 5.4.4.3 presents an estimate of intake for both freshwater and estuarine species. Although the intake estimate is based on the freshwater fish methylmercury concentrations only, EPA believes that the freshwater fish concentrations are similar to the concentrations in these estuarine species presented in the MSRC. EPA, therefore, believes that calculating an intake estimate using the freshwater/estuarine default consumption rates provides a reasonable approximation of combined freshwater/estuarine fish methylmercury exposure. A more accurate estimate of marine fish methylmercury intake has been made (Section 5.4.4.7) since this source of exposure is included in the RSC estimate that is factored into the final water quality criterion calculation.

Table 5-10. Predicted Methylmercury Intake from Dietary Items Based on Five Hypothetical High-End Exposure Scenarios

Parameter	Exposure Scenario[a]										
	Rural Subsistence Farmer		Rural Home Gardener		Urban				High End Fisher		Recreational Angler
	Adult	Child	Adult	Child	Adult Average	Adult High-end	Child Average	Child High-end	Adult	Child	Adult
Body Weight (kg)	70	17	70	17	70	70	17	17	70	17	70
Fraction of Total Mercury From All Sources[b] That Is Methylmercury[c] (%)	10	13	6	6	2	6	2	2	99	99	100
Total Methylmercury Ingestion-All Modeled Sources[b] (mg/kg-day)	4.1E-06	6.9E-06	5.9E-07	7.8E-07	4.0E-09	2.4E-07	3.2E-08	1.2E-06	1.1E-03	1.6E-03	5.6E-04
Fraction of Total Mercury in Produce That Is Methylmercury[d] (%)	6	6	6	6	NA	6	NA	NA	6	6	NA
Methylmercury Intake From Produce (mg/kg-day)	1.7E-06	1.4E-06	5.8E-07	6.6E-07	NA	2.3E-07	NA	NA	5.8E-05	6.6E-07	NA
Fraction of Total Mercury in Animal Products that is Methylmercury[e] (%)	19	19	NA	NA	NA	NA	NA	NA	NA	NA	NA
Methylmercury Intake From Animal Products (mg/kg-day)	2.1E-06	5.3E-06	0	0	NA	NA	NA	NA	0	NA	NA

[a]Data based on ISC simulation for receptors at a humid site 2.5 km from a large hospital medical waste incinerator (HMI) and input from RELMAP (East 50th Percentile)
[b]All sources includes intake from fish, water, soil, produce, and animal products.
[c]Predicted fraction of total mercury that is ingested from all sources as methylmercury.
[d]Predicted fraction of total mercury that is ingested from produce as methylmercury.
[e]Predicted fraction of total mercury that is ingested from animal products as methylmercury.
NA Not available
Source: U.S. EPA (1997c)

5.4.4.1 Measured Concentrations in Freshwater Fish

Data for mercury concentrations in freshwater fish have been previously compiled and evaluated by EPA in Volume IV of the MSRC (U.S. EPA, 1997c). The discussion below provides information on the national studies considered and the database selected by U.S. EPA after careful consideration of data quality issues to provide concentration data for estimating human exposure to methylmercury (U.S. EPA, 1997c).

Two national studies were considered by U.S. EPA (1997c) for estimation of mercury concentrations in freshwater finfish populations. Lowe et al. (1985) reported mercury concentrations in fish from the National Contaminant Biomonitoring Program. The freshwater fish data were collected between 1978-1981 at 112 stations located across the United States. Mercury was measured by a flameless cold vapor technique, with a detection limit of 0.01 μg/g wet weight. Most of the sampled fish were taken from rivers (93 of the 112 sample sites were rivers); the other 19 sites included larger lakes, canals, and streams. Fish weights and lengths were consistently recorded. The mercury concentrations measured in this study are shown in Table 5-11. Several varieties of fish were sampled. Carp, large mouth bass, and white sucker were most common. The geometric mean mercury concentration of all sampled fish was 0.11 μg/g wet weight; the minimum and maximum concentrations reported were 0.01 and 0.77 μg/g wet weight, respectively. The highest reported mercury concentrations (0.77 μg/g wet weight) occurred in a northern squawfish collected from the Columbia River. Mean mercury concentrations (whether geometric or arithmetic mean not specified) by species are reported in the MSRC (U.S. EPA, 1997c).

A national study of chemical residues in freshwater fish was conducted by U.S. EPA (1992b) and also reported by Bahnick et al. (1994). As reported in the MSRC (U.S. EPA, 1997c), five bottom-feeding species (e.g., carp) and five game fish species (e.g., bass) were sampled at each of the 314 sampling sites in the United States. These sites were selected based on proximity to either point or nonpoint pollution sources. Thirty-five "remote" sites among the 314 total sites were included to provide nonimpacted background pollutant concentrations. The study primarily targeted sites that were expected to be impacted by increased dioxin levels. The point sources proximate to sites of fish collection included the following: pulp and paper mills, Superfund sites, publicly owned treatment works (POTWs), and other industrial sites. Data describing fish age, weight, and sex were not consistently collected. Whole body mercury concentrations were determined for bottom feeders, and mercury concentrations in fillets were analyzed for the game fish. Total mercury levels were analyzed using flameless atomic absorption,

with reported detection limits of 0.05 μg/g early in the study (465 samples analyzed prior to 1990) and 0.0013 μg/g later in the study (195 samples), as the analytical technique improved. Nondetects were reported as a zero value and averaged as zeros. The estimated standard deviation for replicate samples was 0.047 μg/g in the concentration range of 0.08 to 1.79 μg/g. Mercury was detected in fish collected from 92% of the sample sites. Concentration data are provided in Table 5-12. The maximum mercury level detected was 1.8 μg/g, and the mean concentration in 669 fish samples across all sites was 0.26 μg/g. The highest measurements occurred in walleye, largemouth bass, and carp. The mercury concentrations in measured in fish around POTWs were the highest among all point source data; the median value for mercury concentration was 0.61 μg/g.

The intake estimates presented in this document, similar to the MSRC, are based on the mean concentration values from the studies described above; that is, the fish mercury concentration data based on the Bahnick et al. (1994) and Lowe et al. (1985) studies were used for the estimates. However, the MSRC also includes summary data from numerous other studies that indicate significantly higher levels of methylmercury in freshwater fish. For example, concentrations of methylmercury in bass, crappie, northern pike, and trout of 2.0, 1.39, 1.71, and 1.19 μg/g, respectively, represent a few of the higher species concentrations reported (see U.S. EPA, 1997d, Table 4-48).

Measurements of elevated levels of mercury in fish have been reported elsewhere. For example, the North East States Coordinated Air Use Management (NESCAUM) summarized data from New England's freshwater fish in the "Mercury Study: A Framework for Action" by the Northeast States and Eastern Canadian Provinces (1998) (see Table 5-11).

Additional data are available for New York State (Simonin and Meyer, 1998). In New York State, maximum mercury concentrations over 2 ppm were seen for the following species: walleye (3.2 ppm), striped bass (5.4 ppm), white perch (3.2 ppm) Northern pike (2.1 ppm), smallmouth bass (3.34 ppm), largemouth bass (2.39 ppm), rock bass (2.7 ppm), drum (1.4 ppm), channel catfish (2.0 ppm), sunfish (1.2 ppm), American eel (1.6 ppm), Lake trout (2.7 ppm), white sucker (1.2 ppm), black crappie (1.4 ppm), and carp (5.8 ppm).

5.4.4.2 Predicted Concentrations in Freshwater Fish

As previously indicated, the MSRC included numerous computer-simulated estimates of mercury exposure for selected population scenarios (U.S. EPA, 1997c). These included predicted concentrations

in Tier 4 (predatory) fish based on exposure modeling. The Industrial Source Code air dispersion model (ISC3) was used for simulation of methylmercury concentrations in water and biota near mercury emissions sources. Model plants (large and small municipal waste combustors, large and small hazardous materials incinerators, coal and oil-fired utility boilers, chlor-alkali plant), defined as hypothetical facilities which were developed to represent actual emissions from existing industrial processes and combustion sources, were situated in hypothetical locations intended to simulate a site in either the Western or Eastern United States (U.S. EPA, 1997b). Input values for air concentrations consisted of simulated concentration results (50^{th} and 90^{th} percentile values) obtained using the regional Lagrangian model of air pollution (RELMAP). The assumptions and inputs utilized in this modeling effort are described in detail in Volume III of the MSRC (U.S. EPA, 1997b).

Fish tissue methylmercury concentrations of 5.3×10^{-1} µg/g and 9.7×10^{-2} µg/g were predicted for the simulated Eastern and Western sites, respectively, in scenarios where the hypothetical emission sources had zero percent impact on local mercury levels (i.e., the predicted concentration resulted only from background levels of mercury in the environment and regional anthropogenic sources). These levels are of the same order of magnitude as the mean measured values of 0.11 and 0.26 µg/g (1.1×10^{-1} and 2.6×10^{-1} µg/g) reported by Lowe et al. (1985) and Bahnick et al. (1994) respectively. The maximum predicted tissue concentration of 68 µg/g was associated with the Eastern site chlor-alkali plant scenario.

5.4.4.3 Intake Estimates from Freshwater/Estuarine Fish

The mercury concentration data reported in U.S. EPA (1992b) and Bahnick et al. (1994) were selected to determine a rough estimate of methylmercury intake from freshwater and estuarine fish. In contrast to the data reported by Lowe et al. (1985), the selected study provides an arithmetic mean as a measure of central tendency. These data have previously been used by U.S. EPA (1997d) to calculate methylmercury intake estimates under different fish ingestion scenarios. In this section, new estimates of methylmercury intake are calculated in accordance with technical guidance provided in the 2000 Human Health Methodology (U.S. EPA, 2000a). Using the mean mercury concentration of 0.26 µg mercury/g fish wet weight (or mg/kg) reported by U.S. EPA (1992b) and Bahnick et al. (1994), and assuming that approximately 100 percent is methylmercury (U.S. EPA, 1997d), the average estimated methylmercury concentration in freshwater/estuarine fish is 0.26 mg/kg.

Table 5-11. Freshwater Fish Mercury Concentrations from Lowe et al. (1985) and Northeast States and Eastern Canadian Provinces (1998)

Lowe et al. (1985)	
Fish Species	***Mean Mercury Concentration (µg/g Wet Wt)***
Bass	0.157
Bloater	0.093
Bluegill	0.033
Smallmouth Buffalo	0.096
Carp, Common	0.093
Catfish (channel, largemouth, rock, striped, white)	0.088
Crappie (black, white)	0.114
Freshwater Drum	0.117
Northern Squawfish	0.33
Northern Pike	0.127
Perch (white and yellow)	0.11
Sauger	0.23
Sucker (bridgelip, carpsucker, klamath, largescale, longnose, rivercarpsucker, tahoe)	0.114
Trout (brown, lake, rainbow)	0.149
Walleye	0.1
Mean of Measured Fish	0.11[a]
Northeast States and Eastern Canadian Provinces (1998)	
Fish Species	***Maximum Mercury Concentration in ppm***
Largemouth bass	8.94
Smallmouth bass	5.0
Yellow perch	3.15
Chain pickerel	2.81
Lake trout	2.70
Walleye	2.04
Brown bullhead	1.10
Brook trout	0.98

[a]Geometric mean; U.S. EPA (1997c) did not specify whether means for individual species or species categories were geometric or arithmetic means.

Source: U.S. EPA (1997c), Northeast States and Eastern Canadian Provinces (1998).

To estimate daily exposure from methylmercury in freshwater/estuarine fish, average body weights and high-end fish ingestion rates (90th percentile) for the populations of concern are estimated, as recommended in the 2000 Human Health Methodology. Default intake values for fish intake by children, women of child-bearing age, and adults in the general population are provided in U.S. EPA (2000a). These intake values were estimated from the on-going, nationally based Continuing Survey of Food Intake for Individuals (CSFII) conducted by the U.S. Department of Agriculture. The CSFII is conducted annually, and dietary data from all 50 States are collected (U.S. EPA, 2000a). The estimates of intake based on CSFII incorporated data for both consumers and nonconsumers of fish, and represent intake of all fish whether store-bought or sport-caught (U.S. EPA, 2000a). The freshwater/estuarine fish ingestion rates for children, women of child-bearing age, and adults in the general population are estimated to be 156.3 g/day, 165.5 g/day, and 17.5 g/day, respectively (U.S. EPA, 2000a). Note that the estimates for both children and women of childbearing age are based on short-term consumption, whereas the estimate for adults in the general population is based on average long-term consumption.

Table 5-12. Freshwater Fish Mercury Concentrations from Bahnick et al. (1994).

Species	**Mean Mercury Concentration (µg/g Wet Wt)**
Carp	0.11
Sucker (white, redhorse, spotter)	0.167
Catfish (channel and flathead)	0.16
Bass (white, largemouth, smallmouth)	0.38
Walleye	0.52
Northern Pike	0.31
Crappie	0.22
Brown Trout	0.14
Mean of Measured Fish	0.26

Source: U.S. EPA (1997c)

The recommended body weights for children 0 to 14 years, women of childbearing age, and adults in the general population are 30 kg, 67 kg, and 70 kg, respectively (U.S. EPA, 2000a). Based on these exposure assumptions, the daily exposure estimates of methylmercury intake from ingestion of freshwater/estuarine fish for children, women of childbearing age, and adults in the general population are 1.4×10^{-3} mg/kg-day, 6.4×10^{-4} mg/kg-day, and 6.5×10^{-5} mg/kg-day, respectively. Input assumptions and calculated daily exposure estimates for freshwater/estuarine fish are summarized in Table 5-13.

5.4.4.4 Measured Concentrations in Marine Fish and Shellfish

The MSRC (U.S. EPA, 1997b,c) has summarized data on concentrations of total mercury and methylmercury in marine fish and shellfish. Analyses of total mercury concentrations in marine fish and shellfish have been carried out over the past two to three decades. Data describing methylmercury concentrations in marine fish are predominantly based on the National Marine Fisheries Service (NMFS) database, the largest publicly available database on mercury

Table 5-13. Freshwater/Estuarine Fish Intake Assumptions and Estimates

Population of Concern	Mercury in Fish[a] (mg/kg)	Methyl-mercury/ Mercury in Fish[b] (%)	Methyl-mercury in Fish (mg/kg)	Ingestion Rate[c] (kg/day)	Body Weight[c] (kg)	Daily Exposure Estimate (mg/kg-day)
Children	0.26	100	0.26	0.1563	30	1.4×10^{-3}
Women of Childbearing Age	0.26	100	0.26	0.1655	67	6.4×10^{-4}
Adults in the General Population	0.26	100	0.26	0.0175	70	6.5×10^{-5}

[a] U.S. EPA (1992b) and Bahnick et al. (1994)
[b] U.S. EPA (1997c)
[c] U.S. EPA (2000a)

concentrations in marine fish. In the early 1970s, the NMFS conducted testing for total mercury in more than 200 seafood species of commercial and recreational interest (Hall et al., 1978). The determination of mercury in fish was based on flameless (cold vapor) atomic absorption spectrophotometry following chemical digestion of the fish sample. These analytical methods are described in Hall et al. (1978).

The NMFS Report provides data on number of samples, the number of samples where mercury was not detected ("nondetects"), and mean, standard deviation, minimum, and maximum detected mercury levels (in parts per million wet weight) for 1,333 combinations of fish/shellfish species, variety, location caught, and tissue (Hall et al., 1978). This database consists of 777 fish/shellfish species for which mercury concentration data are provided. This represents 5,707 analyses of fish and shellfish tissues for total mercury, of which 1,467 or 26%, were reported at nondetectable levels. A discussion of the issues associated with evaluation and use of nondetect data for methylmercury in the NMFS database is provided in the MSRC (U.S. EPA, 1997c). A summary of NMFS concentration data is provided in Table 5-14.

Two additional databases for mercury concentration in marine fish and shellfish are cited in the MSRC (U.S. EPA, 1997d). These are the *Report on the Chance of U.S. Seafood Consumers Exceeding "The Current Daily Intake for Mercury and Recommended Controls"* (U.S. FDA, 1978) and a report by Stern et al. (1996) that examined exposure of New Jersey residents to mercury via fish consumption. Although concentration data from these databases are reported in the MSRC (U.S. EPA, 1997c), detailed descriptions and evaluations of study quality are not provided.

The intake estimates presented in this document, similar to the MSRC, are based on the mean concentration values from the studies described above; that is, the fish mercury concentration data based on the NMFS, Stern et al., and U.S. FDA studies were used for the estimates. However, the MSRC also includes summary data from numerous other studies that indicate significantly higher levels of methylmercury in marine fish. For example, concentrations of methylmercury in mackerel, pompano, shark, snapper, and swordfish of 2.9, 8.42, 4.53, 2.17, and 2.72 μg/g, respectively, represent a few of the higher species concentrations reported (see U.S. EPA, 1997c).

5.4.4.5 Other Measured Concentration Data for Marine Fish and Shellfish

Additional national-scope information on methylmercury in marine biota is available from Project Mussel Watch. Project Mussel Watch measures concentrations of organic and trace metal contaminants

in fresh, whole soft-parts of bivalve mollusks (i.e., mussels and oysters) at more than 240 coastal and estuarine sites. Data are currently available from 1986 through 1993 and are summarized in the MSRC (U.S. EPA, 1997b). Average concentrations along the North Atlantic, Eastern Gulf, and Pacific coasts (0.15, 0.14, and 0.11 μg/g dry weight, respectively) are higher than those collected along the Middle Atlantic, South Atlantic, and Western Gulf coasts (0.06, 0.09, and 0.08 μg/g dry weight, respectively). The highest concentrations exceeded 1.0 μg/g dry weight and were collected along the Western Gulf and Pacific coasts (1.80 and 1.01 μg/g dry weight, respectively).

Annual Mussel Watch data on mercury concentrations in bivalve mollusks at specific sites have been aggregated to national geometric means for the purpose of analyzing temporal trends (O'Conner and Beliaeff, 1995). The national means do not show any temporal trend in mercury concentrations in mussels and oysters for the period 1986-1993. Temporal trend analysis was also conducted on a site-by-site basis for 154 Mussel Watch sites for which there were data for at least 6 years during the period of 1986-1993 (O'Conner and Beliaeff, 1995). Seven sites exhibited an increasing trend in mercury concentrations, and eight sites exhibited a decreasing trend in mercury concentrations, with 95% statistical confidence.

5.4.4.6 Predicted Concentrations in Marine Fish and Shellfish

The computer simulations conducted by EPA and reported in the MSRC (U.S. EPA, 1997c) did not provide predictions for methylmercury concentrations in marine fish or shellfish.

5.4.4.7 Intake Estimates from Marine Fish and Shellfish

In accord with technical guidance provided in U.S. EPA (2000a), mean, median, and 90th percentile concentrations of methylmercury in marine fish were used to estimate daily exposure from methylmercury in marine fish. Species-specific mean concentrations of mercury in marine fish from the National Marine Fisheries Service (NMFS, 1978) are presented in EPA's MSRC (U.S. EPA, 1997c). These data are summarized in Table 5-14. For species where concentration was not reported in NMFS (1978), concentrations were estimated from data reported by Stern et al. (1996), U.S. FDA Compliance Testing data, or U.S. FDA (1978) as cited in U.S. EPA (1997c).

Table 5-14. Average Mercury Concentrations in Marine Fish and Shellfish

Species	Concentration[a] (μg Hg/g Wet Wt.)	Species	Concentration (μg Hg/g Wet Wt.)
Finfish			
Anchovy	0.047	Pompano*	0.104
Barracuda, Pacific	0.177	Porgy*	0.522[b]
Cod*	0.121	Ray	0.176
Croaker, Atlantic	0.125	Salmon*	0.035
Eel, American	0.213	Sardines*	0.1
Flounder*[,e]	0.092	Sea Bass*	0.135
Haddock*	0.089	Shark*	1.327
Hake	0.145	Skate	0.176
Halibut*	0.25	Smelt, Rainbow*	0.1
Herring	0.013	Snapper*	0.25
Kingfish	0.10	Sturgeon	0.235
Mackerel*	0.081	Swordfish*	0.95[c]
Mullet	0.009	Tuna*	0.206
Ocean Perch*	0.116	Whiting (silver hake)*	0.041
Pollock*	0.15	Whitefish*	0.054[d]
Shellfish			
Abalone	0.016	Oysters	0.023
Clam*	0.023	Scallop*	0.042
Crab*	0.117	Shrimp	0.047
Lobster*	0.232	Other shellfish*	0.012[b]
Molluscan Cephalopods			
Octopus*	0.029	Squid*	0.026

Source: U.S. EPA (1997c).

*Denotes species used in calculation of methylmercury intake from marine fish for one or more populations of concern, based on existence of data for consumption in the CSFII (U.S. EPA, 2000b).

[a] Mercury concentrations are from NMFS (1978) as reported in U.S. EPA (1997d) unless otherwise noted, measured as μg of total mercury per gram wet weight of fish tissue.

[b] Mercury concentration data are from Stern et al. (1996) as cited in U.S. EPA (1997c).

[c] Mercury concentration data are from U.S. FDA Compliance Testing as cited in U.S. EPA (1997c).

[d] Mercury concentration data are from U.S. FDA (1978) as cited in U.S. EPA (1997c).

[e] Mercury data for flounder were used as an estimate of mercury concentration in marine flatfish in marine intake calculations

A consumption-weighted mean concentration of mercury for all marine fish was calculated as follows. Each of the marine species selected for inclusion in the analysis was weighted based on species-specific U.S. population intake rates among the three populations of concern (U.S. EPA, 2000b). This weighting system accounts for variability of consumption among different species and across different populations of concern. The consumption weighting factor for each of the selected marine species was calculated as follows. The consumption rates for individual marine species were summed to give a total consumption rate for a particular population of concern. The weighting factor was then calculated as the quotient of the species-specific consumption rate divided by the total consumption rate:

$$\text{Weighting factor for species A} = \frac{\text{Species A consumption rate (g/day)}}{\text{Sum of consumption rates for all selected species (g/day)}}$$

For each population of concern, the average mercury concentration for each species was multiplied by its consumption weighting factor. This product was then summed across all selected marine species to estimate the mean concentration of mercury in all marine fish for that particular population of concern:

$$\text{Mean conc}(\mu g/g) = \sum [\text{species-specific conc}(\mu g/g) \times \text{species-specific weighting factor}]$$

Assuming that approximately 100% of the mercury in marine fish is present as methylmercury (U.S. EPA, 1997c), the weighted-average methylmercury concentrations in marine fish consumed by each of the populations of concern are 0.167 mg/kg, 0.147 mg/kg, and 0.157 mg/kg for children (aged 0-14 years), women of childbearing age, and adults in the general population, respectively.

Specific body weights and several fish ingestion rates (arithmetic mean, median and 90th percentile) for the populations of concern were used to estimate daily exposure from methylmercury in marine fish. Marine fish intake values for children, women of childbearing age, and adults in the general population are provided in U.S. EPA (2000b). For children and women of childbearing age, these intake values were estimated using 3 years of "consumers only" data (1994-1996) from the on-going, nationally based Continuing Survey of Food Intake for Individuals (CSFII) conducted by the U.S. Department of Agriculture. Intake values for adults in the general population were obtained using all survey respondents to derive an estimate of long-term consumption. The marine fish ingestion rates for children, women of childbearing age, and adults in the general population are presented in Table 5-15.

The current default body weights for children 0 to 14 years, women of childbearing age, and adults in the general population are 30 kg, 67 kg, and 70 kg, respectively (U.S. EPA, 2000a). Based on these exposure assumptions, the mean daily exposure estimates of methylmercury intake from ingestion of marine fish for children, women of childbearing age, and adults in the general population are 4.1×10^{-4} mg/kg-day, 2.0×10^{-4} mg/kg-day, and 2.7×10^{-5} mg/kg-day, respectively. The median daily exposure estimates of methylmercury intake from ingestion of marine fish for children, women of childbearing age, and adults in the general population are 3.2×10^{-4} mg/kg-day, 1.6×10^{-4} mg/kg-day, and 0 mg/kg-day, respectively. In addition, the 90th percentile daily exposure estimates of methylmercury intake from ingestion of marine fish for children, women of childbearing age, and adults in the general population are 8.5×10^{-4} mg/kg-day, 4.1×10^{-4} mg/kg-day, and 1.1×10^{-4} mg/kg-day, respectively. Input assumptions and calculated daily exposure estimates for marine fish are summarized in Table 5-16.

5.4.5 Respiratory Exposures

5.4.5.1 Measured Concentrations in Air

Outdoor Air. Vapor-phase elemental mercury is the predominant form of mercury in the atmosphere and constitutes up to 98% of the total mercury concentration (U.S. EPA, 1997b). Increased

Table 5-15. Marine Fish Ingestion Rates

Population of Concern	Mean Intake (kg/day)	Median Intake (kg/day)	90th Percentile Intake (kg/day)
Children	0.07490	0.05971	0.15229
Women of Childbearing Age	0.09104	0.07548	0.18835
Adults in the General Population	0.01246	0	0.04916

Source: U.S. EPA (2000b)

concentrations of the divalent form of mercury may be present near emission sources. Small fractions of particulate mercury and methylmercury may also be present. Atmospheric mercury concentrations in the United States are generally very low (U.S. EPA, 1997b). U.S. EPA (1993a) as cited in the MSRC summarized information on total mercury concentrations in outdoor air and reported ranges of 1 to 4 ng/m^3 for rural areas and 10 to 170 ng/m^3 for urban areas. Methylmercury concentrations from these samples constituted 0% to 21% of the total mercury concentration, with percentage values reported to generally be on the low end of this range. A measure of central tendency was not provided with this estimate. Particulate mercury typically constituted less than 4% of total atmospheric mercury in rural areas, although this fraction was increased in urban areas. The current background mercury concentration over the Northern Hemisphere is considered to be between 1.5 and 2.0 ng/m^3 (Expert Panel on Mercury Atmospheric Processes, 1994). A background concentration of 1.6 ng/m^3 was reported by Fitzgerald (1994). This value was subsequently used by U.S. EPA (1997b) to model mercury fate in watershed soils and surface waters.

Bloom and Fitzgerald (1988) measured vapor-phase mercury concentrations in outdoor air samples collected from Long Island Sound, CT. Total mercury concentrations ranged from 1.4 to 5.3 ng/m^3. The fraction of total mercury present as methylmercury was estimated to be 0% to 1%. During the month of October, the mean methylmercury concentration was 12 pg/m^3 (range 4 to 38 pg/m^3). This concentration represented 0.7% of the total gaseous mercury concentration. During the month of November, the measured methylmercury concentration was less than 10 pg/m^3 and from December through August, the concentration was below the detection limit of 5 pg/m^3.

Indoor Air. No data were identified for indoor air concentrations of methylmercury.

5.4.5.2 Predicted Concentrations in Air

EPA has modeled mercury air concentrations for the continental United States using RELMAP simulation, meteorological data for the year 1989, and current mercury emission data. The background level of mercury in the atmosphere was assumed to be 1.6 ng/m^3. The results of this simulation are reported in (U.S. EPA, 1997b). Predicted concentrations for total mercury are given in Table 5-17. The predicted total mercury concentrations ranged from approximately 1.6 to 1.9 ng/m^3, with the highest concentrations predicted for the Eastern United States. The tabulated results indicate that total

Table 5-16. Intake Assumptions and Estimates for Marine Fish

Population of Concern[a]	Mercury in Marine Fish (mg/kg)	Methylmercury/ Mercury in Marine Fish %	Methylmercury in Marine Fish (mg/kg)	Body Wt. (kg)	Mean Daily Exposure Estimate (mg-kg-day)	Median Daily Exposure Estimate (mg-kg-day)	90th%Daily Exposure Estimate (mg-kg-day)
Children	1.67E-01	100%	1.67E-01	30	4.1E-04	3.2E-04	8.3E-04
Women of Childbearing Age	1.47E-01	100%	1.47E-01	67	2.0E-04	1.6E-04	4.1E-04
Adults in the General Population	1.57E-01	100%	1.57E-01	70	2.7E-05	0.0E+00	1.1E-04

[a] Marine fish intake assumptions for the populations of concern from U.S. EPA (2000b) are summarized in Table 5-15.

Table 5-17. Percentile Analysis of RELMAP Predicted Total Mercury Concentration Results (ng/m^3) for the Continental United States

Region	Min	10th	50th	90th	Max
Continental U.S.	1.602	1.607	1.624	1.685	1.995
East of 90° W longitude	1.616	1.640	1.668	1.720	1.995
West of 90° W longitude	1.602	1.606	1.616	1.642	1.743

Source: U.S. EPA (1997b)

mercury concentration never exceeded the background level by a large percentage (25% maximum) under the conditions of this simulation. Methylmercury concentration estimates were not provided in the model output as reported in the MSRC (U.S. EPA, 1997b) but, again, is presumed to be present predominantly as elemental mercury.

5.4.5.3 Intake Estimates for Air

The primary species of mercury to which humans are exposed through inhalation is vapor-phase elemental mercury (U.S. EPA, 1997g). Thus, inhalation exposure to methylmercury is not expected to be a significant route of concern when compared to intake via fish consumption.

Assuming the background mercury concentration of 0.0016 $\mu g/m^3$ (or 1.6 ng/m^3) reported by Fitzgerald (1994), of which approximately one percent is methylmercury (Bloom and Fitzgerald, 1988), the average methylmercury concentration in air is 0.000016 $\mu g/m^3$ (or 1.6×10^{-8} mg/m^3). Estimates of daily exposure from methylmercury in air were calculated using inhalation rates and body weights specific to the populations of concern. The long-term inhalation rate based on a time-weighted average for children 0 to 14 years is estimated to be 10.4 m^3/day (U.S. EPA, 1997h). The average, long-term inhalation rates for women of childbearing age and adults in the general population are estimated to be 11 m^3/day and 20 m^3/day, respectively (U.S. EPA, 1994, 1997h). The recommended body weights for children 0 to 14 years, women of childbearing age, and adults in the general population are 30 kg, 67 kg, and 70 kg, respectively (U.S. EPA, 2000a). Based on these exposure input assumptions, the daily exposure estimates from methylmercury in air for children 0 to 14 years, women of childbearing age, and adults in the general population are 5.5×10^{-9} mg/kg-day, 2.6×10^{-9} mg/kg-day, and 4.6×10^{-9} mg/kg-day, respectively. These input assumptions and calculated daily exposure estimates for air are presented in Table 5-18.

U.S. EPA (1997c) reported inhalation exposure estimates based on ISC simulation for a humid site 2.5 km from a large hospital medical waste incinerator (HMI) and input from RELMAP (Eastern U.S., 50th percentile) (Table 5-19). The inhalation parameters used in the simulation for children (16 m^3/day) differed from the rate adopted from U.S. EPA (1997h) for calculation of inhalation intake from measured concentrations (see Table 15-1). Estimated intake for all five exposure scenarios was zero mg/kg-day. This prediction supports the finding of low methylmercury intake via inhalation as calculated from measured concentrations.

Table 5-18. Inhalation Exposure Intake Assumptions and Estimates

Population of Concern	Mercury in Air[a] (mg/m^3)	Methyl-mercury/ Mercury in Air[b] (%)	Methyl-mercury in Air (mg/m^3)	Inhalation Rate[c] (m^3/day)	Body Weight[d] (kg)	Daily Exposure Estimate (mg/kg-day)
Children (0-14 yr)	1.6×10^{-6}	1	1.6×10^{-8}	10.4	30	5.5×10^{-9}
Women of Childbearing Age	1.6×10^{-6}	1	1.6×10^{-8}	11	67	2.6×10^{-9}
Adults in the General Population	1.6×10^{-6}	1	1.6×10^{-8}	20	70	4.6×10^{-9}

[a] Fitzgerald (1994) as cited in U.S. EPA (1997b).
[b] Bloom and Fitzgerald (1988) as cited in U.S. EPA (1997b).
[c] Inhalation rates from U.S. EPA (1994, 1997h).
[d] Current default body weight values from U.S. EPA (2000a).

Table 5-19. Predicted Methylmercury Intake from Air for Five Hypothetical High-End Exposure Scenarios

Parameter	Exposure Scenario[a]										
	Rural Subsistence Farmer		Rural Home Gardener		Urban				High End Fisher		Recrea-tional Angler
	Adult	Child	Adult	Child	Adult Aver-age	Adult High-end	Child Aver-age	Child High-end	Adult	Child	Adult
Inhalation Rate (m^3/day)	20	16	20	16	20	20	16	16	20	16	20
Contact Rate for Inhalation (hr/day)	24	24	24	24	24	16	24	24	24	24	24
Body Weight (kg)	70	17	70	17	70	70	17	17	70	17	70
Methylmercury Intake (mg/kg-day)	0	0	0	0	0	0	0	0	0	0	0

[a]Data based on ISC simulation for a receptors at a humid site 2.5 km from a large Hospital medical waste incinerator (HMI) and input from RELMAP (East 50th Percentile).
Source: U.S. EPA (1997c)

5.4.6 Soil/Sediment Exposures

5.4.6.1 Measured Concentrations in Soil/Sediment

The available data for measured methylmercury and total mercury concentrations in soils and sediments are summarized in Table 5-20, including a small number of studies that provide some data that are national in scope. In general, soil mercury levels are usually less than 200 ng/g in the top soil layer, but values exceeding this level are not uncommon, especially in areas affected by anthropogenic activities (U.S. EPA, 1997b). Soil mercury levels vary greatly with depth, with nearly all the mercury found in the top 20 cm of soil. Mercury levels are positively correlated with the percentage of organic matter in soil (Nriagu, 1979).

Some information is available on estimated typical or background levels of total mercury in U.S. soils and may be used with speciation data to estimate soil methylmercury concentrations. The MSRC (U.S. EPA, 1997b) states that approximately 1 to 3% of the total mercury in surface soil is methylmercury. The other 97% to 99% of total soil mercury can be considered to be largely Hg(II) complexes, although a small fraction of mercury in typical soil will be Hg^0 (Revis et al., 1990). The methylmercury percentage has been observed to exceed 3% in garden soil with high organic content under slightly acidic conditions (Cappon, 1987). Computer simulations of mercury fate and transport predict that methylmercury constitutes 2% of the total mercury in watershed soils (U.S. EPA, 1997b).

Davis et al. (1997) reported a range of 50 to 200 ng/g for total mercury concentrations in nonmercuriferous soils and sediments in background areas not directly impacted by volcanic emissions or anthropogenic releases. The authors stated that methylmercury typically constitutes 0.01% to 2 % of the total mercury concentration. Supporting information on the derivation of this estimate was not provided by the authors. The MSRC (U.S. EPA, 1997b) cited data from NJDEPE (1993) that indicates that typical U.S. soils contain 8 to 117 ng/g of total mercury. Neither an estimate of mean mercury concentration nor speciation data were provided in the description of this study as summarized in the MSRC. Assuming that approximately 2% of the total mercury concentration is present as methylmercury, these data suggest that typical U.S. soils contain 0.16 to 2.3 ng/g as methylmercury.

Shacklette and Boerngen (1984) reported mean concentrations, geometric standard deviations, and ranges for total mercury in soils and other surficial materials based on samples collected at 1318 sites across the conterminous United States. The geometric mean concentration for the conterminous United States was 58 ± 2,520 ng/g (ppb), and the estimated arithmetic mean was 89 ng/g. Additional data

indicate that the mean concentration of mercury in soils varies by region. In soils from the Western United States (west of the 96th meridian), the geometric mean concentration was 46 ± 2,330 ng/g (range <10 to 4,600 ng/g) and the estimated arithmetic mean was 65 ng/g. In soils from the Eastern United States (east of the 96th meridian), the geometric mean concentration was 81 ± 2,520 ng/g (range 10 to 3,400 ng/g), with an estimated arithmetic mean of 120 ng/g. Speciation data were not reported by these authors. Assuming that methylmercury constitutes approximately 2% of the total mercury concentration, the geometric and arithmetic mean levels of mercury present as methylmercury in soils in the conterminous United States would be approximately 1.2 ng/g and 1.8 ng/g, respectively.

Additional data are available on soil mercury and methylmercury concentrations for sites in the United States. As reported in the MSRC (U.S. EPA, 1997b), methylmercury concentrations in soil samples at locations in New York and Washington ranged from 0.3 to 22.9 ng/g dry weight and constituted 0.5% to 5.3% of the total soil mercury content. No other information on these studies was provided.

As characterized in the MSRC (U.S. EPA, 1997b), sediment mercury levels are typically higher than soil levels, and concentrations exceeding 200 ng/g are not unusual. Sediment mercury levels follow the same trends as soil in regards to depth, humic matter, and methylmercury percentage. There is some evidence suggesting that the methylmercury percentage increases with increasing total mercury contamination (Parks et al., 1989). Concentrations of mercury and (where available) methylmercury are tabulated in Table 5-20.

Table 5-20. Concentrations of Total Mercury and Methylmercury in Soil and Freshwater Aquatic Sediments

Location	Total Mercury (ng/g dry wt)	Methylmercury (ng/g dry wt)	% Methylmercury	Reference
Soils				
Discovery Park, Seattle, WA	29-133	0.3 - 1.3	0.6 - 1.5	Lindqvist et al. (1991)[a]
Wallace Falls, Cascades, WA	155 - 244	1.0 - 2.6	0.5 - 1.2	Lindqvist et al. (1991)[a]
Control Soil New York State	117	4.9	4.2	Cappon, (1981)[a]
Compost New York State	213	7.3	3.3	Cappon, (1987)[a]
Garden Soil New York State	406	22.9	5.3	Cappon, (1987)[a]
Soil and Other Surficial Materials in Conterminous U.S.	Conterminous U.S. 58 (geo mean) 89 (arith mean) Western U.S. 46 (geo mean) 65 (arith mean) Eastern U.S. 81 (geo mean) 120 (arith mean)	NA	NA	Shacklette and Boerngen (1984)
Typical U.S. Soils	8 - 117	NA	NA	NJDEPE (1993)[a]
Typical background levels in nonmercurifer-ous soils	50 - 200	0.01 - 2	NA	Davis et al. (1997)
Freshwater Aquatic Sediments				
80 Minnesota Lakes	34 -753 mean 174	NA	NA	Glass et al. (1990)[a]
North Central Wisconsin lakes	90 -190	NA	NA	Rada et al. (1989)[a]
Little Rock Lake, Wisconsin	10 - 170	NA	NA	Wiener et al. (1990)[a]
U.S. Lake sediment mean ranges	70 - 310	NA	NA	NJDEPE (1993)[a]

Location	Total Mercury (ng/g dry wt)	Methylmercury (ng/g dry wt)	% Methylmercury	Reference
U.S. GS National Pilot Study	105 Background 211 All sites	2.1 Background 1.87 All sites	0.1 1	Krabbenhoft et al. (1999)

[a] As cited in U.S. EPA (1997b)

5.4.6.2 Predicted Concentrations in Soil

The MSRC (U.S. EPA, 1997b) reported the results of watershed fate and transport modeling conducted to predict the concentration of mercury in watershed soils. Atmospheric concentrations and deposition rates were used as inputs to the IEM-2M model. The IEM-2M model is composed of two integrated models that simulate mercury fate using mass balance equations which describe processes in watershed soils and a shallow lake. Using this approach, total mercury concentrations of 47 and 8 ng/g were predicted for soils at hypothetical Eastern and Western U.S. sites, respectively. These predicted concentrations for total mercury in soils are lower than the measured concentrations reported by Shacklette and Boergen (1984) for conterminous and regional U.S. soils. More than 90% of the total mercury in soil was predicted to occur as the inorganic divalent species. The fraction of the predicted background concentration occurring as methylmercury was 2% for the Eastern site (U.S. EPA, 1997c), suggesting a soil methylmercury concentration of 0.9 ng/g based on modeling predictions for speciation. Corresponding speciation data was not reported for the Western site.

5.4.6.3 Intake Estimates for Soil/Sediment

The primary species of mercury in soil is largely considered to be Hg(II) complexes, although a small fraction of mercury in typical soil will be Hg^0 (Revis et al., 1990). Thus, ingestion exposure to methylmercury in soil is not expected to be a significant route of concern when compared to exposure via fish ingestion.

Assuming the background mercury arithmetic mean concentration of 89 ng/g (or 0.089 mg/kg) reported by Shacklette and Boerngen (1984), of which approximately 2% is methylmercury (U.S. EPA, 1997b,c; Cappon, 1987; Davis et al., 1997), the average estimated methylmercury concentration in soil is 1.78 ng/g (or 0.00178 mg/kg). To estimate daily exposure from methylmercury in soil, ingestion rates and body weights for populations of concerns must also be estimated. The average incidental soil ingestion rate for children is estimated to be 1×10^{-4} kg/day (U.S. EPA, 1997h). In addition, the average soil ingestion rate for pica children is estimated to be 1×10^{-2} kg/day (U.S. EPA, 1997h). The average soil ingestion rates for women of child-bearing age and the general adult population are both estimated to

be 5 x 10^{-5} kg/day (U.S. EPA, 1997h). The default body weights for children 0 to 14 years, women of child-bearing age, and adults in the general population are 30 kg, 67 kg, and 70 kg, respectively (U.S. EPA, 2000a). Based on these exposure input assumptions, the daily exposure estimates from methylmercury in soil for children, pica children, women of child-bearing age, and adults in the general population are 5.9 x10^{-9} mg/kg-day, 5.9 x 10^{-7} mg/kg-day, 1.3 x10^{-9} mg/kg-day, and 1.3 x10^{-9} mg/kg-day, respectively. These input assumptions and calculated daily exposure estimates for soil are presented in Table 5-21.

Table 5-21. Summary of Soil Ingestion Intake Assumptions and Estimates

Population of Concern	Mercury in Soil[a] (mg/kg)	Methyl-mercury/ Mercury in Soil[b] (%)	Methyl-mercury in Soil (mg/kg)	Ingestion Rate[c] (kg/day)	Body Weight[d] (kg)	Daily Exposure Estimate (mg/kg-day)
Children	0.089	2	0.00178	0.0001	30	5.9 x10^{-9}
Pica Children	0.089	2	0.00178	0.01	30	5.9 x10^{-7}
Women of Childbearing Age	0.089	2	0.00178	0.00005	67	1.3 x10^{-9}
Adults in the General Population	0.089	2	0.00178	0.00005	70	1.3 x10^{-9}

[a] Shacklette and Boerngen for the conterminous U.S. (1984).
[b] U.S. EPA (1997b,c); Cappon (1987) as cited in U.S. EPA (1997b); Davis et al. (1997).
[c] U.S. EPA (1997h).
[d] U.S. EPA (2000a).

Estimates of soil ingestion based on exposure modeling reported in the MSRC (U.S. EPA, 1997c) are summarized in Table 5-22. Predicted exposures are based on an ISC model simulation for a receptors at a humid site 2.5 km from a large hospital medical waste incinerator (HMI) and input from RELMAP (East 50th Percentile). Soil intake among the hypothetical receptors was highest for the urban pica child (1.2×10^{-6} mg/kg-day). The remaining estimates ranged from 3×10^{-9} to 2.4×10^{-8} mg/kg-day. These approximations are comparable to exposure estimates based on measured concentrations of mercury in soils in Table 5-21 when the twofold difference in assumed soil ingestion rate is considered.

5.4.7 Occupational and Other Exposures

Occupational Exposure. Occupational exposures are not routinely factored into the derivation of water quality criterion but may be considered on a chemical-specific basis. Information on occupational exposure to mercury has been summarized in the MSRC (U.S. EPA, 1997c). OSHA (1975) estimated that approximately 150,000 U.S. workers are exposed to mercury in at least 56 occupations. More recently, Campbell et al. (1992) reported that about 70,000 workers are annually exposed to mercury. Occupational settings in which exposure to mercury may occur include chemical and drug synthesis, hospitals, laboratories, dental practices, instrument manufacture, and battery manufacture (NIOSH, 1977). Jobs and processes involving mercury exposure include manufacture of measuring instruments (barometers, thermometers, etc.), mercury arc lamps, mercury switches, fluorescent lamps, mercury broilers, mirrors, electric rectifiers, electrolysis cathodes, pulp and paper, zinc carbon and mercury cell batteries, dental amalgams, antifouling paints, explosives, photographs, disinfectants, and fur processing.

Inorganic mercury accounts for nearly all occupational exposures (U.S. EPA, 1997c). Airborne elemental mercury vapor is the main pathway of concern, particularly in those industries with the greatest number of mercury exposures. Occupational exposure to methylmercury appears to be insignificant or rare. Thus, occupational exposures are not considered relevant to the derivation of ambient water criteria for methylmercury.

Table 5-22. Predicted Mercury Intake from Soil for Five Hypothetical High-End Exposure Scenarios

Parameter	Exposure Scenario[a]										
	Rural Subsistence Farmer		Rural Home Gardener		Urban				High-End Fisher		Recreational Angler
					Adult		Child				
	Adult	Child	Adult	Child	Average	High-end	Average	Pica	Adult	Child	Adult
Soil Ingestion Rate (g/day)	0.1	0.2	0.1	0.2*	0.1	0.1	0.2*	7.5	0.1	0.2	0.1
Body Weight (kg)	70	17	70	17	70	70	17	17	70	17	70
Total Mercury Intake (mg/kg/day)	1.5E-07	1.2E-06	1.5E-07	1.2E-06	2.0E-07	2.0E-07	1.6E-06	6.1E-05	1.5E-07	1.2E-06	1.5E-07
Fraction of Total Mercury That Is Methylmercury (%)	2	2	2	2	2	2	2	2	2	2	2
Methylmercury Intake (mg/kg/day)	3.0E-09	2.4E-08	3.0E-09	2.4E-08	4.0E-09	4.0E-09	3.2E-08	1.2E-06	3.0E-09	2.4E-08	3.0E-09

[a]Data based on ISC simulation for a receptors at a humid site 2.5 km from a large hospital medical waste incinerator (HMI) and input from RELMAP (East 50th Percentile).

*Soil ingestion rates for rural home gardener and urban child (average) were not available. An ingestion rate of 0.2 g/day was assumed based on the soil ingestion rates for the rural subsistence farmer and high-end fisher children.

Source U.S. EPA (1997c)

Exposure from Dental Amalgam. Gradual erosion of dental amalgam represents a pathway by which many people are routinely exposed to extremely small amounts of mercury. Dental amalgam fillings contain approximately 50% mercury by weight. The mercury in the amalgam is continuously released over time. Speciation data indicate that release occurs primarily as elemental mercury vapor (Begerow et al., 1994). Exposure to methylmercury via this route is thus expected to be insignificant. Therefore, exposure to methylmercury via this pathway is not considered relevant to RSC analysis for derivation of the water quality criterion.

5.5 EXPOSURE DATA ADEQUACY AND ESTIMATE UNCERTAINTIES

After identifying relevant exposure pathways and obtaining available data for quantifying exposure via each pathway, it is important to consider whether the data are adequate to describe exposure estimates for each exposure medium. The adequacy of the contaminant concentration data, in part, determines the specific method with which the RSC estimates will be determined. Important factors include sample size, accurate representation of the sample (e.g., whether sample selection was biased and whether data are current), the accuracy in the sample analysis procedures (i.e., whether errors occurred during measurement), and the sensitivity of the measurement relative to the environmental levels of concern (i.e., whether detection limits are low enough such that the concentration can be detected in most samples within a data set). Additional discussion on data adequacy is provided in the 2000 Human Health Methodology (U.S. EPA, 2000a).

5.5.1 Adequacy of Intake Estimate for Drinking Water

Ground water. Nationally distributed data for methylmercury or total mercury in ground water were not located. The MSRC (U.S. EPA, 1997b) reports data from three local studies in the United States. However, supporting information on sample size, detection limits, analytical methodology, and other information relevant to data adequacy are not provided in the MSRC. Therefore, these data (as presented in the MSRC) do not satisfy the adequacy requirements of the 2000 Human Health Methodology.

Drinking Water. The MSRC (U.S. EPA, 1997b) cited a typical level of 25 ng/L for total mercury concentration in drinking and tap water (Lindqvist and Rodhe, 1985). A range of 0.3 to 25 ng/L for total mercury in drinking water was also reported (NJDEPE, 1993). The presentation of these data in the MSRC did not provide information on the composition of this water (e.g., fraction from ground water and surface water) or treatment status. Furthermore, the presentation of data in the MSRC did not

provide information on the method of calculation or a detailed description of data quality (including source of data, sample size, detection limits, and analysis procedures) for this estimate. Thus, the data for drinking water (as presented in the MSRC) are considered sufficient only for a rough estimate of intake. Yet, using the higher-end value of 25 ng/L results in an estimate within the range estimated for surface water.

Raw surface water. National data for surface water concentrations (primarily stream data) are available from the U.S. Geological Survey National Pilot Study of Mercury Contamination (Krabbenhoft et al., 1999). Water samples were collected in the summer and fall of 1998 and thus are representative of current concentrations. Sampling occurred at 106 sites clustered in 21 basins across the United States, including Alaska and Hawaii. Data from 104 sites were used to determine values for mean, median, maximum, and minimum methylmercury concentrations. The sampling sites spanned the dominant east-to-west mercury deposition gradient and represented a wide range of environmental settings. Total mercury and methylmercury were measured using sensitive analytical methodology (U.S. EPA Method 1631). The detection limits for total mercury and methylmercury were reported in a separate document (Olson and DeWild, 1999) referenced in the report. Some samples were collected at sites impacted by mining activity. The high concentration of mercury in samples collected at those sites resulted in a positively skewed distribution, and this is reflected in the difference between the arithmetic mean and median values for samples collected at all sites (0.15 $\pm$ 0.26 ng/L vs. 0.06 ng/L, respectively). The measures of central tendency from this study compare favorably to a methylmercury concentration of 0.07 ng/L in surface water predicted by IEM-2M computer simulation (U.S. EPA, 1997b). The data reported by Krabbenhoft et al. (1999) are therefore considered to be adequate to estimate intake from surface water.

5.5.2 Intake from Nonfish Dietary Sources

Data for measured methylmercury concentrations in nonfish foods are available from several local studies and one national study. Estimates of methylmercury concentration in selected produce and animal products are also available from computer simulations (U.S. EPA, 1997c). Data from the local studies provide supporting information on methylmercury speciation and concentration in a variety of foods, but are considered too limited in scope for estimation of intakes for use in RSC analysis. Information on mercury content of fish and nonfish foods is available from the Total Diet Study (1991-1997) conducted by U.S. FDA (1999). This is an on-going, nationally based study conducted for determining intake of nutrients and contaminants by the U.S. population. Based on data adequacy requirements of the 2000 Human Health Methodology (U.S. EPA, 2000a), the sample size of the U.S.

EPA study is sufficient for calculation of central tendency and 90th percentile values. Detection limits and the number of samples with mercury concentrations below detection the limit are reported by food item. The procedure for treating these samples for statistical analysis is reported. These data are thus considered adequate to estimate central tendency and high-end intakes from nonfish food items.

5.5.3 Intake From Fish

The MSRC (U.S. EPA, 1997c) assessed data sources for estimates of both freshwater and marine fish intake. Reliable mercury concentration data are available from databases maintained for marine fish and shellfish by the National Marine Fisheries Service (NMFS, 1978) and two databases for freshwater fish (Lowe et al., 1985; Bahnick et al., 1994). These studies are national in scope, in contrast to many studies that have a local or regional focus. In addition, the studies were not initiated in response to specific incidents of mercury contamination, and thus may avoid potential bias toward high values. Results in these studies are reported as total mercury. However, the MSRC concluded, based on research conducted by Bloom (1992) and Morgan et al. (1994), that over 90% of the mercury present in fish and seafood is methylmercury. Thus, total mercury concentrations are considered appropriate for evaluation of methylmercury exposure in human populations. Detailed information on mercury concentration by species and statistical considerations in use of the available data are presented in U.S. EPA (1997c).

Issues relating to data adequacy for methylmercury concentrations in marine fish and shellfish have been addressed in the MSRC (U.S. EPA, 1997c). Although the NMFS data were initially compiled beginning in the 1970s, comparisons of the mercury concentrations identified in the NMFS database with compliance samples obtained by the U.S. FDA indicate that the NMFS data are appropriate to use in estimating intake of mercury from marine fish at the national level of data aggregation. Cramer (1994) reported on *Exposure of U.S. Consumers to Methylmercury from Fish* and noted that recent information from NMFS indicated that the fish mercury concentrations reported in the 1978 report do not appear to have changed significantly. The U.S. FDA also monitors methylmercury concentration in seafood. Cramer (1994) observed that results of recent U.S. FDA surveys indicate results parallel to earlier findings by U.S. FDA and NMFS. The National Academy of Sciences' National Research Council's Subcommittee on Seafood Safety (1991) also assessed the applicability of the NMFS 1978 database to current estimates of mercury concentrations in fish. This subcommittee similarly concluded that the mercury concentrations in the 1978 database differed little in from the U.S. FDA compliance samples estimating mercury concentrations in fish. An assessment of the NMFS database by persons with expertise in analytical chemistry and patterns of mercury contamination in the environment indicates that temporal patterns of mercury concentrations in fish do not preclude use of this database in current risk

assessment activities (EPA's Science Advisory Board's ad hoc Mercury Subcommittee; Interagency Peer Review Group, External Peer Review Group).

An issue raised by some reviewers of the MSRC (U.S. EPA, 1997c) concerned use of data in the NMFS database where mercury concentration was below the analytical detection limit. A detailed analysis of the methods for reporting and analyzing nondetect data (U.S. EPA, 1997c, Appendix C) indicated that differences among methods used to handle nondetect samples had negligible impact on the reported mean concentrations in marine fish tissue. Additional information on analytical and statistical considerations in use of the NMFS data is available in EPA's MSRC (U.S. EPA, 1997d). Overall, EPA finds that these data are adequate for estimating exposure from marine fish for derivation of the methylmercury water quality criterion.

Two compilations of data on mercury concentrations in freshwater fish were considered for use in development of the water quality criterion for methylmercury. The strengths and weaknesses of these studies have been evaluated and reported in the MSRC (U.S. EPA, 1997c). The studies reported by Lowe et al. (1985) and by Bahnick et al. (1994) appear to be systematic, national collections of fish pollutant concentration data. However, higher mercury concentrations in fish have been detected in other studies, and the values obtained in the Lowe et al. (1985) and Bahnick et al. (1994) studies should be interpreted as approximations of the mean concentrations in freshwater finfish (U.S. EPA, 1997c). The mean mercury concentrations for each study in all fish sampled vary by a factor of two. The mean mercury concentration reported by Lowe et al. (1985) was 0.11 μg/g, whereas the mean mercury concentration reported by Bahnick et al. (1994) was 0.26 μg/g. The basis for these differences in methylmercury concentrations is unknown. Differences in sampling of fish by trophic position, size, or age might have been responsible for the differences in mean mercury concentrations reported in the two studies. Older and larger fish, which occupy higher trophic positions in the aquatic food chain, would be expected to have higher mercury concentrations. The type of water body from which fish were collected may also influence fish mercury concentrations. Most of the fish collected by Lowe et al. (1985) were from rivers. The fate and transport of mercury in river systems is not as well characterized as in small lakes. In comparison, most of the data reported by Bahnick et al. (1994) were collected with a bias toward more contaminated/industrialized sites, although sampled sites were not specifically contaminated with mercury. Thus, it is possible that there is more mercury available to the aquatic food chains at the sites sampled by Bahnick et al. (1994). Another possibility is that the higher mercury concentrations reported by Bahnick et al. (1994) when compared with those reported by Lowe et al. (1985) reflect increases in mercury contamination over the time period between the studies. Trend data for methylmercury concentrations in freshwater fish over time do not exist, although there are data for fish

collected from coastal and estuarine sites (U.S. EPA, 1997c) as discussed above and in Section 5.4.4.5. Those data suggest that there are no clear temporal trends in tissue mercury concentrations in fish and shellfish over the past two decades. Overall, the data from either study were considered adequate for calculating central tendency and high-end estimates of methylmercury intake from freshwater fish.

5.5.4 Intake from Air

The MSRC (U.S. EPA, 1997b) reported concentration ranges for mercury in urban and rural air. Information on geographic location, sample sizes, and detection limits were not provided. A range of 0 to 21% for methylmercury speciation was presented without an estimate of central tendency. Thus, these data as presented in the MSRC do not satisfy the adequacy requirements of the 2000 Human Health Methodology. A value of 1.6 ng/m^3 was presented in the MSRC as representative of national background levels for total mercury. Details on the derivation of this concentration were not provided; however, this value was considered of sufficient reliability to be used as input for fate and transport modeling reported in the MSRC (U.S. EPA, 1997b,c). Concentration measurements and exposure modeling data presented in the MSRC (U.S. EPA, 1997c) were also evaluated as an alternative estimate of methylmercury concentration in air. Many factors (including selection of modeling equations, input assumptions, and source data) in the modeling analysis affect the predicted concentrations and resulting exposures. These factors are summarized and discussed in U.S. EPA (1997b,c,g). No data were located for methylmercury concentrations in indoor air. Thus, this potential source of exposure was not considered in the estimate of intake from air.

The information available on both measured and predicted air concentrations of methylmercury from the MSRC is insufficient to fully determine data adequacy for estimating central tendency and high-end exposures to methylmercury via inhalation. Estimates of inhalation exposure are presented, although they are considered to represent rough approximations of actual (or likely) intake. Yet, the available data summarized in the MSRC (including the computer-simulated estimates) indicate that exposure to methylmercury in ambient air is negligible.

5.5.5 Intake From Soil

Three studies report aggregate values for measured soil mercury concentration. Shacklette and Boerngen (1984) reported arithmetic and geometric mean concentrations, geometric standard deviations, and ranges for total mercury in soils and other surficial materials based on samples collected at 1,318 sites across the conterminous United States. Sample size for these estimates is adequate, and the data are

representative of concentrations in the United States, although detailed information on analytical methodology, detection limit, and the number and statistical treatment of samples below detection limit was not provided.

Davis et al. (1997) reported a range of 50 to 200 ng/g for total mercury concentration and an estimate of the percent present as methylmercury in nonmercuriferous soils and sediments in background areas not directly impacted by volcanic emissions or anthropogenic releases. However, supporting information on the derivation of this estimate was not provided by the authors. The MSRC (U.S. EPA, 1997b) cited data from NJDEPE (1993) which indicates that typical U.S. soils contain 8 to 117 ng/g of total mercury. Information necessary for assessment of data adequacy was not provided in the summary of this study.

Additional data are available on soil mercury and methylmercury concentrations for sites in the United States. The MSRC (U.S. EPA, 1997b) summarized two reports on methylmercury speciation in soils collected at sites in New York and Washington state. Because each of these studies addressed soil concentrations in only one state, they were not considered adequate for estimating methylmercury exposure from soil.

Computer simulation data for predicted soil concentration, methylmercury speciation, and exposure estimates are available for comparison to measured values. Predicted concentrations were calculated on a regional (Eastern and Western U.S.) basis. As noted by U.S. EPA (1997b,c,g), many factors in the simulation analysis (including modeling equations, input assumptions, and source data) potentially affect the predicted concentrations.

Overall, the currently available soil concentration data are considered adequate to obtain central tendency and high-end estimates of exposure. Although some information was not readily available from the summarized studies in the MSRC (e.g., detection limits), the estimates of exposure from soil ingestion presented in this document are considered adequate given the sampling size (especially the Shacklette and Boerngen study) and geographic representativeness. There is also a clear indication from all available studies that the amount of methylmercury in soil that is methylmercury is approximately 2%.

5.6 TOTAL EXPOSURE ESTIMATES

Total exposure (calculated as the sum of exposure from water, freshwater and estuarine fish, marine fish, nonfish foods, air, and soil) for the three population groups in comparison to the RfD is shown in

Table 5-23. To evaluate potential differences in exposure from ambient water and drinking water, total exposure was calculated using methylmercury exposure estimates for each source. Because the contribution of ambient water or drinking water intake to total exposure is negligible in comparison to the sum of intake from other sources, there is no difference in the total exposure estimated using these two alternatives.

The contribution of exposure from different media as a percentage of total exposure for three types of individuals is summarized in Tables 5-24 through 5-26. Daily exposure estimates on a mg/kg-day basis are presented in Tables 5-27 through 5-29. The information in these tables reflects use of three different intake assumptions for consumption of marine fish: mean, median and 90^{th} percentile.

Table 5-23. Total Exposure Compared with the RfD for Methylmercury

Population of Concern	Exposure Parameters								Total Exposures with Ambient Water (mg/kg-day)			Total Exposures with Drinking Water (mg/kg-day)		
	Body Weight (kg)	Drinking Water Intake (L/day)	Fresh/ Estuarine Fish Intake (kg/day)	Inhalation (m^3/day)	Soil Ingestion (kg/day)	Mean Marine Fish Intake (kg/day)	Median Marine Fish Intake (kg/day)	90% Marine Fish Intake (kg/day)	Marine Mean[a]	Marine Median[b]	Marine 90%[c]	Marine Mean[a]	Marine Median[b]	Marine 90%[c]
Adults in the General Population	70	2.0	0.0175	20	0.00005	0.01246	0	0.04916	9.2×10^{-5}	6.5×10^{-5}	1.8×10^{-4}	9.2×10^{-5}	6.5×10^{-5}	1.8×10^{-4}
Women of Childbearing Age	67	2.0	0.1655	11	0.00005	0.09104	0.07548	0.18835	8.4×10^{-4}	8.0×10^{-3}	1.1×10^{-3}	8.4×10^{-4}	8.0×10^{-3}	1.1×10^{-3}
Children Age 0-14 Years	30	1.0	0.1563	10.4	0.0001 0.01[d]	0.0749	0.05971	0.15229	1.7×10^{-3}	1.6×10^{-3}	2.1×10^{-3}	1.7×10^{-3}	1.6×10^{-3}	2.1×10^{-3}
RfD									1.0×10^{-4} mg/kg-day			1.0×10^{-4} mg/kg-day		

[a] For adults in the general population, intake rates are estimates of all survey respondents to derive an estimate of long-term consumption.
[b] For children and women of childbearing age, intake rates are estimates of "consumers only" data (as described in U.S. EPA, 2000b).
[c] All freshwater/estuarine fish intake rates are based on the 90th percentile from the CSFII data (U.S. EPA, 2000b).
[d] Total exposure calculated using marine mean exposure estimate.
[e] Total exposure calculated using marine median exposure estimate.
[f] Total exposure calculated using marine 90th percentile exposure estimate.
[g] Pica child soil ingestion

Table 5-24. Percent of Total Exposures Using Marine Mean Intakes and Default Exposure Percentages for Three Types of Individuals [a]

Exposure Route	Fish and Water Criterion[b]			Fish-Only Criterion[c]		
	Exposure as a Percent of Total Exposure			Exposure as a Percent of Total Exposure		
	Adults in the General Population	Women of Childbearing Age	Children Age 0-14 Years	Adults in the General Population	Women of Childbearing Age	Children Age 0-14 Years
Freshwater/Estuarine Fish	70.6490	76.1903	76.0230	70.6047	76.1848	76.0200
Water				0.0608	0.0069	0.0038
Marine Fish	29.3446	23.8093	23.9764	29.3281	23.8078	23.9755
Diet (Nonfish foods)	0	0	0	0	0	0
Air	0.005	0.0003	0.0003	0.005	0.0003	0.0003
Soil	0.0014	0.0002	0.0003	0.0014	0.0002	0.0003

[a] Exposures are based upon default body weight values (Table 5-1) and do not include pica child soil ingestion.
[b] Ambient surface water exposure estimates used in the fish and water criterion.
[c] Drinking water exposure estimates used in the fish only criterion.

Table 5-25. Percent of Total Exposures Using Marine Median Intakes and Default Exposure Percentages for Three Types of Individuals [a]

Exposure Route	Fish and Water Criterion[b]			Fish-Only Criterion[c]		
	Exposure as a Percent of Total Exposure			Exposure as a Percent of Total Exposure		
	Adults in the General Population	Women of Childbearing Age	Children Age 0-14 Years	Adults in the General Population	Women of Childbearing Age	Children Age 0-14 Years
Freshwater/Estuarine Fish	99.9909	79.9997	80.2464	99.9047	79.9938	80.2431
Water				0.0862	0.0073	0.0040
Marine Fish	0	19.9998	19.7539	0	19.9984	19.7522
Diet (Nonfish foods)	0	0	0	0	0	0
Air	0.0071	0.0003	0.0003	0.0071	0.0003	0.0003
Soil	0.0020	0.0002	0.0004	0.0020	0.0002	0.0004

[a] Exposures are based upon default body weight values (Table 5-1) and do not include pica child soil ingestion.
[b] Ambient surface water exposure estimates used in the fish and water criterion.
[c] Drinking water exposure estimates used in the fish only criterion.

Table 5-26. Exposure from Various Routes as a Percent of Total Exposure Using Marine 90th % Intakes and Default Exposure Percentages for Three Types of Individuals [a]

Exposure Route	Fish and Water Criterion[b]			Fish-Only Criterion[c]		
	Exposure as a Percent of Total Exposure			Exposure as a Percent of Total Exposure		
	Adults in the General Population	Women of Childbearing Age	Children Age 0-14 Years	Adults in the General Population	Women of Childbearing Age	Children Age 0-14 Years
Freshwater/Estuarine Fish	37.1431	60.9523	61.0326	37.1297	60.9488	61.0307
Water				0.0319	0.0055	0.0031
Marine Fish	62.8535	39.0473	38.9668	62.8349	39.0453	38.9657
Diet (Nonfish foods)	0	0	0	0	0	0
Air	0.0026	0.0002	0.0003	0.0026	0.0002	0.0003
Soil	0.0007	0.0001	0.0003	0.0007	0.0001	0.0003

[a] Exposures are based upon default body weight values (Table 5-1) and do not include pica child soil ingestion.
[b] Ambient surface water exposure estimates used in the fish and water criterion
[c] Drinking water exposure estimates used in the fish only criterion.

Table 5-27. Daily Exposure Estimates from All Media Using Marine Mean Intakes for Individuals From Three Populations of Concern

Population of Concern	Summary of Exposure (mg/kg-day)[a]							
	Ambient Water	**Drinking Water[b]**	**Nonfish Dietary Items**	**Freshwater/ Estuarine Fish**	**Marine fish**	**Air**	**Soil**	**Total Exposure**
Children Age 0-14 Years	5.0×10^{-9}	6.5×10^{-8}	0	1.3×10^{-3}	4.2×10^{-4}	5.5×10^{-9}	5.9×10^{-9}	1.7×10^{-3}
%Total Exposure	0.0003%	0.0038%	0.0000%	76.0198%	23.9755%	0.0003%	0.0003%	
Women of Childbearing Age	4.5×10^{-9}	5.8×10^{-8}	0	6.4×10^{-4}	2.0×10^{-4}	2.6×10^{-9}	1.3×10^{-9}	8.4×10^{-4}
%Total Exposure	0.0005%	0.0069%	0.0000%	76.1844%	23.8076%	0.0003%	0.0002%	
Adults in General Population	4.3×10^{-9}	5.6×10^{-8}	0	6.5×10^{-5}	2.7×10^{-5}	4.6×10^{-9}	1.3×10^{-9}	9.2×10^{-5}
%Total Exposure	0.0047%	0.0608%	0.0000%	70.6014%	29.3267%	0.005%	0.0014%	

[a] Refer to exposure parameters listed in Table 5-1.

[b] Upper-bound concentration for methylmercury used in calculation.

Table 5-28. Daily Exposure Estimates From All Media Using Marine Median Intakes for Individuals From Three Populations of Concern

Population of Concern	Exposure (mg/kg-day)[a]							
	Ambient Water	**Drinking Water[b]**	**Nonfish Dietary Items**	**Freshwater/ Estuarine Fish**	**Marine fish**	**Air**	**Soil**	**Total Exposure**
Children Age 0-14 Years	5.0×10^{-9}	6.5×10^{-8}	0	1.3×10^{-3}	3.2×10^{-4}	5.5×10^{-9}	5.9×10^{-9}	1.6×10^{-3}
%Total Exposure	0.0003%	0.0040%	0.0000%	80.2429%	19.7521%	0.0003%	0.0004%	
Women of Childbearing Age	4.5×10^{-9}	5.8×10^{-8}	0	6.4×10^{-4}	1.6×10^{-4}	2.6×10^{-9}	1.3×10^{-9}	8.0×10^{-4}
%Total Exposure	0.0006%	0.0073%	0.0000%	79.9933%	19.9983%	0.0003%	0.0002%	
Adults in General Population	4.3×10^{-9}	5.6×10^{-8}	0	6.5×10^{-5}	0	4.6×10^{-9}	1.3×10^{-9}	6.5×10^{-5}
%Total Exposure	0.0066%	0.0861%	0.0000%	99.8983%	0.0000%	0.0071%	0.0020%	

[a] Refer to exposure parameters listed in Table 5-1.
[b] Upper-bound concentration for methylmercury in drinking used in calculation.

Table 5-29. Daily Exposure Estimates from All Media Using Marine 90th Percentile Intakes for Individuals from three Populations of Concern

Population of Concern	Exposure (mg/kg-day)[a]							
	Ambient Water	**Drinking Water[b]**	**Nonfish Dietary Items**	**Freshwater/ Estuarine Fish**	**Marine fish**	**Air**	**Soil**	**Total Exposure**
Children Age 0-14 Years	5.0×10^{-9}	6.5×10^{-8}	0	1.3×10^{-3}	8.5×10^{-4}	5.5×10^{-9}	5.9×10^{-9}	2.1×10^{-3}
%Total Exposure	0.0002%	0.0031%	0.0000%	61.0305%	38.9656%	0.0003%	0.0003%	
Women of Childbearing Age	4.5×10^{-9}	5.8×10^{-8}	0	6.4×10^{-4}	4.1×10^{-4}	2.6×10^{-9}	1.3×10^{-9}	1.1×10^{-3}
%Total Exposure	0.0004%	0.0055%	0.0000%	60.9485%	39.0451%	0.0002%	0.0001%	
Adults in General Population	4.3×10^{-9}	5.6×10^{-8}	0	6.5×10^{-5}	1.1×10^{-4}	4.6×10^{-9}	1.3×10^{-9}	1.8×10^{-4}
%Total Exposure	0.0025%	0.0319%	0.0000%	37.1288%	62.8333%	0.0026%	0.0007%	

[a] Refer to exposure parameters listed in Table 5-1.
[b] Upper-bound concentration for methylmercury used in calculation.

5.7 RELATIVE SOURCE CONTRIBUTION (RSC) ESTIMATES

5.7.1 RSC Policy Summary

As described in Section 5.1, water quality criteria for noncarcinogens account for anticipated exposures from sources other than drinking water and freshwater/estuarine fish ingestion. These exposures can include other dietary intakes, air, and soil. By accounting for other exposures, the entire RfD is not attributed to drinking water and freshwater/estuarine fish consumption alone. The relative source contribution (RSC) approach apportions the RfD to ensure that the water quality criterion is sufficiently protective, given the other anticipated sources of exposure. Thus, accounting for nonwater exposure sources results in a more stringent water quality criterion than if those sources were not considered. Details of the RSC approach (the Exposure Decision Tree) are described in more detail in the 2000 Human Health Methodology (U.S. EPA, 2000a).

The RSC determination differs from chemical to chemical depending on several factors: (a) the magnitude of total exposure compared with the RfD; (b) the adequacy of data available; (c) whether more than one guidance or criterion is to be set for the chemical in question; and (d) whether there is more than one significant exposure source for the chemical and population of concern. The target population for this methylmercury criterion is discussed in Section 5.2; the sources of methylmercury exposure, exposure estimates, and data adequacy are discussed in Sections 5.3 through 5.5.

5.7.2 Target Population for RSC/Rationale for Approach to Methylmercury

The target population for the RSC estimate is the general population. The health risk measure, the RfD, is intended to be protective of the whole population, including (but not restricted to) sensitive subpopulations. This is not a developmental RfD *per se*. Even though the critical endpoint was neurotoxic effects observed in children exposed *in utero*, application of the RfD is not restricted to pregnancy only, or to developmental periods only.

As discussed in the 2000 Human Health Methodology, the RSC policy approach allows for use of a subtraction method to account for other exposures when one health-based criterion is relevant for the chemical in question. In this circumstance, other sources of exposure can be considered "background" and can be subtracted from the RfD. Such is the case with methylmercury; that is, there are no health-based criteria, pesticide tolerances, or other regulatory activities to warrant apportionment using the alternate percentage method.

5.7.3 Data Adequacy for RSC Estimate

Section 5.4 describes information on levels of occurrence and provides estimates of exposure to methylmercury in ambient surface water, drinking water, fish, nonfish foods, air, soil, and sediment. The information in Section 5.4 indicates that, for almost all media sources, the sampling data meet the adequacy requirements (e.g., sample sizes, representativeness) for describing both central tendency and high-end concentrations for those sources (Box 3 of the Methodology Decision Tree approach [U.S. EPA, 2000a]). Thus, the data summarized for ambient surface water concentrations, nonfish dietary concentrations, marine fish concentrations, and soil concentrations are adequate to use for estimating overall exposure and RSC. Available data on methylmercury in ground water and estimates of methylmercury in drinking water are not as adequate, as defined by the data adequacy requirements in the 2000 Human Health Methodology. However, the estimates made for both ground water and drinking water in Section 5.4.2.3 indicate levels no higher in magnitude than the surface water estimates, even when using most high-end values. Information on ambient air concentrations summarized from the MSRC failed to indicate sample sizes, geographic representativeness, or detection limits and, thus, are not considered adequate in terms of the Methodology's Decision Tree (Box 3) requirements. However, 98% of mercury in ambient air occurs in the form of vapor-phase elemental mercury, according to the MSRC. Therefore, exposures to methylmercury in ambient air are probably negligible. This assumption is supported by the estimates presented in Section 5.4.5, including the MSRC model simulations predicting exposures of zero near a waste incinerator.

5.7.4 RSC Estimate/Apportionment of the RFD

Once it has been determined that the data are adequate to describe exposure intakes for relevant exposure sources and that there are no other health-based criteria to apportion, exposure intakes from sources other than the source addressed by the criterion are subtracted from the RfD (Box 12 of the Decision Tree, see U.S. EPA, 2000a). Based on the available data, human exposures to methylmercury from all media sources except freshwater/estuarine and marine fish are negligible, both in comparison to exposures from fish and compared to the RfD. Estimated exposure from ambient water, drinking water, nonfish dietary foods, air, and soil are all, on average, at least several orders of magnitude less than those from freshwater/estuarine fish intakes. Nonfish sources of intake are in the range of 10^{-5} to 10^{-9} μg methylmercury/kg body weight-day for adults in the general population. The combined methylmercury exposure intakes from water ingestion, (nonfish) diet, air, and soil represent approximately 0.07% of total estimated exposure to methylmercury (and less than 1/100 of 1% of the RfD) for adults in the general

population. Therefore, these exposures are not factored into the RSC because they will not quantitatively affect the final criterion value.

Ingestion of marine fish is a significant contributor to total methylmercury exposure. The MSRC (U.S. EPA, 1997c) indicates that in the general population of fish consumers, those that consume freshwater/estuarine species of fish are also consumers of marine species of fish. EPA has, therefore, made the assumption in the derivation of the methylmercury fish tissue criterion. In making this assumption, EPA does not believe that, by and large, the high-end consumer of freshwater/estuarine fish is also a high-end consumer of marine fish. The Agency believes that it is more appropriate, and a reasonably conservative assumption, to use the average intake rate (approximately 12.5 g/day) for the marine fish component of the RSC estimate.

The marine fish exposure source is estimated using species-specific mean methylmercury fish tissue data from NMFS (see Section 5.4.4.4) and calculating species-weighted intakes from the CSFII consumption rates (see Section 5.4.4.7). Following the MSRC (U.S. EPA, 1997c), nearly 100% of the mercury in marine fish was assumed to be present as methylmercury. The RSC estimate from marine fish has been calculated with an overall assumed average intake of 12.46 g/day of marine fish based on the CSFII, for all respondents aged 18 and over. The estimated weighted-average methylmercury concentration in marine fish is 0.157 mg methylmercury/kg fish, and the estimated average exposure to methylmercury from marine fish is 2.7×10^{-5} mg methylmercury/kg body weight-day. This exposure represents 27% of the RfD.

All exposure intake values estimated for methylmercury are presented in Table 5-30. The RSC factor in this case is determined by adding the estimated intakes that are quantitatively relevant for methylmercury; that is, only the intake from marine fish consumption of 2.7×10^{-5} mg/kg-day has any affect on the calculation. This amount is subtracted from the RfD of 0.1 μg methylmercury/kg body weight-day or 1.0×10^{-4} mg methylmercury/kg body weight-day. The remainder of the RfD is used to calculate the fish tissue residue concentration in terms of the assumed body weight and freshwater/estuarine fish ingestion. This results in an amount of methylmercury that is allowable in freshwater/estuarine fish and that will not exceed the RfD, considering the additional exposure from marine fish consumption.

Table 5-30. Exposure estimates for methylmercury and percent of total exposure based on adults in the general population

Exposure Source	Exposure Estimate (mg/kg-day)	Percent of Total Exposure	Percent of RfD
Ambient water intake	4.3×10^{-9}	0.0047	0.004
Drinking water intake[a]	5.6×10^{-8}	0.0605	0.006
Nonfish dietary intake	0	0	0
Marine fish intake	2.7×10^{-5}	29.33	27
Air intake	4.6×10^{-9}	0.005	0.005
Soil Intake	1.3×10^{-9}	0.0014	0.001
Total intake	9.2×10^{-5}	100	27.01

[a] This represents the high-end of the range of estimates. Because the contribution of ambient water or drinking water intake to total exposure is so negligible in comparison to the sum of intake from other sources, there is no difference in the total exposure estimated using either of these two alternatives.

6.0 METHYLMERCURY BIOACCUMULATION

6.1 INTRODUCTION

Aquatic organisms can accumulate and retain certain chemicals in their bodies when exposed to these chemicals through water, their diet and other sources. This process is called bioaccumulation. In order to prevent harmful exposures to waterborne pollutants through the consumption of contaminated fish and shellfish, national 304(a) water quality criteria for the protection of human health must address the process of chemical bioaccumulation in aquatic organisms. For deriving national 304(a) ambient water column criteria to protect human health, EPA accounts for potential bioaccumulation of pollutants in fish and shellfish through the use of national bioaccumulation factors (BAFs). A national BAF is a ratio (in L/kg) which relates the concentration of a chemical in water to its expected concentration in commonly consumed aquatic organisms in a specified trophic level. The magnitude of bioaccumulation by aquatic organisms varies widely depending on the chemical but can be extremely high for some highly persistent and hydrophobic chemicals. For such highly bioaccumulative chemicals, concentrations in aquatic organisms may pose unacceptable human health risks from fish and shellfish consumption even when concentrations in water are too low to cause unacceptable health risks from drinking water consumption alone. These chemicals may also biomagnify in aquatic food webs, a process whereby chemical concentrations increase in aquatic organisms of each successive trophic level due to increasing dietary exposures (e.g., increasing concentrations from algae, to zooplankton, to forage fish, to predator fish). Methylmercury is a chemical that bioaccumulates and biomagnifies to a relatively high extent. Methylmercury BAFs for upper trophic level freshwater and estuarine fish and shellfish typically consumed by humans generally range between 500,000 and 10,000,000 (Glass et al., 1999; Lores et al., 1998; Miles and Fink, 1998; Monson and Brezonik, 1998; Watras et al., 1998; Mason and Sullivan, 1997).

6.2 ISSUES IN DEVELOPING METHYLMERCURY BAFS

The fates of mercury and methylmercury in the environment are complex processes affected by numerous biotic and abiotic factors that are subjects of ongoing research by various government, private, and academic groups around the world. Methylation of mercury is a key step in the entrance of mercury into food chains. The biotransformation of inorganic mercury species to methylated organic species in water bodies can occur in the sediment and the water column. Inorganic mercury can be absorbed by aquatic organisms but is generally taken up at a slower rate and with lower efficiency than is

methylmercury. Methylmercury continues to accumulate in fish as they age. Predatory organisms at the top of aquatic and terrestrial food webs generally have higher methylmercury concentrations because methylmercury is typically not completely eliminated by organisms and is transferred up the food chain when predators feed on prey; for example, when a largemouth bass feeds on a bluegill sunfish, which fed on aquatic insects and smaller fish, all of the prey could contain some amount of methylmercury that gets transferred to the predator. Nearly 100% of the mercury that bioaccumulates in upper trophic level fish (predator) tissue is methylmercury (Bloom, 1992; Akagi, 1995; Kim, 1995; Becker and Bigham, 1995).

Numerous factors can influence the bioaccumulation of mercury in aquatic biota. These include, but are not limited to, the acidity (pH) of the water, length of the aquatic food chain, temperature, and dissolved organic material. Physical and chemical characteristics of a watershed, such as soil type and erosion or proportion of area that is wetlands, can affect the amount of mercury that is transported from soils to water bodies. Interrelationships among these factors are poorly understood and are likely to be site-specific. No single factor (including pH) has been correlated with extent of mercury bioaccumulation in all cases examined. Two lakes that are similar biologically, physically, and chemically can have different methylmercury concentrations in water, fish, and other aquatic organisms (Cope et al., 1990; Grieb et al., 1990; Jackson, 1991; Lange et al., 1993). For more in-depth discussions about the chemical, physical, and biological interactions affecting methylmercury bioaccumulation in aquatic organisms see the *Mercury Study Report to Congress* (MSRC), Volume III and Volume III Appendix D (U.S. EPA, 1997c), and the compilation of papers in *Mercury Pollution: Integration and Synthesis* (Watras and Huckabee, 1994).

To derive section 304(a) water quality criteria for the protection of human health, EPA needs to conduct a human health risk assessment on the pollutant in question and to gather information on the target population's exposure to the pollutant. Traditionally, EPA has expressed its section 304(a) water quality criteria guidance to protect human health in the form of pollutant concentrations in ambient surface water. To account for human exposure through the aquatic food pathway when deriving a water column-based water quality criterion, EPA uses national BAFs (U.S. EPA 2000). A BAF is a ratio (in L/kg) that relates the concentration of a chemical in water to its expected concentration in commonly consumed aquatic organisms in a specified trophic level (U.S. EPA 2000). A national BAF is meant to be broadly applicable to all waters in the United States, whereas a site-specific BAF is based on local data and integrates local spacial and temporal factors that can influence bioaccumulation. For pollutants that biomagnify, such as methylmercury, EPA's preferred approach for deriving national BAFs for use in deriving section 304(a) water quality criteria is to use empirical field data collected in the natural

environment. EPA prefers this approach because BAFs derived with field data integrate the chemical, biological, and physical factors that can affect bioaccumulation in fish and shellfish. With this preference in mind, EPA explored the feasibility of developing field-derived national methylmercury BAFs for each trophic level of the aquatic food chain consumed by humans (i.e., trophic levels 2-4). Using Agency guidance on BAFs contained in the 2000 Human Health Methodology and procedures outlined in Volume III, Appendix D of the peer-reviewed MSRC (U.S. EPA, 1997c), EPA empirically derived draft national methylmercury BAFs for each trophic level of the aquatic food chain. The draft national BAFs were single value trophic level-specific BAFs calculated as the geometric mean of field data collected across the United States and reported in the open literature as well as other publically available reports. These draft methylmercury BAFs were compiled in a draft internal report and submitted to a panel of external scientific experts for peer review. The Appendix contains a summary of the internal BAF report and BAF peer review report. The entire internal draft methylmercury BAF report and peer review report can be obtained from the Water Docket W-00-20.

Within any given trophic level, the individual empirically derived draft methylmercury BAFs generally ranged up to two orders of magnitude. This range in BAFs reflects the various biotic factors (such as food chain interactions and fish age/size) and abiotic factors (such as pH and dissolved organic carbon). The large range in the individual empirically derived draft methylmercury BAFs results in uncertainty as to the ability of single trophic level-specific national methylmercury BAFs to accurately predict bioaccumulation of methylmercury in general across the waters of the United States. Presently, it is EPA's understanding that the mechanisms that underlie many of the influencing factors are not well understood and can not be accurately predicted. As the science of methylmercury improves, in the future it may be possible predict or model these processes and use such information to more accurately predict bioaccumulation. Until such time, EPA is unable to improve the predictive power of the methylmercury BAFs by universally accounting for influencing factors. This is not the case for other highly bioaccumulative pollutants; for example polychlorinated biphenyls (PCBs). For such pollutants, EPA has methods that improve the predictive capability of empirically derived or model predicted BAFs (such as normalizing fish tissue concentrations to lipid and normalizing ambient water concentrations to dissolved and particulate organic carbon). EPA is actively involved in, and will continue to support, various types of research aimed at better understanding the fate of mercury in the environment and the processes that underlie methylmercury bioaccumulation. EPA hopes that results of new research will enable better predictions of methylmercury bioaccumulation.

The BAF peer reviewers recognized the need for methylmercury BAFs and were supportive of most aspects of the methodology used to derive the draft national methylmercury BAFs. The peer reviewers did have issues with certain data used to derive the methylmercury BAFs and certain assumptions about food chain relationships. Overall, most of the peer reviewers believed that derivation of single-value trophic level-specific national BAFs for methylmercury that would be generally applicable to all waters of the United States under all conditions is difficult at best, and perhaps impossible. This opinion was based on consideration of the highly site-specific nature of methylmercury bioaccumulation in aquatic environments and the large range in the empirically derived draft methylmercury BAFs. These peer reviewers recommended developing methylmercury BAFs on a more local or regional scale, if not on a site-specific basis. Although EPA generally agrees with this suggestion, the data needed to derive BAFs at more localized scales across the U.S. are not available. See Appendix A for a summary of the internal BAF report and the BAF peer review report.

6.3 CONSIDERATION OF A FISH TISSUE RESIDUE CRITERION

After considering the various issues about mercury fate in the environment, the recent report by the National Research Council (NRC, 2000) on the toxicological effects of mercury, and the methylmercury BAF peer review comments, EPA concluded that it is more appropriate at this time to derive a fish tissue (including shellfish) residue water quality criterion for methylmercury rather than a water column-based water quality criterion. EPA believes a fish tissue residue water quality criterion for methylmercury is appropriate for many reasons. A fish tissue residue water quality criterion integrates spatial and temporal complexity that occurs in aquatic systems and that affect methylmercury bioaccumulation. A fish tissue residue water quality criterion in this instance is more closely tied to the CWA goal of protecting the public health because it is based directly on the dominant human exposure route for methylmercury. The concentration of methylmercury is also generally easier to quantify in fish tissue than in water and is less variable in fish and shellfish tissue over the time periods in which water quality standards are typically implemented in water quality-based controls, such as NPDES permits. Thus, the data used in permitting activities can be based on a more consistent and measurable endpoint. Finally, this approach is consistent with the way in which fish advisories are issued. Fish advisories for mercury are also based on the amount of methylmercury in fish tissue that is considered acceptable, although such advisories are usually issued for a certain fish or shellfish species in terms of a meal size. A fish tissue residue water quality criterion should enhance harmonization between these two approaches for protecting the public health.

Because EPA did not use national, empirically derived methylmercury BAFs to establish today's section 304(a) recommended methylmercury water quality criterion, EPA has deferred further efforts to derive national BAFs for methylmercury at this time. EPA notes, however, that there may be adequate field data for some waterbodies or geographical regions on which to base accurate predictive, site-specific methylmercury BAFs. EPA may reconsider developing national methylmercury BAFs in the future once more field data is available for a broader range of species and aquatic ecosystems, or once more information is available describing the mechanisms that affect bioaccumulation. Such information could enable EPA to more accurately predict methylmercury bioaccumulation on a broader scale given a certain total mercury concentration in water.

7.0 WATER QUALITY CRITERION CALCULATION

7.1 EQUATION FOR TISSUE RESIDUE CONCENTRATION AND PARAMETERS USED

The equation for calculating the methylmercury fish tissue residue criterion is:

$$TRC = \frac{BW \times (RfD - RSC)}{\sum_{i=2}^{4} FI_i}$$

Where:

TRC	=	Fish tissue residue criterion (mg methylmercury/kg fish) for freshwater and estuarine fish
RfD	=	Reference dose (based on noncancer human health effects) of 0.0001 mg methylmercury/kg body weight-day
RSC	=	Relative source contribution (subtracted from the RfD to account for marine fish consumption) estimated to be 2.7×10^{-5} mg methylmercury/kg body weight-day
BW	=	Human body weight default value of 70 kg (for adults)
FI	=	Fish intake at trophic level (TL) i (i = 2, 3, 4); total default intake is 0.0175 kg fish/day for general adult population. Trophic level breakouts for the general population are: TL2 = 0.0038 kg fish/day; TL3 = 0.0080 kg fish/day; and TL4 = 0.0057 kg fish/day.

This yields a methylmercury TRC value of 0.3 mg methylmercury/kg fish (rounded to one significant digit from 0.288 mg methylmercury/kg fish).

This equation is essentially the same equation used in the 2000 Human Health Methodology to calculate a water quality criterion, but is rearranged to solve for a protective concentration in fish tissue rather than in water. Thus, it does not include a BAF or drinking water intake value (as discussed above, exposure from drinking water is negligible). The TRC of 0.3 mg methylmercury/kg fish is the concentration in fish tissue that should not be exceeded based on a total consumption of 0.0175 kg fish/day.

7.2 SITE-SPECIFIC OR REGIONAL ADJUSTMENTS TO CRITERIA

Several parameters in the Water Quality Criterion equation can be adjusted on a site-specific or regional basis to reflect regional or local conditions and/or specific populations of concern. These include the fish consumption rates and the RSC estimate. States and authorized Tribes can also choose to apportion an intake rate to the highest trophic level consumed for their population or modify EPA's default intake rate based on local or regional consumption patterns. EPA strongly encourages States and authorized Tribes to consider developing a criterion using local or regional data over the default values if they believe that they would be more appropriate for their target population. States and authorized Tribes are encouraged to make such adjustments using the guidance provided in the 2000 Human Health Methodology (U.S. EPA, 2000a).

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APPENDIX A

SECTION I. DRAFT NATIONAL METHYLMERCURY BIOACCUMULATION FACTORS

This appendix is a brief summary of the initial effort conducted to determine the feasibility of deriving draft National bioaccumulation factors for methylmercury. This appendix is based on the draft bioaccumulation report. The complete version of the original draft bioaccumulation factor report, with more in-depth discussions of the methodology, a list of the references cited, rationales for using data, and an uncertainty discussion can be obtained from the Water Docket W-00-20.

This appendix does not reflect comments or changes suggested by the peer reviewers. No changes were made to the draft report that served as the basis for this appendix. Data interpretations, findings, or conclusions discussed in this appendix are preliminary and may be changed in the future.

Introduction

The methylmercury bioaccumulation factors (BAFs) were estimated using guidance presented in the *Methodology for Deriving Ambient Water Quality Criteria for the Protection of Human Health* (U.S. EPA, 2000a; hereafter "the 2000 Human Health Methodology") and supplemented with methods presented in the Mercury Study Report to Congress (MSRC; U.S. EPA, 1997c). The generalized equation for estimating a BAF is as follows:

$$BAF = \frac{C_t}{C_w} \qquad \textbf{Equation-1}$$

where:

C_t = Concentration of the chemical in the wet tissue (either whole organism or specified tissue)

C_w = Concentration of chemical in water

Literature searches were conducted to obtain data on bioaccumulation, concentrations of different forms of mercury in water, percent methylmercury in tissue, and mercury predator-prey data. The data sources primarily included articles from peer reviewed journals published between 1990 and April of 1999 and publicly available reports (e.g., State, Federal, or trade/industry group reports; dissertations;

proceedings from professional meetings). Data from a variety of aquatic ecosystems (i.e., lakes, rivers, estuaries) and on lower trophic levels was specifically looked for since the MSRC focused only on lakes (primarily northern oligotrophic lakes) and trophic levels 3 and 4 fish.

BAFs are used in the ambient water quality criteria (AWQC) equation to estimate human mercury exposure from consumption of contaminated fish. Equation 2 is the generalized AWQC equation for a noncarcinogen and shows where the BAF fits into the calculation.

$$AWQC = RfD \times RSC\left[\frac{BW}{DI + \sum_{i}^{x}(FI \times BAF_i)}\right] \qquad \textbf{Equation 2}$$

Where:

RfD = reference dose for noncancer human health effects

RSC = relative source contribution to account for non-water sources of exposure

BW = human body weight

DI = drinking water intake

FI = fish intake

BAF_i = bioaccumulation factor for chemical "i".

The methylmercury BAFs that would be used in the above equation are presented in the accompanying table A-9, and are calculated as the geometric mean BAF of all BAFs calculated for a given trophic level.

Attachment A at the end of this appendix also contains the general comments made by the external peer reviewers on the draft national methylmercury BAFs.

Methods for Estimating Bioaccumulation Factors

Three approaches were used to derive draft BAFs that could be used to derive draft national methylmercury BAFs. These are direct, indirect, and conversion (modified direct) approaches. Each of

these approaches has its own limitations, biases, and uncertainties associate with it. These approaches and the BAFs derived using them are summarized below.

EPA's BAF derivation guidance is based on a data hierarchical preference approach. Under the hierarchy, the preferred method for deriving a BAF for an organometallic compound such as methylmercury is to use field-measured data to directly calculate a BAF (i.e., the direct method). BAFs estimated using this direct approach are calculated using the simple ratio of the chemical concentration in tissue and water. When such field data do not exist, or if the available field data are considered unreliable, the next preferred method in the hierarchy estimates a BAF by multiplying a bioconcentration factor (BCF) by a food chain multiplier (FCM) (i.e., the indirect method). The FCM is a factor used to account for food chain interactions and biomagnification. EPA has used this indirect method to estimate BAFs to support the development of wildlife criteria values in the Great Lakes Water Quality Initiative or GLWQI (EPA, 1993) and in the MSRC (EPA, 1997). With few exceptions, field-derived FCMs were calculated using concentrations of methylmercury in predator and prey species using the following equations:

$$FCM_{TL2} = BMF_{TL2} \qquad \text{Equation-3}$$

$$FCM_{TL3} = (BMF_{TL3})\,(BMF_{TL2}) \qquad \text{Equation-4}$$

$$FCM_{TL4} = (BMF_{TL4})\,(BMF_{TL3})\,(BMF_{TL2}) \qquad \text{Equation-5}$$

where:

FCM = Food chain multiplier for designated trophic level (TL2, TL3, or TL4)
BMF = Biomagnification factor for designated trophic level (TL2, TL3, or TL4)

The basic difference between FCMs and BMFs is that FCMs relate back to trophic level one, whereas BMFs always relate back to the next lowest trophic level. Biomagnification factors are calculated from methylmercury tissue residue concentrations determined in biota at a site according to the following equations:

$$BMF_{TL2} = C_t, TL2) / (C_t, TL1) \qquad \text{Equation-6}$$

$$BMF_{TL3} = (C_t, TL3) / (C_t, TL2) \qquad \text{Equation-7}$$

$$BMF_{TL4} = (C_t, TL4) / (C_t, TL3) \qquad \text{Equation-8}$$

where:

C_t = concentration of chemical in tissue of appropriate biota that occupy the specified trophic level (TL2, TL3, or TL4).

With the indirect BAF approach, it is important that when either selecting predator prey field data from the literature or when conducting a site-specific field study to obtain such data, that the feeding relationships between predator and prey are based on functional feeding relationships. It should be verified that a given predator is feeding on a given prey item at the location in question so that the BMFs and FCMs reflect actual trophic transfer of the chemical as close as possible. Usually, it is not enough to simply know that organisms are from two different trophic levels. Unfortunately, for the analyses presented here, much of the available data obtained from the published literature were insufficient to document functional feeding relationships. Thus, BAFs derived using the indirect approach were not used in determining the draft national methylmercury BAFs, but are presented only for comparison purposes.

In the MSRC, in cases where the direct empirical BAF derivation method could be used, but the available data was for a form of mercury other than dissolved methylmercury, a modified direct approach was also used. The modified direct approach was used when either the water data or organism tissue data was not in the methylmercury form (e.g., total mercury, dissolved total mercury, total methylmercury) but could be converted to methylmercury using translating factors. Data for mercury in water was converted to dissolved methylmercury by using chemical translators (see Section II of this Appendix). Mercury in tissue reported as total mercury was converted to methylmercury by multiplying by a factor that estimates the fraction of total mercury present in the methylated form (i.e., fmmf translator). The fmmfs were developed from field studies where both total mercury and methylmercury were measured in biota tissue.

Using the methods outlined above, BAFs were estimated initially by trophic level for lakes (lentic aquatic systems), rivers and streams (lotic aquatic systems), and estuaries. An ecosystem-based approach to deriving the BAFs was used because differences in general bioaccumulation trends would be expected among the aquatic ecosystems due to inherent differences in methylation processes, food web dynamics,

mercury loadings, and watershed interactions, among other factors. However, due to the lack of data in terms of both quality and quantity, no clear differences in bioaccumulation trends were observed between lentic and lotic ecosystems based on the available data (see Figure A-3). Based on qualitative and semi-quantitative comparisons of the data, no significant difference was found between the lentic and lotic BAFs. Thus, they were combined for each trophic level to obtain the trophic level-specific draft national BAFs. A near complete lack of adequate data prohibited derivation of draft national BAFs for estuarine systems.

Summary of BAFs for Methylmercury in Lentic Ecosystems

Table A-1 compares the BAFs estimated using the two primary approaches (direct and indirect) methods for estimating BAFs for trophic levels 2, 3, and 4 species. Although the BAFs based on the indirect approach are not used in the national draft BAF calculations because they are not based on verifiable functional predator-prey feeding relation ships, they are nonetheless useful for comparing and assessing general tends in bioaccumulation. Other than the BAF_2, the BAFs are within a factor of two of one another. Both the direct and indirectly estimated BAFs show an expected increase in methylmercury bioaccumulation with increasing trophic position. This suggests that if functional predator-prey feeding relationships can be developed, that indirect BAFs could provide reasonably good approximations of methylmercury bioaccumulation in organisms in the field.

Table A-1: Summary of Bioaccumulation Factors for Methylmercury Mercury in Lentic Ecosystems

Parameter	Methylmercury (1)	
	Direct ($L \cdot kg^{-1}$)	Indirect ($L \cdot kg^{-1}$)
BCF	5.9×10^4	NA
BAF_2	8.6×10^4	3.1×10^5
BAF_3	1.3×10^6	2.2×10^6
BAF_4	6.8×10^6	1.1×10^7

(1) All values are geometric means

Summary of BAFs for Methylmercury in Lotic Ecosystems

Table A-2 compares the lotic BAFs estimated using the direct and indirect methods. The BAFs based on the indirect approach are not used in the draft national BAF calculation because they are not based on verifiable functional predator-prey feeding relation ships; they are nonetheless useful for comparing and assessing general tends in bioaccumulation. As was the case with the lentic indirectly estimated BAFs, the indirect lotic BAFs are close approximations of the directly estimated BAFs (within a factor of 3 or less). Also, as was observed for lentic ecosystems, both the direct and indirectly estimated lotic BAFs show an expected increase in methylmercury bioaccumulation with increasing trophic position. This suggests that if functional predator-prey feeding relation ships can be developed,

Table A-2: Summary of Dissolved Methylmercury Bioaccumulation Factors for Lotic Ecosystems

Parameter	Methylmercury (1)	
	Direct ($L \cdot kg^{-1}$)	Indirect ($L \cdot kg^{-1}$)
BCF	1.2×10^4	NA
BAF_2	4.4×10^5	1.9×10^5
BAF_3	1.6×10^6	5.6×10^5
BAF_4	2.5×10^6	3.2×10^6

(1) values are geometric means

that indirect BAFs could provide reasonably good approximations of methylmercury bioaccumulation in organisms in the field.

Methylmercury BAFs Translated from Other Mercury Forms

Converted BAFs (that is, in terms of other mercury forms) were derived for dissolved methylmercury using translator factors (see Section II, Chemical Translators for Mercury and Methylmercury) and by using factors to convert total mercury measured in organism tissues to methylmercury in tissues.

Mercury Translators

For those studies that met the data quality objectives but did not analyze or report water mercury concentrations in the dissolved methylmercury form, the reported form of mercury was converted to the mean fraction of dissolved methylmercury (f_d $MeHg_d$) by using one or more of the "translators" listed in Table A-3. Section II below discusses the methodology and data used to derive the translators. Section II of this appendix also provides partition coefficients (K_D) that were not necessary for this analysis, but that can be used along with total suspended solids information to estimate the desired fraction of mercury in water.

Table A-3: Summary of Mercury Translators for Mercury in Water

f_d value	Lentic	Lotic
f_d Hg_d/Hg_t	0.600	0.370
f_d $MeHg_d/Hg_t$	0.032	0.014
f_d $MeHg_d/MeHg_t$	0.613	0.490

Conversion Factors for Mercury in Organism Tissue

Similar to the water data, if mercury in biota tissue (muscle or whole body) was reported as total mercury then the appropriate mean (arithmetic) estimate of the fraction present in the methylated form (fmmf) for the respective trophic level was used to convert it to methylmercury. Table A-4 summarizes the fmmfs used to estimate converted BAFs.

Table A-4: Summary of fmmfs for Lentic and Lotic Ecosystems

Trophic Level	Lentic	Lotic
1	0.18	0.05
2	0.44	0.49
3	1.00	1.00
4	1.00	1.00

Summary and Comparison of Converted BAFs and BCFs derived for Lentic and Lotic Ecosystems

Methylmercury translator factors (see Section II, *Chemical Translators for Mercury and Methylmercury*) were used to estimate dissolved methylmercury BCFs and BAFs in lotic and lentic ecosystems. Table A-5 summarizes the converted BAFs. The converted lentic BAFs range from approximately 2 to 37 times greater than the converted lotic BAFs.

Figures A-1 and A-2 compare the direct and converted estimates of BAFs and BCFs for lentic and lotic ecosystems, respectively. Although the data sets are relatively small, the ranges of converted BAFs are in agreement with BAFs directly estimated. Tables A-6 and A-7 summarize and compare the point estimates of each data set. In lentic ecosystems, the difference between the mean directly estimated BAFs and mean converted BAFs is generally less than a factor of two. For lotic ecosystems, the difference is slightly larger, ranging from a factor of two to a factor of seven, with an overall mean difference of four. This information suggests that the converted BAFs in each ecosystem are good estimates of directly measured BAFs for all trophic levels. However, because the set of BAFs estimated using the two different approaches are small for each ecosystem, insufficient data were available to perform any rigorous statistical evaluation to determine if a significant difference exists between the BAFs of each system. Nonetheless, graphically the data suggest that the direct and converted BAFs can be combined to derive overall BAFs for each trophic level in each ecosystem. The BAFs based on the combined data sets are presented in Table A-8.

Figure A-3 compares the combined data sets (e.g., directly-measured and converted BAFs and BCFs) for lentic and lotic ecosystems. While the lotic BAFs clearly span a greater range than the lentic BAFs, the differences between the mean lotic BAFs and the mean lentic BAFs for each trophic level are fairly small (differences range between 1 and 5). To investigate if there were significant differences between the BAFs for the two ecosystems significant, a student's T-test was performed on the combined data for each trophic level-specific BAF and BCF using the computer software WINKS (Texasoft, 1999). Although differences in mercury bioaccumulation between lentic and lotic ecosystems could be expected due to differences in mercury loading characteristics, bioavailability, food web dynamics, and methylation processes, among other factors, no significant statistical differences ($p>0.05$) were found between the lentic and lotic BAFs and BCFs. Furthermore, a closer inspection of the converted lentic BAF_4 data for several Minnesota Lakes (Glass et al., 1999) suggests that, given a larger sample size, the lower range of field-measured lentic BAF_4 values could be similar to the lower range of values observed for lotic ecosystems. Whether these observations are artifacts of the available data or trends due to real

Table A-5: Comparison of Converted Bioaccumulation Factors for Methylmercury in Lotic and Lentic Ecosystems

Parameter	Lentic ($L \cdot kg^{-1}$)	Lotic ($L \cdot kg^{-1}$)
BCF	4.3×10^4	6.1×10^3
$_{MD}BAF_2$	1.5×10^5	6.2×10^4
$_{MD}BAF_3$	1.3×10^6	3.5×10^4
$_{MD}BAF_4$	4.1×10^6	1.4×10^6

Table A-6: Comparison of Direct and Converted Methylmercury BAFs and BCFs for Lentic Ecosystems

	$_{MD}BCF$		$_{MD}BAF_2$		$_{MD}BAF_3$		$_{MD}BAF_4$	
Value[a]	direct	converted	direct	converted	direct	converted	direct	converted
5th	12,300	13,400	16,700	47,500	322,000	466,000	3,270,000	3,800,000
50th (GM)	58,700	43,000	85,600	150,000	1,260,000	1,330,000	6,800,000	4,080,000
95th	281,000	138,000	439,000	474,000	4,900,000	3,820,000	14,200,000	4,380,000
GSD	2.59	2.26	2.70	2.01	2.29	1.90	1.56	1.04

[a] GM = geometric mean; GSD = geometric standard deviation.

Table A-7: Comparison of Direct and Converted Methylmercury BAFs and BCFs for Lotic Ecosystems

	$_{MD}BCF$		$_{MD}BAF_2$		$_{MD}BAF_3$		$_{MD}BAF_4$	
Value[a]	direct	converted	direct	converted	direct	converted	direct	converted
5th	340	1,200	15,600	3,400	261,800	45,800	283,000	55,400
50th (GM)	5,400	6,000	179,000	61,900	1,640,000	346,000	2,520,000	1,380,000
95th	85,800	29,800	2,000,000	1,130,000	10,200,000	2,620,000	22,500,000	30,300,000
GSD	5.38	2.63	4.40	3.39	3.05	3.42	3.78	6.80

[a] GM = geometric mean; GSD = geometric standard deviation.

processes is not distinguishable. Because the range of available BAF values for lentic and lotic systems overlap one another, the individual BAFs for the two systems were combined in one data set to derive the trophic level-specific draft national methylmercury BAFs.

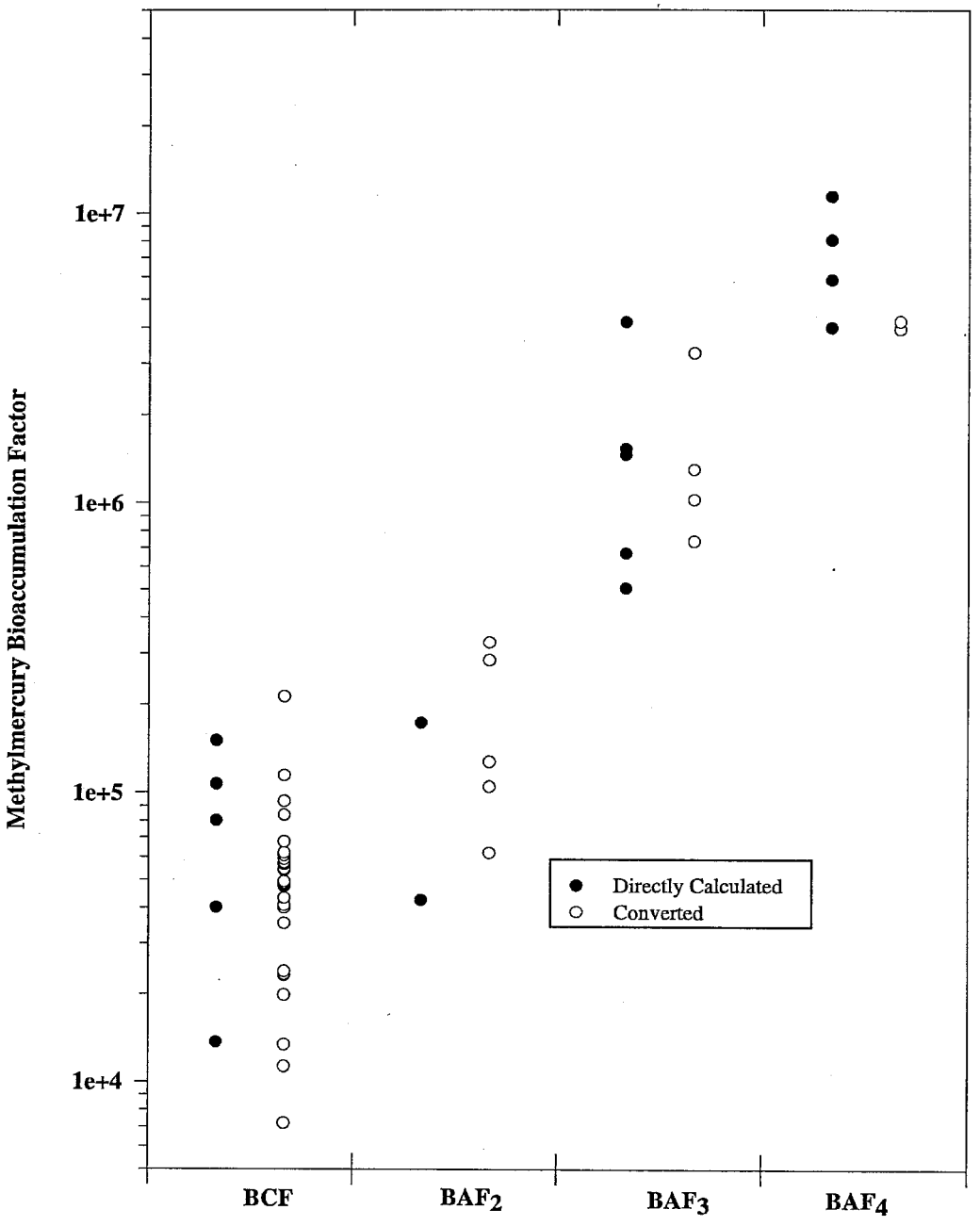


Figure A-1. Comparison of direct field-measured and converted field-measured methylmercury BCFs and BAFs for lentic ecosystems.

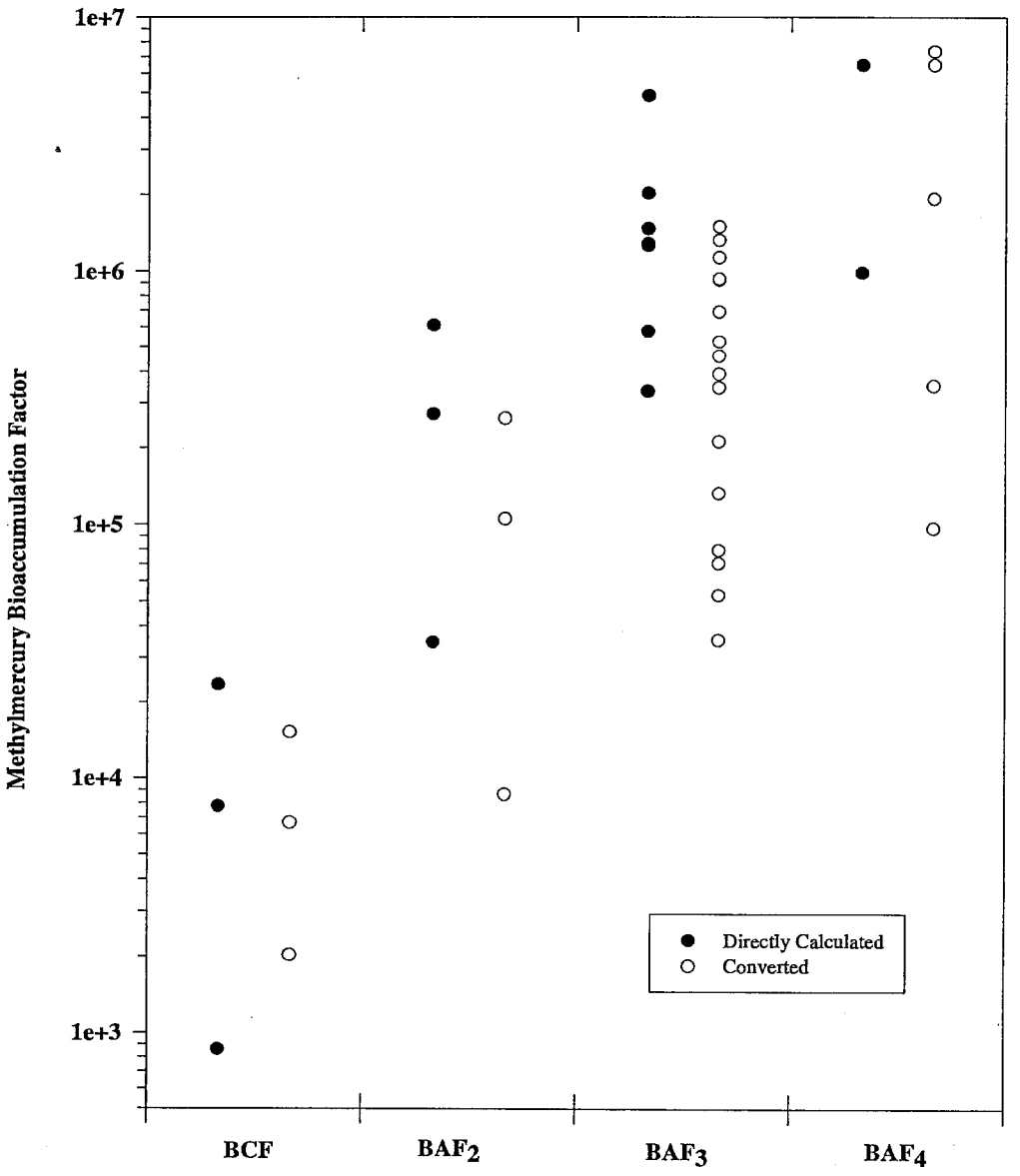


Figure A-2. Comparison of direct field-measured and converted field-measured methylmercury BCFs and BAFs for lotic ecosystems.

Table A-8: Summary of Lentic and Lotic Methylmercury BAFs and BCFs

	$_{MD}BCF$		$_{MD}BAF_2$		$_{MD}BAF_3$		$_{MD}BAF_4$	
Value[(1)a] (%)	Lentic	Lotic	Lentic	Lotic	Lentic	Lotic	Lentic	Lotic
5th	13,300	800	37,000	8,000	423,000	46,000	2,800,000	73,400
50th (GM)	45,000	5,700	127,800	105,000	1,115,000	517,000	5,740,000	1,240,000
95th	153,000	43,200	440,000	1,390,000	2,930,000	5,820,000	11,800,000	20,900,000
GSD	2.10	5.14	2.12	4.80	2.02	4.36	1.55	5.57

(1) Values are based on combined direct and converted BAFs and BCFs.

[a] GM = Geometric Mean; GSD = geometric standard deviation.

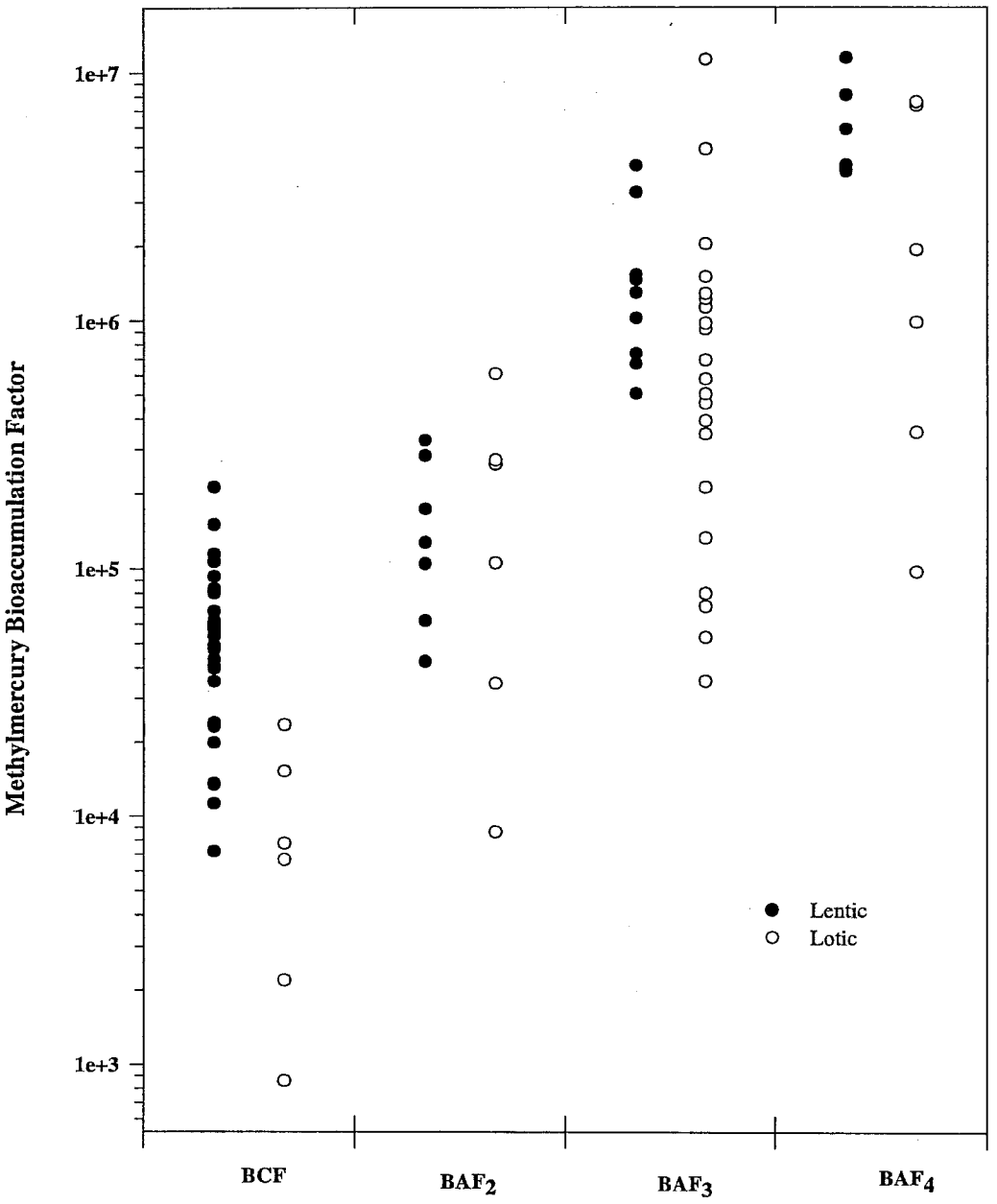


Figure A-3. Comparison of lentic and lotic methylmercury BAFs. Data includes both direct field-measured BAFs and converted field-measured BAFs.

Draft National Bioaccumulation Factors for Methylmercury

Based on the data presented above, and because the goal of the draft national BAFs is to be applicable under as many circumstances and to as many water bodies as possible, the BAFs based on the combined data sets (e.g., direct and converted, lentic and lotic) were chosen to be the empirically-derived draft national BAFs for methylmercury. The draft National BAFs, along with the draft BCF, and their empirical distributions are presented in Table A-9.

Table A-9: Summary of Draft National BAFs and BCF for Dissolved Methylmercury

Value[a]	BCF	BAF_2	BAF_3	BAF_4
5th percentile	5,300	18,000	74,300	250,000
50th (GM) percentile	33,000	117,000	680,000	2,670,000
95th percentile	204,000	770,000	6,230,000	28,400,000
GSD	3.03	3.15	3.84	4.21
Draft National Values	**3.3×10^4**	**1.2×10^5**	**6.8×10^5**	**2.7×10^6**

[a]GM = geometric mean; GSD = geometric standard deviation.

Discussion of Uncertainty and Variability in the BAF Estimates

The BAFs in this document were designed to estimate the central tendency of the concentration of mercury in fish of a given trophic level from an average concentration of dissolved mercury for water bodies located in the continental U.S. As shown in figures A1-A3, there is at least an order of magnitude in the variability of the individual BAF estimates for a given trophic level, which leads to uncertainty in the overall central tendency estimate. This is further reflected in the range of 90 percent (5th and 95th percentiles) confidence intervals. Although the empirical range of any given 90 percent confidence interval may largely overestimate the true extent of variability, the distributions do provide a rough estimate of the total uncertainty in the aggregate processes and an idea of the precision (or lack thereof) of the BAF estimates. The uncertainty in the BAF estimates is related to two basic sources. First is the uncertainty arising from natural variability, such as size of individual fish or differences in metabolic processes. Second is the uncertainty due to measurement error, such as error in measurements of mercury in water and fish samples or lack of knowledge of the true variance of a process (e.g., methylation). These two sources of uncertainty are generally referred to as "variability" and

"uncertainty", respectively. In this analysis, there was no distinction made between variability and uncertainty; they are aggregated in the final BAF distributions and point estimates. Thus, it cannot be determined where natural variability stops and uncertainty starts. However, some of the more important sources of variability and uncertainty are highlighted below in order to assist risk managers in understanding what the limitations are surrounding the BAFs, to see how the uncertainty in the BAF estimates might be reduced should they derive more data, and to assist them in decisions on development of site-specific BAFs.

Uncertainty Due to Sampling and Chemical Analysis

In many cases, water methylmercury concentrations reported in the available studies incorporated limited or no cross-seasonal variability, incorporated little or no spacial variability, and were often based on a single sampling event. Because fish integrate exposure of mercury over a life time, comparing fish concentrations to a single sample or mean annual concentrations introduces bias to the estimates. The geographic range represented by the water bodies is also limited. The available lentic data are biased towards northern oligotrophic lakes, primarily located in the Great Lakes region. The lotic BAFs are primarily based on data from canals of the Everglades (assumed to act as flowing aquatic ecosystems) and from a point-source-contaminated stream in Tennessee. Because of this general lack of data, a few studies on water bodies in other countries were included in the analysis, requiring one to assume that biotic and abiotic processes in these lakes are similar to lakes in the continental U.S.

The same sampling and analytical methods for water and tissue samples were not used in each acceptable study. Although all studies used met general requirements for data quality, studies with different analytical detection limits were combined to estimate the BAFs. The range of species used in the BAF estimates is relatively small compared to the suite of fish and invertebrates consumed by the general human population. Much of the available trophic level 4 data for both lentic and lotic ecosystems is limited to walleye, pike, or bass. For trophic level 3 much of the data is for bluegill and perch. For trophic level 2, most of the data was for zooplankton in lentic waters and for planktivorous fish in lotic waters. The lack of data complicated comparisons between the two aquatic ecosystems and introduces uncertainty into application of the BAFs.

Uncertainty Due to Estimation Method

Each of the approaches use to estimate BAFs have their own inherent uncertainties. Both the direct and indirect approaches assume that the underlying process and mechanisms of mercury bioaccumulation are the same for all species in a given trophic level and for all water bodies. The indirect approach deals with this assumption more specifically by assuming that the translators and fmmfs used to convert BAFs are equally applicable to all ecosystems. In reality, these factors are based on a limited set of data. Although the translators and fmmfs used in the analysis are consistent with those reported elsewhere (Porcella, 1994), they may over- or underestimate bioavailability and bioaccumulation in specific water bodies. Ideally, site-specific conversion factors would be used to estimate BAFs more reflective of conditions in a given water body. The approach used here aggregates all of the species-specific BAFs into a single trophic level-specific BAF; this also increases the over all variability in the BAF estimates.

Uncertainty Due to Biological Factors

Other than deriving BAFs based on organism trophic level, and initially by general water body type (i.e., lentic and lotic), there were no distinctions in the BAFs as to size/age of fish, water body trophic status, or underlying mercury uptake processes. It has been shown that methylmercury bioaccumulation for a given species can vary as a function of the ages (body size) of the organisms examined (Glass et al, 1999; Watras et al., 1998; Suchanek et al., 1993; Lange et al. 1993). As a result, it has been suggested that to reduce some of the lake-to-lake variability seen in BAFs for a given species, comparisons between water bodies should be made using "standardized" fish values (i.e., a value for a hypothetical 1 kg northern pike; Glass et al., 1999). Typically such data "normalization" is derived by linear regression of residue data collected from individuals of varying size and/or age. However, the currently available data are too limited to perform this kind of normalization; most of the water body-specific BAFs, and resulting trophic level distributions, are based on "opportunity" (whatever you catch, you include) and do not report age or size of individuals sampled.

Uncertainty Due to Universal Application of BAFs

Perhaps the greatest source of variability is that of model uncertainty. That is, uncertainty introduced by failure of the model (in this analysis a single trophic level-specific BAF) to represent significant real-world processes that vary from water body to water body. The simple linear BAF model relating methylmercury in fish to total mercury in water simplifies a number of nonlinear processes that

lead to the formation of bioavailable methylmercury in the water column and subsequent accumulation. Much of the variability in field data applicable to the estimation of mercury BAFs can be attributed to differences in biotic factors (e.g., food chain, organism age/size, primary production, methylation/demethylation rates), and abiotic factors (e.g., pH, organic matter, mercury loadings, nutrients, watershed type/size) between aquatic systems. As an example, in lake surveys conducted within a relatively restricted geographic region, large differences can exist between lakes with respect to mercury concentrations in a given species of fish (Cope et al., 1990; Grieb et al., 1990; Sorenson et al., 1990; Jackson, 1991; Lange et al., 1994; Glass et al., 1999). These observations have led to the suggestion that a considerable portion of this variability is due to differences in within-lake processes that determine the percentage of total mercury that exists as the methylated form. Limited data also indicate that within a given water body, concentrations of methylmercury are likely to vary with depth and season. Unfortunately, while the concentration of methylmercury in fish tissue is presumably a function of these varying concentrations, published BAFs are generally estimated from a small number of measured water values, whose representativeness of long-term exposure is poorly known. Furthermore, although it is known that biotic and abiotic factors control mercury exposure and bioaccumulation, the processes are not well understood, and the science is not yet available to accurately model bioaccumulation on a broad scale.

Summary

Three different approaches were use to estimate methylmercury bioaccumulation factors for use in deriving national 304(a) ambient water quality criteria for mercury. All three approaches resulted in BAFs with central tendency point estimates in good agreement with one another. Based on data comparability and EPA's national guidance for deriving BAFs, methylmercury BAFs estimated using directly measured and converted field data were used as the basis for deriving the draft national BAFs. Given the large range in the data, at this time lotic BAFs can not be distinguished from lentic BAFs, though the data suggests slightly reduced methylmercury accumulation may occur in higher trophic level organisms in lotic/wetland environments. The same trend is observed when BAFs are compared on a total mercury basis. Some of this difference might be accounted for by the lower accumulation of methylmercury at the base of the food chain in lotic/wetland ecosystems. A plausible explanation for this difference is the observation that the bioavailability of methylmercury in lentic environments (usually a low dissolved organic carbon content) may exceed the bioavailability of methylmercury in lotic/wetland environments (usually a high dissolved organic carbon content). Methylmercury and mercury have a high binding capacity to dissolved organic carbon which can affect their bioconcentration in

phytoplankton/periphyton. Watras et al. (1998) used modeling to show that BAFs based on the bioavailable fraction of methylmercury in water exceed BAFs based on the operationally defined (filtered) dissolved methylmercury in water. Bioavailability is perhaps the single most important factor affecting BAFs for mercury.

EPA fully recognizes that the approach taken to derive mercury BAFs collapses a very complicated non-linear process, which is affected by numerous physical, chemical, and biological factors, into a rather simplistic linear process. EPA also recognizes that uncertainty exists in applying a National BAF universally to all water bodies of the United States. Therefore, in the revised 2000 Human Health Methodology (EPA , 2000) we encourage and provide guidance for States, Territories, Authorized Tribes, and other stakeholders to derive site-specific field-measured BAFs when possible. In addition, should stakeholders believe some other type of model may better predict mercury bioaccumulation on a site-specific basis they are encouraged to use one, provided it is scientifically justifiable and clearly documented with sufficient data.

SECTION II. CHEMICAL TRANSLATORS FOR MERCURY AND METHYLMERCURY

Introduction

By regulation (40 CFR 122.45(c)), the permit limit, in most instances, must be expressed as total recoverable metal. Because chemical differences between the discharged effluent and the receiving water are expected to result in changes in the partitioning between dissolved and adsorbed forms of metal, an additional calculation using what is called a translator is required.
The translator is used to convert the dissolved concentration of a metal to a total metal concentration for use in waste load limit calculations. The translator is the fraction of the total recoverable metal in the downstream water that is dissolved, f_d. The translator can be used to estimate the concentration of total recoverable metal in a water body.

Methods

Two procedures were used to develop site-specific translators. The most straightforward approach for translating from a dissolved water quality criterion to a total recoverable effluent concentration is to analyze directly the dissolved and total recoverable fractions. The translator is the fraction of total recoverable metal that is dissolved. It may be determined directly by measurements of dissolved and total recoverable metal concentrations in water samples taken from the well mixed effluent and receiving water (i.e., at or below the edge of the mixing zone). In this approach, a number of samples are taken over time and an f_d value is determined for each sample:

$$f_d = Cd/Ct \quad [Eqn. 1]$$

where:

Cd = the dissolved concentration, and
Ct = the total metal concentration.

The translator is then calculated as the geometric mean (GM) of the dissolved fractions.

The second approach derives an f_d from the use of a partition coefficient K_D where usually the coefficient is determined as a function of total suspended solids (TSS) (although some other basis such as humic substances or particulate organic carbons may be used). The partition coefficient is the ratio of the particulate-sorbed and dissolved metal species multiplied by the adsorbent concentration, i.e. Cd + TSS $\rightleftharpoons$ Cp, where Cp is the bulk particulate-sorbed concentration, and is expressed as:

$$K_D = Cp/(Cd \cdot TSS) \qquad [Eqn.2].$$

The dissolved fraction and the partition coefficient are related as shown in equation 3.

$$f_d = (1 + K_D \cdot TSS)^{-1} \qquad [Eqn.3]$$

As in the first approach, numerous samples are collected over time, and the f_d and TSS values found at the site are fit to a least squares regression, the slope of which is K_D. The established K_D is then used to determine the translator using Eqn. 3 with a TSS value representative of some critical condition, e.g., low flow conditions.

Although development of site-specific translators is recommended, EPA also envisions the possible need for national or default translators for use in translating dissolved mercury and dissolved methylmercury criteria into total mercury and methylmercury water quality permit limitations. Translators and/or related K_D values can be generated from an acceptable existing literature-derived data base. EPA's MSRC (U.S. EPA, 1997) contains extensive data, obtained primarily from lake systems, that are relevant to developing translators for mercury (e.g., percent total as methylmercury, percent total as dissolved mercury). Supplementation of these translators with additional, acceptable data from lotic and estuarine systems and update of lentic systems provides the necessary data base for the translators. To gather this data base, peer-reviewed literature papers from 1990 to present, were searched and reviewed. Since awareness of the contamination problems with mercury at low levels and the existence of analytical methods capable of accurately and precisely measuring mercury and methylmercury at low levels are relatively recent, the literature review was not conducted for publications prior to 1990. All data from the literature for use in developing the translators were required to meet the following criteria:

- Clean techniques, or equivalent, to reduce contamination were used in sampling and analysis.
- Adequate QA/QC procedures were used.
- Analytical methods used provided sufficiently low enough detection level.

Draft Translators

Table A-10 summarizes the numerous tables from the EPA internal draft BAF report (see Water-Docket W-00-20). These results are presented separately for lake, river and estuarine systems, and for each system, where sufficient data were available, both f_d and K_D values were tabulated. The K_D values were calculated using Eqn. 2. The K_D values could not be derived using the f_d-TSS correlation approach due to the limited data, i.e., multiple sampling events over time with measurements of both f_d and TSS were not conducted in most of the studies. The results are presented separately for both mercury and methylmercury. Table A-10 provides a summary of the GM values calculated for each system for f_d and K_D values, again for both mercury and methylmercury.

It is possible to calculate a "pseudo" K_D value for the partitioning of dissolved methylmercury with particulate total mercury using f_d and K_D data for a waterbody utilizing the following equation (see Attachment B for derivation and example calculation):

$$\text{"Pseudo" } K_D \text{ MeHgd/Hgt} = K_D \text{ MeHgd} \cdot \text{MeHgt} \bullet \text{Ratio Hgd/MeHg}_p \bullet \text{Ratio MeHgd/MeHg}_p$$

[Eqn. 4]

Table A-10: Summary of F_d and K_d Values for Lakes, Rivers, and Estuaries[a]

f_d and K_D Values	Lakes	Rivers	Estuaries
f_d Hg	0.60	0.37	0.353
f_d $MeHg_d/Hg_t$	0.032	0.014	0.190[b]
f_d $MeHg_d/MeHg_t$	0.613	0.49	0.612[b]
Log K_D Hg	5.43	5.06	5.52
Log K_D MeHg	5.53	4.81	NF[c]
"pseudo" Log K_D $MeHg_d/Hg_t$	6.83	6.44	NC[d]

a Values calculated as GM
b Only two sites
c No data found from the literature search
d Not able to calculate due to insufficient data

The K_D so derived is a "pseudo" value since dissolved methylmercury partitioning with particulate total mercury is just a synthetic or functional type description. These values are also given in Table A-10. The "pseudo" K_D values, however, allow for direct translation of dissolved methylmercury criteria to total mercury permit limits employing some designated TSS level. Insufficient data were found, e.g., K_DMeHg, to allow for calculation of "pseudo" K_Ds for estuaries. It should be understood that all values in Table A-10 represents values generated from the above-described literature-gleaned data base. Insufficient data were obtained to provide either reliable f_d (translator) or K_D "default" values for methylmercury for estuarine systems (only two sites). Examination of the translator values for lakes and rivers shows that in all instances the river values for both f_ds and K_Ds are lower than the lake values. The lower translator values can be generally explained by the generally higher TSS levels found in rivers as compared to lakes. For example, typical TSS values for eastern Washington state lakes are 0.5 to 5 mg/L, whereas river levels can be typically 5-50 mg/L (Pankow and McKenzie, 1991). Higher TSS levels lead to lower f_d values.

The lower K_D values for rivers vs. lakes are not as readily explainable. K_D values are not constant and are sensitive to environmental conditions and water chemistry (Sung, 1995). Inclusion of the colloidal fraction in the dissolved phase that is used in determining the K_D has been used to explain variation of K_D values and for deviation of the values from any true K_D (Pankow and McKenzie, 1991; Sung, 1995). Higher colloidal contents or higher DOC levels in the river samples compared with lake samples would produce lower apparent (as measured) K_D values. However, the following other factors have been suggested to play major roles in K_D determinations, and one or all of these may contribute significantly to the reason why the river K_Ds are less than the lake K_Ds for both mercury and methylmercury:

- Biotic or organic content of the TSS
- Dissolved organic content of the water
- Geochemistry and residual metal content of the TSS
- TSS particle size
- Pollution level existing in the waters

Regardless of the reason(s) for the differences between the lake and river values, differences do exist and are sufficiently significant that it is recommended that the two systems be treated separately with regard to translator values. Until additional data are available for estuarine systems, and a satisfactory comparison to lake and river systems can be made, it is recommended that separate values be retained for estuaries also.

One can estimate the TSS level that is represented by the f_d values for each system through the use of Eqn. 3 and employing the default K_D values provided in Table A-10. The results of calculations of these estimated levels and an example calculation are presented in Table A-11. The data show the following:

- In lakes, the f_d for mercury (0.60) would reflect TSS levels of 2.5 mg/L. The f_d for methylmercury (0.032) would reflect TSS levels of 1.8 mg/L. At TSS levels lower than these values, a greater fraction of the mercury and methylmercury would be expected to be dissolved than indicated by the f_d.

- In rivers, the f_d for mercury (0.37) would reflect TSS levels of 14.8 mg/L. The f_d for methylmercury (0.014) would reflect TSS levels of 16.3 mg/L. At TSS levels lower than these values, a greater fraction of the mercury and methylmercury would be expected to be dissolved than indicated by the f_d.

- In estuaries, the f_d for mercury (0.35) would reflect TSS levels of 5.5 mg/L.

Existing TSS levels less than those above would, in any instance, that the dissolved fraction present in the water could be greater than the value suggests.

Use of the partition coefficient approach may provide advantages over the dissolved fraction. EPA suggests (EPA, 1996) that when using dynamic simulation for Waste Load Allocation (WLA) or the Total Maximum Daily Load (TMDL) calculations and permit limit determinations, K_D allows for greater mechanistic representation of the effects that changing environmental variables have on f_d (the significance of the TSS variable has been shown in Table A-11 data and discussed above, and this variable is addressed or can be handled in the K_D approach).

Table A-11: Estimation of TSS Level at f_d Values

	Lakes		Rivers		Estuaries	
	f_d	Est. TSS, mg/L	f_d	Est. TSS, mg/L	f_d	Est. TSS, mg/L
Mercury[a]	0.60	2.5*	0.37	14.8	0.35	5.5
Methylmercury[b]	0.032	1.8	0.014	16.3	0.190	NC[c]

(a) Calculated using default K_D values and equation: $f_d = 1/(1+K_D \times TSS)$

(b) Calculated using default "pseudo" K_D values and equation: $f_d = 1/(Hg_d/HgMe_d + K_D \times TSS)$

(c) Not able to calculate; insufficient data.

* Calculation:

$f_d = 1/(1 + K_D \times TSS \times 10^{-6})$ note: 10^{-6} used to provide TSS in mg/L units

default K_DHg (lakes) = 269,153

substituting: $0.60 = 1/(1 + 269{,}153 \times TSS \times 10^{-6})$

$0.60 + 0.161 \times TSS = 1$

$0.161 \times TSS = 0.40$

$TSS = 2.5$

Although the K_D approach may be advantageous in use, employment of a default K_D value has inherent problems as does the use of a f_d. For example, mercury K_Ds have been shown to range from about 10^4 to about 10^6 (Watras et al., 1995). At an average K_D value of about 10^5 (the value found for rivers), and a critical TSS level of 10 mg/L, a translator value of 0.5 is derived from the K_D approach.

However, if the site K_D, for example, is close to the lower end of the K_D range, the translator value should be about 0.9. Thus the value is inaccurate at this site. Only at sites where the existing K_D is 10^5 or greater (at 10 mg/L TSS) would the use of the default K_D yield a translator value that does not underestimate the dissolved mercury level.

An additional problem with the use of the K_D approach is that even at a given site, K_D values can vary. Usually, K_D values decrease at a site as TSS increases, as has been shown recently for mercury and methylmercury in a Virginia river (Mason and Sullivan, 1998). In addition, the K_D translator approach necessitates that f_d correlate with TSS. A poor correlation, however, has been found to exist for many metals in a recent analysis of data obtained from State of Michigan surface waters (MDEQ, 1996).

Although the K_D approach has its advantages, the f_d approach is the most straightforward. Both approaches have their disadvantages, as discussed previously. The K_D is derived from f_d values and so the two approaches are truly linked. Therefore, preferential recommendation of either one approach over the other at present cannot be made.

Use of either f_d or K_D default values can be made as long as one recognizes the short comings of the approach taken. Perhaps the approach taken should be the one with the stronger data base, if a clear difference exists. As additional data appears in the literature, it is reasonable to assume that a fine-tuning of both the f_d and K_D default values will result. EPA recommends that translators be derived from site-specific studies when possible, but the values in Table A-10 could be used in absence of any site-specific data.

ATTACHMENT A: BAF PEER REVIEWERS' GENERAL COMMENTS

The following was excerpted from the BAF Peer Review Comments Report, August 23, 2000. See Water Docket W-00-20 for a complete version of the peer review report.

2.0 REVIEWERS' COMMENTS

2.1 General Comments

Nicolas Bloom

Overall, I found the document quite clear and well written compared to other EPA mercury documents that I have recently reviewed, a fact that made my job considerably easier. On the other hand, it seems quite clear that there is insufficient data currently available for the EPA to make any more than the broadest generalizations about methyl mercury bioaccumulation factors. The current greater than one order of magnitude spread in estimated BAFs will not be very useful in any actual case, although it serves to describe the situation in general terms. The EPA should be impelled to proceed by instigating research and/or requiring site-specific bioaccumulation factors to be developed until such time that a sufficient database is accumulated to allow some meaningful resolution between BAFs from different water body types, climates, and trophic levels.

I oppose the general use of the confusingly similar terms "lentic" and "lotic," which although probably clear to fish ecologists, never-the-less provide endless confusion to the rest of us. I conducted a poll of the 51 employees of our aquatic sciences research company, and no one could define these words correctly, although a few did say that they had heard of them back in college. Additionally, even though physically, the term "lentic" can be used to lump together the Everglades with a swiftly moving glacial stream, I see no logical biogeochemical reason to do so.

There is also the overwhelming sense, in the description of the trophic levels considered, that the only valid food chain model being considered is the water to plankton to zooplankton to fish model. However, many systems (i.e., Lavaca Bay, TX) are dominated by a sediment porewater to benthic invertebrates to fish model, which means that sediment issues (methyl concentrations, methylation depth profiles, redox condition, seasonality, etc.) loom way more important that water column concentrations.

James Hurley

First and foremost, the development of a national AWQC for methylmercury must be based on sound data with strict quality control/quality assurance to ensure that the calculation of bioaccumulation factors (BAFs) is scientifically valid. This is a difficult task when conducting literature searches for data that form the backbone of the report. Among the data chosen, methods must be comparable to allow transferability. Individual investigators also apply different definitions of biological assemblages and food chain pathways. This makes the task of synthesizing appropriate data a difficult task at best.

My overall concern with data used for determination of the national BAF is that not one study from which data was obtained for this report was actually with the specific purpose of generating MeHg-based BAFs through all trophic levels. I fully understand that EPA also recognizes this problem and commend them for assembling the data presented. However, I do think that EPA should consider a research effort designed to produce results directly related to their MeHg BAF goals. This would ensure that sample types and methodologies were consistent with the overall goal of development of national BAFs for methylmercury. Development of a scientifically sound BAF is a critical step in development of a management plan for this Level I contaminant in the U.S.

In addition to developing a field effort, EPA should also consider development of dedicated laboratory studies that address Hg and MeHg partitioning and transport in trophic levels 1 and 2. Although EPA decided to choose an approach that incorporates field-derived BAFs, laboratory studies using cultures of phytoplankton and zooplankton, coupled with key contrasting water chemistries, would certainly aid in reducing the variability that is inherent in using field-derived data on partitioning. Results of these studies alone would avoid the ambiguity that is inherent in using the terms "seston" and "phytoplankton" interchangeably for BCFs.

The current report divides the data into two environments (lentic and lotic) but then combines BAFs to determine a national BAF in the final section of the report. I strongly encourage EPA to establish a series of National BAFs that are watershed-type based, in slightly more detail than a simple lentic/lotic division. Data from lotic systems in the report combine wetlands with flowing rivers. As a result, the lotic grouping contains high dissolved organic carbon (DOC) systems such as wetlands, with low DOC headwater streams. This type of grouping of sites with such disparate Hg-cycling environments most likely accounts for both the spread of data for directly-calculated BCFs and the lack of agreement between directly calculated and converted BCFs depicted in Figure 5-2.

While I agree that translators are appropriate in some instances, they too should be calculated on a more site-specific basis. Use of the translators to calculate the fraction (f_d) of total Hg as MeHg should be refined to address factors such as trophic state and watershed type. The grand mean of 3.2% for this translator encompasses a range from 0.2% to 13.9% in lake waters. Similarly, the grand mean from rivers of 1.4% encompasses a range from 0.2 to 5.11% in rivers. Better grouping of the data would reduce variability for this data set. For instance, Kd's for several contaminants have been shown to decrease with increasing DOC. The processes controlling methylation and particle partitioning are site-specific, and the current report attempts to define complex chemical and biological processes across gradients by the use of a simple fraction. Since this factor (the amount of inorganic Hg that is converted to the bioaccumulative methyl form) is perhaps the most critical step in developing a BCF, a simple default conversion factor is not the best approach.

Finally, development of an acceptable model is mentioned within the report as a future goal, but I feel that model development and acceptance should be fast-tracked along with development of a National MeHg BAF. Models, such as the recent revisions of the Mercury Cycling Model (MCM), that incorporate processes such as methylation, aquatic speciation, and bioenergetics are keys to validation of the BAFs among contrasting sites. Having worked specifically with the MCM Model, I am confident that is has been tested on a number of contrasting environments (northern Wisconsin lakes, Everglades, Great Lakes) and could be used to validate BAFs for differing aquatic environments.

David Krabbenhoft

Overall, I found the document to be in very good order structurally, grammatically, and was of an appropriate length for the subject matter; my compliments to the authors. A quality manuscript makes the reviewer's job much easier, and a better technical review results when he or she is not "put off" for having to do editorial service too. I heartily support the U.S. EPA's decision to pursue changes to the AWQC for mercury and have methylmercury (MeHg) be the basis for such regulations. Although this has been a long time in coming, I do recognize that the peer reviewed data for this type of proposed change has been limited to just a few study locations until the past few years. That being said, however, I have serious reservations as to whether enough high quality data has been made available by the scientific community for the EPA to make an important decision like assigning "National BAF's". The authors of this report have largely done an admirable job with what is available, but it may be slightly ahead of its time. It may be that with the very recent release of the National Academy of Sciences report on human health and mercury, and the proposed decision time line of the EPA to enact emissions

regulations in the 5-year time frame, that a well-conducted, national-synoptic study to for the proper basis for a MeHg BAF's is in order.

David Maschwitz/Edward Swain

1. An update of the mercury bioaccumulation factor (BAF) is very much needed, for the reasons cited on page 1 of Section I. The new analytical methods that can measure ambient mercury in water at sub-nanogram per liter levels, and the large number of recent studies that provide field measured BAF data make the determination of a new BAF a necessity, if EPA plans to update the human health-based mercury criterion. The BCFs/BAFs used in previous EPA mercury criteria are clearly outdated. A new mercury BAF and criterion will be a great help to states and tribes (hereinafter, state). The determination of a BAF is often the biggest road block to the calculation of a human health-based water quality standard for state regulatory agencies.

2. The following comments are on *National Bioaccumulation Factors for Methylmercury* (Section I) and *Default Chemical Translator for Mercury and Methylmercury* (Section II). We have not reviewed for comment the background document.

3. The overall organization of Sections I and II, is logical, straight forward and easy to follow.

4. The EPA search for both available published and unpublished BAF data uncovered a substantial amount of new information; and, short of carrying out an independent literature search to confirm this comment, it should be reasonably complete and current.

5. The discussion of uncertainty associated with the final recommended BAFs (beginning on page 73, Section I), including a discussion of the limitations associated with reducing highly variable BAF data to a single national BAF (for each trophic level), and the myriad of variables that can affect BAFs, is appropriate. Further, EPA's rationale that, in spite of the uncertainty (actually, because of it), the recommendation of a single default BAF for each trophic level is valid. The recommendation that states should use local BAF data is good as well, but EPA must realize that local BAF data is not likely to be available in many situations. Thus, the default BAFs will get substantial use.

6. The decision to use only the preferred, field measured, BAF data (including the converted direct BAFs) and not use the indirectly determined BAFs (BCFs or BAFs times a FCM or BFM) is appropriate given the quality and quantity of the former. This is consistent with the proposed new EPA human health criteria methodology (EPA, 1998). However, including the comparison of direct and indirect BAFs in Section I (Tables 3-10 and 4-11) is valuable information.

7. To eliminate any uncertainty about the proper application of the translators listed in Tables 5-1 and 5-2, it is suggested that EPA include in Tables 5-3 through 5-10 columns showing the translators used in the conversions, and/or a column showing the "raw" as well as the converted BAFs. An alternative to expanding these tables is to add to the summary information at the beginning of each subsection (i.e., Variable, Definition, Estimate, Distribution) a section on "Translators" or "Conversion" that shows the translator(s) and conversion calculations (this option assumes the translators used and all the conversion calculations are the same for all the individual BCFs/BAFs). A third, but less desirable alternative, is to provide example calculations in the introductory discussion of converted methylmercury BAFs, beginning on page 49 of Section I.

8. Overall, we believe the final recommended BAFs (Table 5-15) are supported and a reasonable conclusion of the data analysis.

9. The introduction to Section II (page 1) talks about EPA's policy to use dissolved analyses for trace metals to measure compliance with the standard. This policy was developed in the context of the toxicity of particulate and chemically bound, versus the toxicity of "dissolved" or ionic forms, of trace metals to aquatic life. The science behind EPA's dissolved metal policy may not be as relevant to a highly bioaccumulative metal like mercury, for which the concern is the methyl form, and the risk is to human health through fish consumption rather than to aquatic life directly. EPA should expand this section to discuss if and how mercury differs from non-bioaccumulative trace metals with regard to the need or desirability of measuring dissolved metal in water.

10. EPA discusses in the "Background" part of Section II, total to dissolved metal conversion factors. Along the lines of comment number nine, the conversion factor of 0.85 for the current mercury criteria (CMC and CCC) are applicable to toxicity-based mercury criteria, not the human health-based chronic criterion (*Federal Register* 63: 68354-68364). The conversion factor for the chronic human health-based mercury criterion is 1.0 (see also *Federal Register* 60: 15392). EPA needs to

revise their discussion of conversion factors to reflect the conversion factor for the human health criterion, and to address the points made in comment number nine.

11. Separate average translators and K_D values for lakes, rivers and estuaries as derived in Section II seem to be reasonable and supported by the data presented.

Darell Slotton

I found the reports to be clear in their intent and in their explanation of approaches used. I especially appreciated the straightforward acknowledgment of the myriad sources of uncertainty and variability. My overall response to the entire exercise is that those sources of uncertainty and variability (geographic, water quality, water trophic status, analytical, individual organism, true trophic "level", food web complexity, etc.) make this a very difficult if not impossible proposition. I strongly support the development of tissue-based mercury criteria as the preferred mechanism for addressing mercury risk assessment and regulatory concerns throughout the huge range of aquatic systems affected. That said, if EPA has a legal charge to also develop the best predictive relationships it can as defaults, etc., the approach being used is probably as good as can be expected. It may be significantly more useful as a regional tool, though (e.g., northern midwestern lake systems, California rivers, Florida, etc). A truly applicable, nation-wide set of factors may be unattainable. I strongly concur with the suggestion that site-specific research is preferable in the event that BAFs are to be used.

ATTACHMENT B: DERIVATION AND CALCULATION OF "PSEUDO" K_DS FOR METHYLMERCURY

Derivation

$MeHg_d + TSS \rightleftharpoons Hg_p$, including $MeHg_p$

$$\text{"Pseudo"}K_D\text{MeHg / Hg} = \frac{Hg_P}{MeHg_D \bullet TSS} \quad \text{[Eqn. A.1]}$$

$$\text{Also: } K_D\text{MeHg} = \frac{MeHg_P}{MeHg_D \bullet TSS} \quad \text{[Eqn. A.2]}$$

Equating TSS and combining Eqn. A.1. and Eqn. A.2 yields:

$$\text{"Pseudo"}K_D\text{MeHg / Hg} \bullet \frac{MeHg_d}{Hg_p} = K_D\text{MeHg} \bullet \frac{MeHg_d}{MeHg_p}$$

Rearranging:

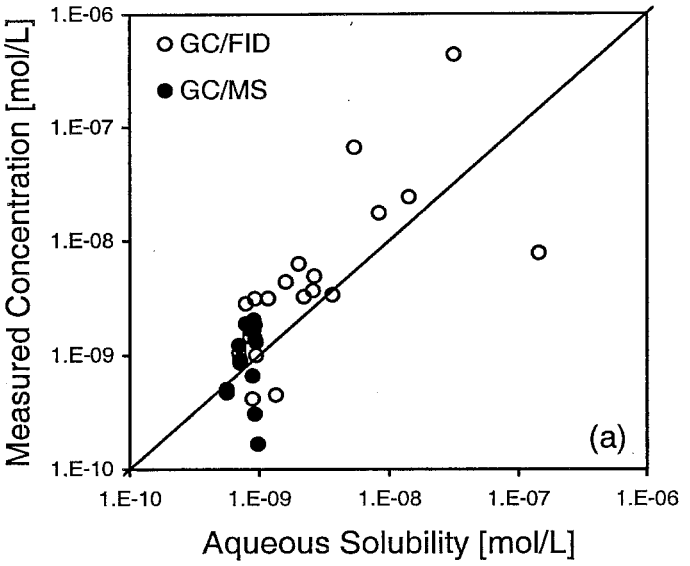
$$\text{"Pseudo"}K_D\text{MeHg / Hg} = \frac{Hg_p}{MeHg_d} \bullet \text{KMeHg} \bullet \frac{MeHg_d}{MeHg_p} \quad \text{[Eqn. A.3]}$$

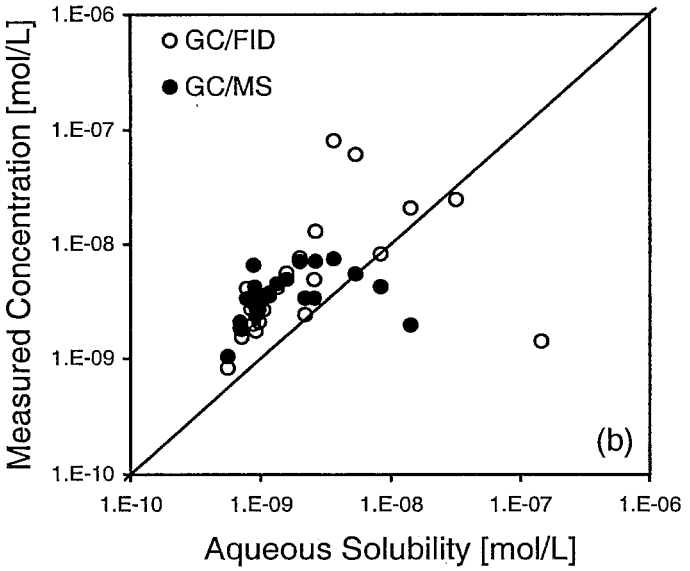
Example Calculation for Lakes (see text of original draft report for source of data)

- K_DMeHg = 338,844
- When $Hg_T = 1$, $MeHg_d = 0.032$, $Hg_d = 0.60$ and therefore $Hg_p = 0.40$
  and the ratio $Hg_p/MeHg_d = 0.40/0.032 = 12.5$
- When MeHg = 1, $MeHg_d = 0.613$, and therefore $MeHg_p = 0.387$
  and the ratio $MeHg_d/MeHg_p = 0.613/0.387 = 1.58$
- Substituting the above values in Eqn. A.3 gives:
  "Pseudo" K_DMeHg / Hg = 12.5 • 338,844 • 1.58 = 6,692,169
  Log "Pseudo" K_DMeHg / Hg = 6.83

Example Calculation for Rivers (see text of draft report for source of data)

- $K_D MeHg = 64{,}565$
- When $Hg_T = 1$, $MeHg_d = 0.014$, $Hg_d = 0.37$ and therefore $Hg_p = 0.63$
  and the ratio $Hg_p/MeHg_d = 0.63/0.014 = 45.0$
- When $MeHg = 1$, $MeHg_d = 0.49$, and therefore $MeHg_p = 0.51$
  and the ratio $MeHg_d/MeHg_p = 0.49/0.51 = 0.96$
- Substituting the above values in Eqn. A.3 gives:
  "Pseudo" $K_D MeHg / Hg = 45.0 \bullet 64{,}565 \bullet 0.96 = 2{,}789{,}208$
  Log "Pseudo" $K_D MeHg / Hg = 6.44$





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